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**TUTORIAL REVIEW**

# Designer peptide surfactants stabilize diverse functional membrane proteins

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Multi-spanning integral membrane proteins, including G-protein coupled receptors (GPCR), ion channels, and ion transporters, comprise a major class of drug targets. However, despite their vital importance, most molecular structures of membrane proteins remain elusive. This is largely due to lack of effective materials and methods to stabilize their functional conformation for sufficient time. Thus finding optimal surfactants and developing new approaches to study fundamental properties of unstable membrane proteins is urgently needed. In this *tutorial review* we summarize designer peptides with surfactant properties and their usefulness to stabilize membrane proteins. These peptide surfactants present new opportunities for the stabilization and characterization of diverse membrane proteins. Previous studies on the interaction between surfactant peptides and membrane proteins revealed strategies to design new peptides tailor-made for the stabilization of specific proteins. We review examples of solubilization, purification, long-term stabilization of membrane proteins, and the design principles of peptide sequences. We discuss future trends for exploiting spatial features, thermodynamic parameters, and self-assembling properties to create peptide surfactant structures to facilitate the characterization of diverse membrane proteins.

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## Introduction

Membrane proteins from families including G-protein coupled receptors (GPCRs), ion channels, cyclooxygenases (*e.g.* COX-2), and membrane-bound protein kinases are involved in key

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regulatory pathways and disease mechanisms and, therefore, are targets for drug development, bioassays, and fabrication of biosensors.[1–3] Integral and peripheral membrane proteins represent about one third of all genes in most genomes.[4,5] However, as of June 2011, membrane protein structures account for only about ~1% (*i.e.* 280 unique proteins among 777 membrane protein structures) of the ~72 000 structures currently deposited in the Protein Data Bank (PDB) (http://www.rcsb.org/pdb/, http://pdbtm.enzim.hu/, http://blanco.biomol.uci.edu/Membrane_Proteins_xtal.html).

As focus turned to membrane protein characterization it was realized that traditional biochemical approaches could not be employed. The difficulties associated with solubilization and stabilization of membrane proteins are mainly due to exposure of the transmembrane hydrophobic protein domains to an aqueous environment, which results in aggregation of the proteins. Synthetic or natural chemical detergents/surfactants can in many cases successfully solubilize membrane proteins following extraction from their native environment in the cell membrane[6–10] but they do not always facilitate long term stability.[11,12] Chemical detergents have denaturing properties that often lead to protein denaturation and can cause disorders in crystals of properly folded membrane proteins, thus hindering structural analyses.[13,14]

The discovery and tailor-made design of novel peptide surfactants presents compelling opportunities for stabilization[15–20] and crystallization of membrane proteins. In this review, we will highlight past development of designer peptide surfactants and recent advances across multiple applications in biotechnology and structural analyses of membrane proteins.

## Peptides for membrane protein stabilization

In general, designer peptide-based surfactants for membrane protein stabilization belong to three categories of peptide prototypes, the amphipathic helical peptides,[16] hybrid, peptide–lipid complexes[18] and various short, lipid-like peptide surfactants[21] (Fig. 1). A common strategy in the design of such peptides is the presence of hydrophobic and hydrophilic domains which, upon association with membrane proteins, results in better stabilization of the membrane proteins in aqueous media.

## Amphipathic α-helical peptide surfactants

Peptides with surfactant properties were first proposed as potential candidates for the stabilization of membrane proteins in 1993.[16] Peptitergent, PD1, a 24-amino acid peptide with sequence ac-EELLKQALQQAQQLLQQAQELAKK-$CONH_2$ was designed and synthesized with the aim to solubilize membrane proteins. PD1 folds into a 35 Å α-helix which is long enough to accommodate the cell membrane spanning domain of a membrane protein. The peptide contains 4 alanine residues at the center of the helix which form a flat hydrophobic surface that interacts with the hydrophobic transmembrane domain of the endogenous membrane protein. On the N- and C-termini of the α-helix, charged residues E and K were added to stabilize the helix with salt-bridges. In the absence of membrane proteins, crystallographic analysis revealed that PD1 self-assembles to form four α-helix bundles with the hydrophobic side of the α-helix in the interior of the bundle and the hydrophilic segment outside (Fig. 1A).

