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Lipopeptides: from self-assembly to bioactivity

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This Feature Article discusses several classes of lipopeptide with important biomedical applications as antimicrobial and antifungal agents, in immune therapies and in personal care applications among others. Two main classes of lipopeptide are considered: (i) bacterially-expressed lipopeptides with a cyclic peptide headgroup and (ii) linear lipopeptides (with one or more lipid chains) based on bio-derived and bio-inspired amino acid sequences with current clinical applications. The applications are briefly summarized, and the biophysical characterization of the molecules is reviewed, with a particular focus on self-assembly. For several of these types of biomolecule, the formation of micelles above a critical micelle concentration has been observed while others form bilayer structures, depending on conditions of pH and temperature. As yet, there are few studies on the possible relationship between self-assembly into structures such as micelles and bioactivity of this class of molecule although this is likely to attract further attention.

Introduction

Lipopeptides are a remarkable class of self-assembling molecule that are able to form peptide-functionalized supramolecular nanostructures. Lipopeptides are amphiphilic molecules which incorporate one or more lipid chains attached to a peptide headgroup. Self-assembly is observed depending on the hydrophile/lipophile balance of the molecules as well as interactions between the peptide units. The self-assembly of synthetic bio-mimetic and bio-inspired lipopeptides has been the subject of previous reviews and this is not covered again here.¹ We present an up-to-date overview on the self-assembly of bio-derived and bio-active lipopeptides, with proven activity in on-the-market applications in medicine and other fields such as crop protection.

Compared to the self-assembly of lipids, that of lipopeptides is relatively unexplored. This is remarkable, given their relevance to important applications in biomedicine, including use as last resort antimicrobial agents to treat for example MRSA (methicillin-resistant *S. aureus*) infections (e.g. daptomycin, Fig. 1),² use in immune disease therapies (e.g. Pam₃CSK4, Fig. 13)³ and in cosmeceuticals (e.g. Matrixyl™, C₁₆-KTTKS).⁴ Other applications of lipopeptides extend beyond human medicine, including use as fungicides for crop treatment (e.g. surfactin, Fig. 7b),⁵ avoiding adverse environmental effects associated with a conventional synthetic pesticides.^{5b,6} For potential biomedical applications, lipopeptides offer advantages compared to peptides of improved amphiphilicity and compatibility with the lipid wall of cell membranes, enabling for example the delivery of actives into

cells *via* endocytosis (*vide infra*). In addition, the self-assembly of lipopeptides facilitates the presentation of peptide functionalities at high density on the surface of nanostructures such as fibrils, micelles and vesicles.

In the main, naturally expressed bioactive lipopeptides contain a cyclic peptide headgroup attached to a single lipid chain. Cyclisation of the peptide unit as in many bacterially expressed lipopeptides enhances the *in vivo* stability of the motif compared to the linear counterpart (and thus can be expected to have evolved to be expressed in the cyclic form). This is due to reduced proteolysis resulting from the absence of free C- and N-termini.⁷ Of course, cyclisation also restricts the peptide conformation which may also be relevant to bioactivity. Expressed lipopeptides usually comprise molecules with a range of lipid chain lengths (typically in the range C₁₄–C₁₈) and conformations. Another class of bioactive lipopeptides is usually produced synthetically based on bio-derived sequences. These lipopeptides often contain a linear peptide headgroup attached to one, two or three



Fig. 1 Molecular structure of daptomycin.¹¹

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hexadecyl (palmitoyl) lipid chains. In the following we use the notation C₁₆ or Pam for a hexadecyl lipid chain, depending on conventional usage for the relevant lipopeptide.

This review is focussed on lipopeptides, and does not cover the fascinating field of lipoproteins which also have a variety of *in vivo* biological roles, for example apolipoproteins are the major component of high density lipoproteins which are a risk factor associated with cardiovascular disease. The structure of apolipoproteins has been reviewed elsewhere.⁸ This is a mini-review aiming to introduce the main classes of bioactive lipopeptides investigated to date, relating their structure to activity and self-assembly properties where these have been investigated (which is only the case for a few materials). It is not our purpose to exhaustively review all studies on the biological activity of lipopeptides, of which there are many hundreds published. We apologise to the authors of papers whose work has not been included.