PD1 has been shown to keep bacteriorhodopsin in solution for at least two days and the majority of rhodopsin for five hours. High concentration of PD1 increased the activity of the trimeric G-protein guanosine triphosphatase, GTPase, of rat brain cortical membranes. However, the effect was reversed at low protein concentrations: a phenomenon that was attributed to binding of the peptide to the C-terminal α-helical region of the $G_\alpha$-subunit of the heterotrimeric protein.[17] Furthermore, PD1 did not stabilize the PhoE porin membrane protein[16] and showed limited effectiveness toward solubilization of two other integral membrane proteins (*i.e.* $Na^+/K^+$-exchanging ATPase and rat cortical membrane G-proteins) compared to typical chemical detergents such as sodium cholate, CHAPS, and Triton X-100.[17,22]

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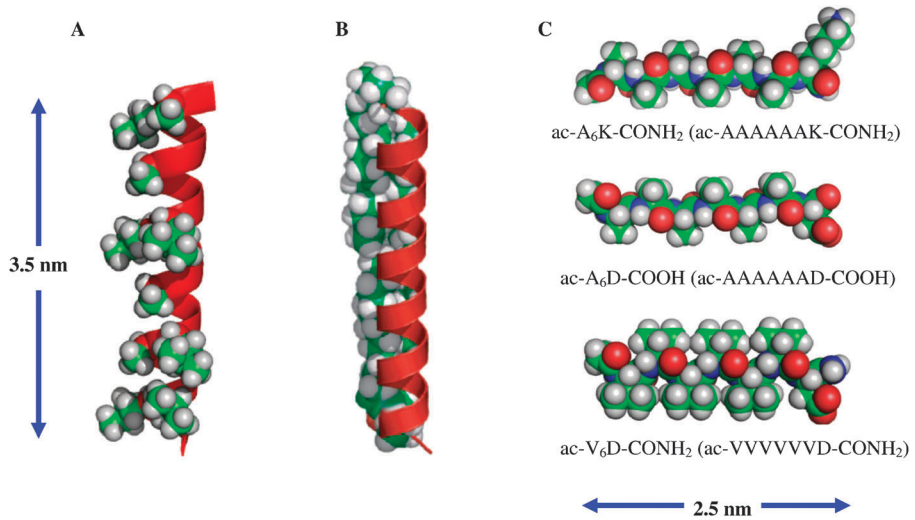


**Fig. 1** (A) Structure of the helical PD1 "peptitergent" with the hydrophobic side consisting of Leu and Ala amino acids depicted as spheres (hydrophilic amino acids are not shown for simplicity), (B) possible structure of the "lipopeptide" LPD with the alkyl chain shown as spheres and the peptide chain as helix, and (C) models of short, lipid-like, self-assembling peptide surfactants with length ≈ 2.5 nm (color code: carbon, green; oxygen, red; nitrogen, blue; hydrogen, white).

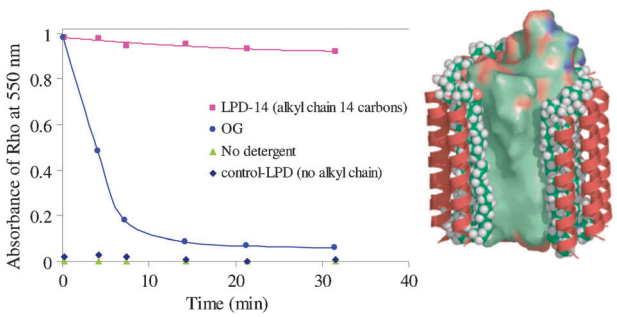


**Fig. 2** Proposed models of rhodopsin stabilization by LPD-14 lipopeptide which consists of a helical 25-amino acid peptide and two aliphatic 14-carbon chains attached to the ornithines at the C- and N-termini of the peptide. The solubilized membrane protein (solid surface) is surrounded by the LPD-14 (represented by the red α-helices and the space-filling aliphatic chain). Data in the graph are adapted from McGregor *et al.* and show the effect of LPD-14, OG, and of the control-LPD (helical peptide without the aliphatic 14-carbon chain) on the stability of rhodopsin's structural integrity which was assessed by the absorbance at 550 nm.[18]

## Lipopeptides

The lipopeptide, LPD, was designed and synthesized in 2003 using principles similar to those employed for the synthesis of PD1 with the addition of two alkyl chains attached to the C- and N-termini of the peptide (Fig. 1B).[18] The sequence of the peptide segment of LPD is ac-AOAEAAEKAAKYAA-EAAEKAAKAOA-$CONH_2$ with aliphatic tails varying in length between 12 and 20 carbons. LPDs in 1 to 15 molar excess were effective to solubilize α-helical and β-barrel type integral membrane proteins directly from cell membranes. It was suggested that membrane proteins should first be extracted, solubilized and purified using a traditional detergent which would be exchanged for LPD at a later stage for the stabilization of the membrane protein. For example, it was shown that bacteriorhodopsin purified in n-octyl-β-D-gluco-pyranoside (OG), detergent and exchanged for LDP at 0.5–2.5 μM was stable for up to a month in solution (Fig. 2).[18] Furthermore, LPD-16 was more efficient than DDM in stabilizing the membrane protein lactose permease and prevented aggregation at 37 °C for 5 days whereas LD-14 stabilized the folded form of the β-barrel membrane protein PhoPQ-activated gene P (PagP) for sufficient time to perform NMR analysis.

Notably, the "control" lipopeptide, which contains the same amino acid sequence but no aliphatic chains, is unstructured and did not show the characteristic α-helical profile in spectroscopic analyses.[18] Therefore, it was postulated that the aliphatic chains assist the formation of the α-helical conformation and the subsequent self-assembly of the LPD monomers. Despite the early success of lipopeptides, the prohibitive cost of large-scale production limited the widespread use of these peptides.

## Self-assembling lipid-like peptides

The first generation of peptide surfactants involved relatively long amino acid sequences (*i.e.* more than 20 amino acids) whose widespread use was largely constrained due to cost limitations. As part of the renewed interest in peptides with surfactant properties for the characterization of membrane proteins shorter peptides were developed. Since 2002, a number of lipid-like self-assembling peptides with amphiphilic properties were designed, synthesized, and characterized.[21,23–28] These short peptides contain 6–8 hydrophobic amino acids and 1–2 hydrophilic amino acids (Fig. 1C). The shorter length of these peptides makes them significantly cheaper than their predecessors.

The possibility of using these short peptides as stabilizing agents for membrane proteins was first evaluated in 2004 for the reaction center from the purple bacteria *Rhodobacter sphaeroides* and Photosystem I (PS-I) from spinach. It was shown that in the dry state the short peptide with sequence acetyl-AAAAAAK-

**Table 1** Peptide surfactants and their properties and applications in membrane protein stabilization and activity enhancement

| Peptide surfactant | Morphology | Applications | Ref. |
|---|---|---|---|
| ac-$A_6$D-COOH, ac-$V_6$D-COOH, ac-$V_6D_2$-COOH, ac-$L_6D_2$-COOH [7- or 8-peptides] | Self-assemble into vesicles, micelles, nanotubes | Stabilization of membrane proteins | 21 |
| ac-EELLKQALQQAQQLLQQAQELAKK-$CONH_2$, PD1 [24 amino acids] | α-Helix | Stabilization of the membrane protein P450 | 35 |
| ac-AOAEAAEKAAKYAAEAAEKAAKAOA-$CONH_2$ [25 amino acids with two 12–20 alkyl chains attached to the terminal ornithines] | α-Helix, assemble into cylindrical micelles | Stabilization of membrane proteins | 18 |
| ac-$V_6$D-COOH, ac-$V_6$K-COOH, ac-$A_6$D-COOH, ac-$A_6$K-COOH [7 amino acids] | Self-assemble into vesicles, micelles, nanotubes | Stabilization of the glycerol-3-phosphate dehydrogenase membrane protein | 15 |
| ac-$A_6$K-COOH and [ac-$A_6$K-COOH/ ac-$V_6$D-COOH]$_{mixture}$ [7 amino acids] | β-Sheet, self-assemble into vesicles, micelles | Stabilization of Photosystem-I (PS-I) in the dry state | 19 and 36 |
| ac-$A_6$D-COOH [7 amino acids] | Self-assemble into vesicles, nanotubes | Stabilization of the GPCR rhodopsin | 20 |
| ac-$I_6K_2$-$CONH_2$, ac-$A_6$K-$CONH_2$, ac-$V_6R_2$-$CONH_2$, ac-$V_6K_2$-$CONH_2$ [7- or 8-amino acids] | β-Sheet, self-assemble into vesicles, micelles | Stabilization and increased activity of Photosystem-I (PS-I) in solution | 29 |
| ac-$V_3$D-COOH, ac-$V_3$K-COOH, ac-$L_3$K-$CONH_2$, ac-$A_6$D-COOH, ac-$A_6$K-$CONH_2$ [4- or 8-amino acids] | β-Sheet | Increased expression and solubilization of the GPCRs hOR17-210 and mOR103-15 olfactory receptors, human formyl peptide receptor 3 (hFPR3), and human trace amine-associate receptor 5 (hTAAR5) in a cell-free expression system | 31 |

$CONH_2$ (ac-$A_6$K-$CONH_2$) stabilized PS-I for up to 3 weeks while for the same period of time ac-$A_6$K-$CONH_2$ mixed with ac-$V_6$D-$CONH_2$ stabilized the reaction center of the protein super-complex in solution.[19] Therefore, it was suggested that the peptide-stabilized proteins could be applied in solid-state electronic devices to generate energy from solar light (Table 1).