Previous reviews have been published covering lipopeptides as a class of biosurfactant.⁹ In addition, reviews focussed specifically on the natural functions of lipopeptides from *Bacillus* and *Pseudomonas* species are available.^{6b,10}

As discussed in the following, lipopeptides may self-assemble into spherical micelles above a critical micelle concentration (cmc) or into other structures (especially nanofibrils and nanotapes), in which case we use the notation critical aggregation concentration (cac) to denote the concentration above which self-assembly is observed.

Bacterially expressed cyclic lipopeptides

Lipopeptides are expressed by many bacteria, having several roles including activity against other microorganisms including bacteria, fungi and viruses, involvement in bacterial motility and swarming behaviour and in attachment to surfaces.^{6b,10e} The host defense mechanism produces lipopeptides in response to other microorganisms including fungi, viruses, mycoplasma and bacteria. Two genera of bacteria, *Bacillus* and *Pseudomonas* have produced lipopeptides that have been particularly extensively studied. They have important applications due to antibacterial, antifungal and antiviral activity as discussed in the following.

Daptomycin (Fig. 1) is a lipopeptide comprising a decanoyl lipid chain attached to a partly cyclised 13-amino acid peptide. Daptomycin is produced by the Gram-positive bacterium *Streptomyces roseoporous*.¹² It has potent antimicrobial properties and is clinically approved for use as an antibiotic for serious infections caused by Gram-positive bacteria such as MRSA (methicillin-resistant *Staphylococcus aureus*) and vancomycin-resistant enterococci.^{12a} It is marketed under the tradename Cubicin by Cubist Pharmaceuticals^{2c,13} (recently acquired by Merck). It incorporates two non-natural amino acids: L-kynurenine, which is unique to daptomycin, and L-3-methylglutamic acid. The former residue, containing an aniline unit, gives rise to interesting fluorescence properties, in particular an emission peak at 460 nm.¹⁴ The mode of activity of daptomycin is still unclear.^{12a,15}

One hypothesis involves the inhibition of lipoteichoic acid synthesis (lipoteichoic acid is a proteoglycan which is a major component of the cell wall of Gram-positive bacteria).¹⁵ Another proposed mechanism involves disruption of bacterial membrane potential, for example *via* pore formation.¹⁵ The latter mechanism seems favoured based on recent work examining ion-dependent pore formation in model membranes,¹⁶ along with the fact that its activity is calcium ion dependent.¹⁷

The existence of a critical aggregation concentration (cac) has been determined for daptomycin by making use of the afore-mentioned fluorescence properties of the lipopeptide, as well as NMR and light scattering techniques.^{14b} The cac has been measured as a function of pH, temperature and calcium ion concentration.^{14b} The aggregation number in aqueous solution at pH 4–6.5 is reported as (18 ± 2). Fig. 2 shows a cryo-TEM recently obtained by our group showing spherical micelles formed by daptomycin.

The echinocandins are a class of cyclic lipopeptide compounds with antifungal activity. Examples of this class of molecule are shown in Fig. 3. The most widely known is caspofungin, marketed as a drug by Merck and Co. The antifungal activity of this class of compound is due to the interaction of lipopeptide molecules with the insoluble polysaccharide component of the cell wall of fungal cells, specifically due to the inhibition of the synthesis of glucans.¹⁹ The interactions of echinocandins with *Candida* fungi such as *C. albicans* has been particularly well studied.^{19,20} The self-assembly properties of these compounds seem to be largely unexamined although Eli Lilly have patented micellar formulations of echinocandins with conventional surfactants.²¹