The stabilization of PS-I by peptide surfactants has also been studied in aqueous media. Matsumoto and colleagues performed a comparative study in which commercial detergents and peptide surfactants were tested for their effectiveness to stabilize functional PS-I (Fig. 4).[29] They showed that the best peptides for the stabilization of PS-I are in order of effectiveness: ac-$A_6$K-$CONH_2$, ac-$V_6K_2$-$CONH_2$, and ac-$V_6R_2$-$CONH_2$, which were significantly better than chemical detergents commonly used for the stabilization of membrane proteins. The ac-$A_6$K-$CONH_2$ peptide surfactant maintained the PS-I activity for more than 3 months in solution.

The effectiveness of short, lipid-like self-assembling peptides was also tested for their stabilization effect on the GPCR rhodopsin.[20] Bovine rhodopsin purified in OG-cell membrane lipid mixed micelles according to a standard protocol[30] has a half-life of ~71 minutes (Fig. 5). Addition of the ac-$A_6$D-$CONH_2$ peptide markedly improved the stability of bovine rhodopsin resulting in a half-life of 277 minutes, a fourfold increase (Fig. 5). Interestingly, when OG was replaced by a mixture of n-dodecyl-β-D-maltoside (DDM) and ac-$A_6$D-$CONH_2$, rhodopsin was significantly more stable at temperatures up to 40 °C.[20]

The possibility of using surfactant peptides during purification to solubilize and stabilize membrane proteins was investigated by Yeh *et al.*[15] Using a 40 : 60 mixture of ac-$A_6$D-$CONH_2$ and ac-$A_6$K-$CONH_2$, glycerol-3-phosphate dehydrogenase (GlpD) was extracted and solubilized from *E. coli* membranes up to *ca.* 60% of the total amount of membrane protein produced by *E. coli*. The effectiveness of the peptide surfactant mix was comparable to the solubilizing effect of traditional detergents such as Triton X-100 and DDM (*i.e.* 50%) but less effective than OG which achieved ~90% solubilization. However, in contrast to chemical detergents, the short, lipid-like peptides provided long-term stabilization of GlpD. Enzymatic activity tests showed that peptide surfactant-solubilized GlpD was active 10 times longer than GlpD solubilized in DDM or OG.

In a recent report, Wang and colleagues used peptide surfactants in a commercial cell-free system to produce the GPCRs human formyl peptide receptor 3 (hFPR3), human trace amine-associate receptor 5 (hTAAR5), and the olfactory receptors (ORs) hOR17-210 and mOR103-15.[31] Therein it was observed that the addition of peptide surfactants during GPCR production (without any added standard chemical detergents) resulted in significantly increased protein expression compared with the amount of GPCR produced without peptide surfactants. Notably, GPCRs expressed in the presence of peptide surfactants were soluble and had α-helical secondary structures which is characteristic of GPCR proteins. Furthermore, functionality assay tests using microscale thermophoresis showed that the mOR103-15 olfactory receptor expressed and stabilized using peptide surfactants was able to bind heptanal, which is its known ligand. The effect of the peptide surfactants on the increased expression, solubilization, and stabilization in the functional form of the GPCRs was

 

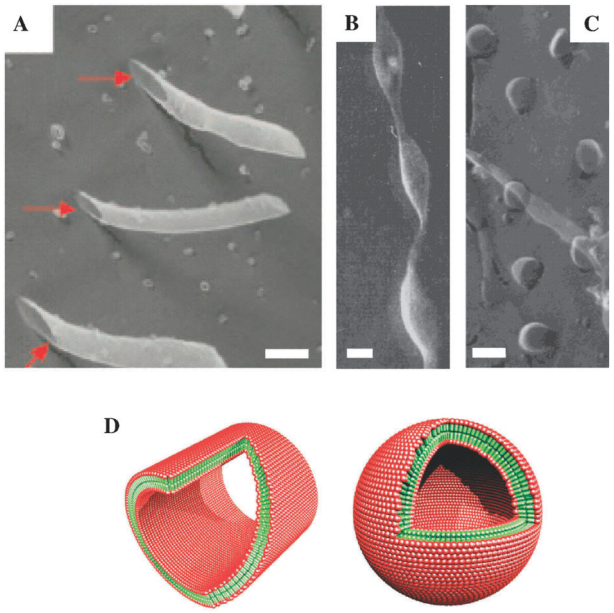


**Fig. 3** (A–C) Quick-freeze/deep-etch TEM images and (D) molecular modeling studies showing the morphology of cationic surfactant-like peptide assemblies: (A) ac-$V_6$D-$CONH_2$ at pH 7 forming nanotubes with diameter 30–50 nm and openings at the end (red arrows),[21] (B) ac-$A_6$K-$CONH_2$ at pH 4,[28] and (C) ac-$V_6K_2$-$CONH_2$ at pH 4.[28] Scale bar is 100 nm. (D) Molecular modeling of the self-assembly of the lipid-like peptide surfactants resulting in the formation of a bilayer, similar to biological phospholipids, to sequester the hydrophobic tails from the aqueous environment.[23,27,28] Unlike lipids in which hydrophobic interactions are responsible for the assembly of the lipids, in the proposed nanotube and nanovesicle bilayers the packed peptides would likely form hydrogen bonds with one another on the backbone.

also assessed by performing control experiments in which the GPCRs were produced in the presence of peptides which did not have surfactant properties.

## Design principles

The self-association of amphipathic helices to shield hydrophobic groups from being exposed to the polar solvent is a characteristic of many natural helices. The PD1 peptide was designed to (i) form an α-helix, (ii) be slightly longer than the thickness of the cell membrane bilayer, (iii) have the C- and N-termini capped to neutralize destabilizing charges and dipole effects of the helix, and (iv) have glutamic acid and lysine amino acids at the helix termini to form salt bridges and stabilize the helix.

Some of the design principles that were employed in the design of PD1 (*e.g.* α-helical conformation, length of the molecule, polar and hydrophobic residues on opposite sides of the α-helix, capped N- and C-termini of the peptide) were also applied in the case of the LPD lipid–peptide hybrid. The novelty of LPDs lies on the addition of two ornithines in positions 2 and 24 of the peptide sequence for the coupling of the aliphatic chains. Ornithines were selected as the residues for the linkage because they can facilitate better orientation of the aliphatic tails. This linkage resulted in a molecule in which the aliphatic chains could associate with the non-polar side of the peptide.

LPD monomers were designed to have a ''wedge'' shape, with the diameter of the LPD's amphipathic peptide helix slightly larger than that of the aliphatic chain that aligns along the hydrophobic face of the helix. Hence, it was assumed that LPDs self-associate into cylindrically shaped micelles whereas traditional detergents and phospholipids form spherical vesicles and micelles. It was postulated that LPDs form more compact and rigid protein–LPD surfactant complexes compared to those observed upon association of chemical detergents with membrane proteins.

Short, lipid-like peptide surfactants were designed to resemble the structure and dimensions of natural lipids. A comparative analysis showed that the design of an effective lipid-like

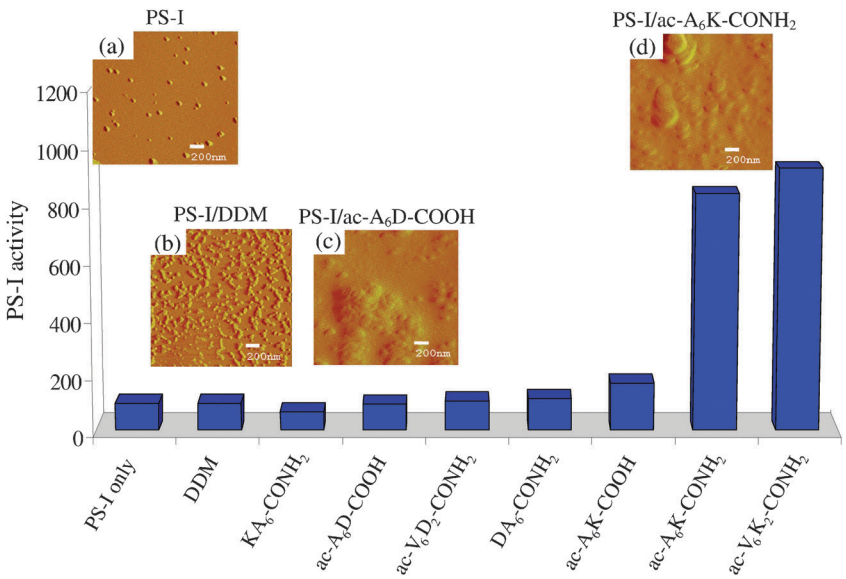


**Fig. 4** Biological activity of PS-I stabilization by short, lipid-like peptide surfactants and in the presence of the chemical detergent DDM. AFM images show (a) the PS-I multi-protein complex which natively appears as dimer or trimer with diameter between 30–50 nm, (b) DDM mixed with PS-I, (c) PS-I with ac-$A_6$D-COOH, and (d) PS-I with ac-$A_6$K-$CONH_2$. DDM dispersed PS-I to small particles whereas PS-I mixed with ac-$A_6$K-$CONH_2$ or ac-$A_6$D-COOH resulted in large and small vesicles, respectively. Scale bar is 200 nm.[29]

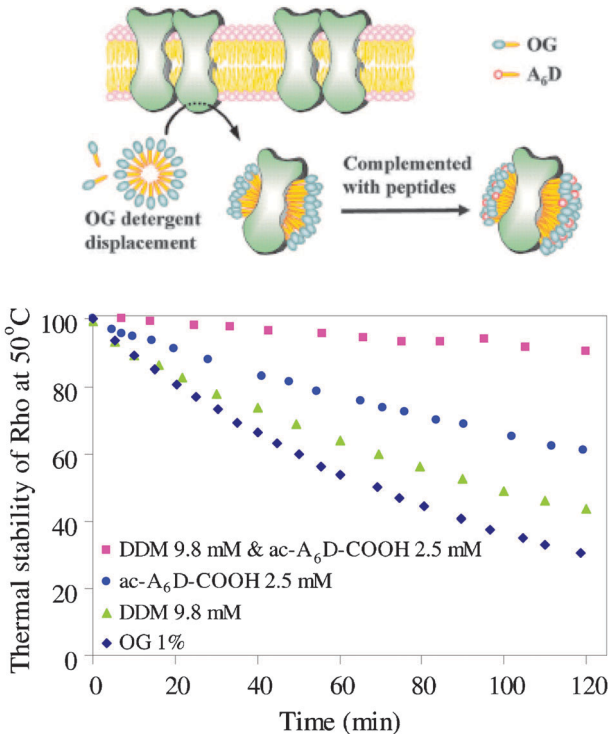


**Fig. 5** GPCR bovine rhodopsin stabilization by short, lipid-like peptide surfactants (Fig. 6). The membrane protein was initially extracted from the cell membrane with OG chemical detergent and subsequently was stabilized by ac-$A_6$D-COOH. Data in the graph are adapted from Zhao *et al.*[20] and show increased stabilization of bovine rhodopsin as a function of time in the presence of a mixture of DDM/ac-$A_6$D-COOH compared to ac-$A_6$D-COOH and DDM alone as well as in the presence of OG.

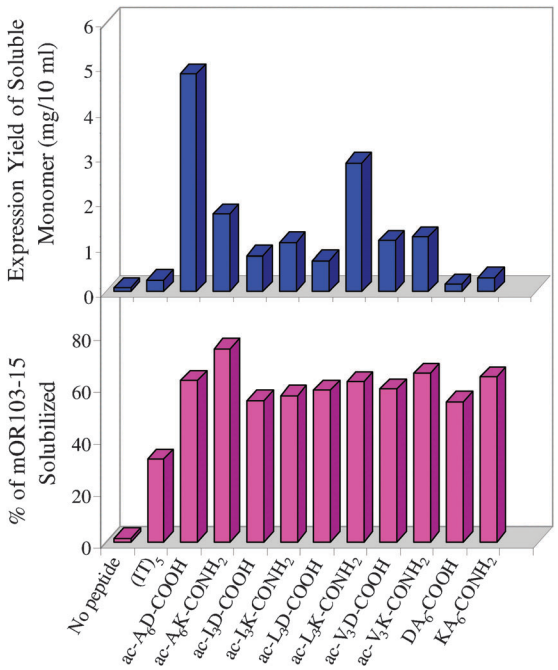


**Fig. 6** Expression yields of the mOR103-15 olfactory receptor (blue bars) using a cell-free expression system in the absence and in the presence of peptide surfactants as well as in the presence of the control peptide $(IT)_5$ which does not have surfactant properties. Following expression of the olfactory receptor, solubilization of the protein pellet was attempted using peptide surfactants (magenta bars). Data in the graph are adapted from Wang *et al.*[31] and show increased production and solubilization of the olfactory receptor in the presence of peptide surfactants compared to controls.

peptide surfactant for the stabilization of membrane proteins should take into consideration two main factors: (i) the type of charge at the hydrophilic head of the peptide and (ii) the charge distribution on the peptide sequence. In particular, it was demonstrated that positive charges on the C-terminal end of the peptide (*e.g.* ac-$V_6K_2$-$CONH_2$ and ac-$A_6K$-$CONH_2$) are essential for the stabilization of the PS-I membrane protein[29] whereas stabilization of bovine rhodopsin was observed when the negatively charged peptide surfactant ac-$A_6$D-COOH was used (Fig. 4 and 5).[20] However, peptides carrying a negative charge in the C-terminus such as ac-$V_6D_2$-$CONH_2$, ac-$A_6$D-COOH and $DA_6$-$CONH_2$ did not have a significant effect on stabilizing functional PS-I.

Furthermore, the amino acid sequence is equally important for the stabilization of functional PS-I (Fig. 4). Experiments showed that the interaction of ac-$A_6K$-$CONH_2$ with PS-I significantly increased the protein activity whereas the $KA_6$-$CONH_2$ peptide had only a minor effect on PS-I activity. To design an efficient membrane protein stabilization surfactant it is important that the conformational characteristics of the peptide match those of the protein complex. Stabilization also depends on the peptide's amino acid sequence, the acetylation and amidation of the N- and C-termini, respectively, the hydrophobicity of the amino acids in the hydrophobic tail of the peptide and the hydrophilicity and polarity of the amino acids in the polar head of the peptide.

The importance of peptide length, charge distribution, and type of charge was also demonstrated in the work of Wang *et al.* in which the yields of the soluble GPCRs hFPR3, hTAAR5, and of the olfactory receptors hOR17-210 and mOR103-15 were compared in the presence of different peptide surfactants.[31] For instance, it was shown that in the case of the mOR103-15 olfactory receptor the negatively (−2) charged ac-$A_6$D-COOH peptide is better for increased expression in the cell-free expression system compared to the $DA_6$-$CONH_2$ (negatively charged but contains one negative charge (−1) and has different charge localization on the peptide sequence compared to ac-$A_6$D-COOH) and the ac-$A_6K$-$CONH_2$ and $KA_6$-$CONH_2$ (positively charged) peptides (Fig. 6). Furthermore, the use of the ac-$A_6$D-COOH peptide, which consists of 7 amino acids, results in significantly increased production compared to shorter peptide surfactants regardless of the charge (*e.g.* the negatively charged ac-$I_3$D-COOH, ac-$L_3$D-COOH, ac-$V_3$D-COOH and the positively charged ac-$I_3K$-$CONH_2$, ac-$L_3K$-$CONH_2$, ac-$V_3K$-$CONH_2$) and hydrophobicity/hydrophilicity of the amino acids in the peptide chain.

## Mechanism of interaction between peptide surfactants and membrane proteins

Although many detergents are available for the stabilization of membrane proteins, their behavior in solution and in the presence of the membrane protein is largely unknown. A better

understanding of the structure of peptide surfactant micelles and of the protein–peptide surfactant complexes is thus crucial for structural biologists and membrane protein biochemists. To date, the mechanism of membrane protein stabilization by peptide surfactants remains unclear. It is generally speculated that cell membrane lipids are substituted by peptide surfactants although the crystal structure of such membrane protein–peptide surfactant complexes has not yet been determined to confirm the assumption.

The structure of PD1 alone, but not in complex with a membrane protein, has been determined. The peptide structure alone, however, is not sufficient to determine the mechanism by which membrane proteins are stabilized by PD1.[16] Stroud and colleagues postulated that the mechanism for PD1 stabilization of rhodopsin and bacteriorhodopsin involves shielding of the peptide's hydrophobic residues from water through interaction with the hydrophobic domains of the membrane protein.

In the case of LPDs, it was estimated that 10–15 peptides per protein molecule are necessary to form the stable LPD–membrane protein complex in which the LPDs self-assemble to form a belt surrounding the membrane protein. In this complex, LPD is oriented so that its aliphatic segments face the membrane protein and the side with polar amino acids is exposed to the solvent (Fig. 2).[18]

A similar mechanism was proposed for the stabilization of rhodopsin by Zhao *et al.* (Fig. 5).[20] It was postulated that short, lipid-like peptides mixed with chemical detergents accumulate around the transmembrane domains of the protein and orient with their hydrophobic tail toward the protein and the hydrophilic head exposed to the solvent. Mixing short, lipid-like peptides with chemical detergents resulted in stabilization of rhodopsin better than the peptides and the chemical detergents alone and prevented aggregation of the protein molecules in water.

On the other hand, the proposed mechanism of PS-I stabilization by short, lipid-like peptide surfactants is different. Experimental evidence showed that peptide surfactants do not bind to the protein in a tight and irreversible mode. Rather integration of the membrane protein into the peptide vesicle bilayer is similar to nature's mechanism of membrane protein stabilization in which membrane proteins are embedded in the cell membrane (Fig. 4).[29] Formation of peptide surfactant vesicles is not sufficient for the stabilization of PS-I. The size of the supramolecular peptide assemblies must be large in order to have PS-I, a multi-subunit trimeric protein with a diameter of 50 nm, properly embedded in the vesicle. Matsumoto and colleagues also proposed that chemical and structural compatibility at the atomic and macroscopic level between peptide surfactant assemblies and PS-I are crucial for increased activity and stabilization for extended periods of time.

## Physical, chemical and morphological properties of peptide surfactants

There have been relatively few systematic studies of peptide surfactants alone or associated with membrane proteins. Consequently, there is little knowledge on the influence of the peptide structure, amino acid sequence, charge distribution, solubility, self-assembling properties, *etc.* on membrane protein stability and function.

Bavec and coworkers attempted to determine the critical micelle concentration (CMC) of PD1 in solution.[17] PD1 stabilized and increased the activity of GTPase at a concentration of 1.5 μM. However, in the concentration range of 0.05–0.10 mM in TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 7.5), PD1 did not form vesicles or micelles. Therefore, it was suggested that the helical peptide surfactant PD1 should not be considered as a typical detergent like Triton X-100 or CHAPS.

In water, the CMC of LPDs with aliphatic chains ranging from 12 to 20 carbon atoms was estimated to be less than 1 μM.[18] These CMC values are significantly lower than that of most chemical detergents. For example, DDM which also has a 12-carbon aliphatic chain has a CMC of ~180 μM. The extremely low CMC of the LPDs may present a difficulty in protein purification because the surfactant could not be readily removed by dialysis.

Relatively mild surfactant properties were observed for the short, lipid-like peptides which, depending on the peptide sequence, were characterized by CMC values between 0.1 and 1.0 mM in water. Lower CMC values were measured in buffer solutions containing 0.15 M NaCl.[29,32]

Microscopic analyses revealed different morphologies for the short, lipid-like peptide surfactants depending on the amino acid sequence, the type and concentration of the electrolyte, the pH, the CMC value and their concentration in solution. Transmission electron microscopy (TEM) analysis of quick-freeze/deep-etch sample preparations resulted in stunning images of nanovesicles, micelles, and nanotubes.[21,23,28] Several structural transitions were observed from spherical to cylindrical to lamellar micelles as the concentration of surfactant was increased. TEM, cryo-TEM, AFM, and small angle X-ray scattering (SAXS) analyses demonstrated that nanotubes consisting of short, lipid-like peptide surfactants are hollow, similar to the lipid microtubes.[21,32,33] TEM imaging of these peptides in a solution with pH above their isoelectric point revealed that the nanotubes collapsed and formed sheets.[28] The dynamic nature of these systems and the morphology of the macroscopic formations were observed for both anionic and cationic short, lipid-like peptide surfactants.[23,28,29,32,34]

Molecular modeling studies in water suggested that peptide monomers self-assembly involves side-by-side and tail-to-tail bilayer formation creating a unilamellar shell with a thickness of approximately 5–6 nm. The hydrophobic tails pack together to avoid water whereas hydrophilic heads are exposed to water in the inner and outer portion of the tube or the vesicle (Fig. 3).

## Peptide surfactants for applications in molecular biology, biotechnology, and nanotechnology

In addition to the considerable interest in stabilizing membrane proteins for structural analyses, membrane protein stabilizers have profound potential applications in molecular biology for studying the interaction of membrane proteins with signaling molecules and drug targets. An interesting approach to stabilize membrane proteins in aqueous media was presented by Schoch and colleagues.[35] In their strategy,

the bases encoding PD1 were fused to the 5′-end of a plant cytochrome P450 enzyme gene. The addition of PD1 resulted in stabilization of the protein in water for several days thus enabling the acquisition of good quality NMR spectra of the substrate bound to the active site of the P450 enzyme.

Zhang and coworkers tested a mixture of the peptide surfactants ac-$A_6K$-$CONH_2$ (cationic) and ac-$V_6D$-$CONH_2$ (anionic) for the construction of solid-state photovoltaic cells.[36] Therein, it was shown that the short, lipid-like peptide surfactants not only stabilized but also increased the functionality of photosynthetic complexes that were integrated in an efficient light-harvesting device.

## Conclusions and future perspectives

Designer peptide surfactants exhibit advantageous properties over traditional chemical detergents for a number of reasons: (i) they can be easily designed and synthesized, (ii) with the exception of the costly LPD lipid–peptide hybrids, production may be scaled-up at modest costs, *e.g.*, short peptides cost about $26 per gram, (iii) they stabilize membrane proteins through mild interactions thus minimizing the risk of protein denaturation, (iv) their properties can be fine tuned to match the specific membrane protein's stabilization requirements simply by changing the amino acid sequence.

The fact that traditional chemical detergents are not sufficient in stabilizing diverse membrane proteins necessitates further research for alternative surfactants, which will lead to rapid advancements in the field of surfactant peptides. A major area of future work will be the expansion of the capabilities of peptide surfactants so that each component of the peptide's amino acid sequence plays a distinct role in the stabilization and crystallization of membrane proteins in their native conformation. Essential to meeting this goal is establishment of guidelines for the design of peptide surfactant sequences that can be fine tuned to stabilize multiple membrane proteins for prolonged periods of time and will not obstruct the crystallization process.

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