Viscosin (Fig. 4) is a cyclic lipopeptide obtained from *Pseudomonas libanensis*²³ or *Pseudomonas fluorescens*.²² It has antibiotic activity as shown against the tubercle bacillus.²⁴ Viscosin is highly surface active^{23a,25} and is able to inhibit the migration of cancer cells.^{23b} Fig. 5 shows surface tension data used to determine a cmc = 0.15 mg ml⁻¹ for viscosin in aqueous solution. Viscosin and the related cyclic lipopeptide massetolide (Fig. 4) also produced by *P. fluorescens* are able to protect it from protozoan predation.²²

Lichenysins,²⁷ pumilacidins²⁸ and polymyxin B (Fig. 6)²⁹ are classes of antimicrobial lipopeptides produced by *Bacillus*

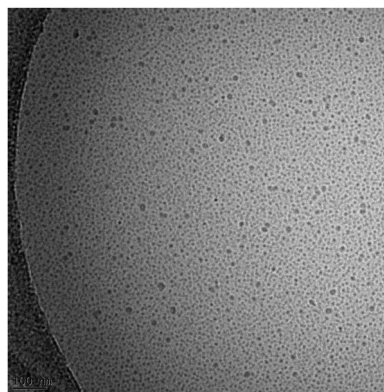


Fig. 2 Cryo-TEM image of micelles formed by daptomycin (2.5 mM in 0.625 mM CaCl₂).¹⁸





Fig. 3 Molecular structure of several echinocandins. Adapted from ref. 19. (a) Caspofungin, (b) micafungin, (c) anidulafungin.



Fig. 4 Viscosin ($R = H$) and massetolide ($R = CH_3$).²²

licheniformis, *Bacillus pumilus* and *Bacillus polymyxa* respectively. Lichenysin A (Fig. 7a) is a powerful surfactant, and antimicrobial activity against several Gram positive and Gram negative bacteria, although not as great as that observed for surfactin.^{27a} Several pumilacidins show antiviral activity against herpes simplex virus as well as anti-ulcer activity.²⁸ Polymyxin B (Fig. 6) and polymyxin E (colistin) show antibiotic activity against a range of Gram-negative bacteria.²⁹ The two molecules are distinguished by the substitution of a D-leucine in colistin with a D-phenylalanine in polymyxin B.^{26b} The mode of action of these lipopeptides has been proposed to be membrane disruption due to interaction between the cationic polymyxin and the anionic bacterial outer membrane leading to a detergent-like activity.²⁹



Fig. 5 Surface tension versus concentration used to determine the cmc for viscosin (shown by arrow). Redrawn from ref. 23a.

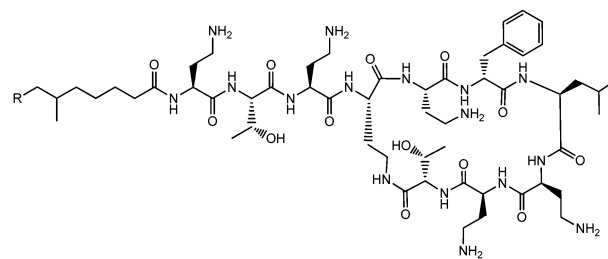


Fig. 6 Structure of polymyxin B.²⁶ Polymyxin B is a mixture of lipopeptides with different lipid chain lengths (here indicated by R).

Fig. 7 shows the structure of several lipopeptides produced by *B. subtilis*.³⁰ Variants of surfactins, iturins and lichenysins can be genetically engineered to incorporate different amino acids (*via* for example, incorporation in the bacterial culture medium). Synthetic variants have also been examined. The engineered/synthetic *Bacillus* lipopeptides have distinct structure–activity relationships as reviewed elsewhere.^{5a,b,10b} All of these classes of lipopeptide are potent antifungal agents with applications in crop protection.^{6a,31} The effect of surfactin on the swarming behaviour of *B. subtilis* has been examined, its absence reducing swarming activity.³²

Cyclic lipopeptides produced by *Pseudomonas* species include the viscosin group (as discussed above), the amphisin group, the syringomycin group and the tolaasin group (Fig. 8).^{10e,33} The biosynthesis of these lipopeptides is discussed elsewhere.^{10e} There are few studies on the aggregation behaviour of these lipopeptides although a report on the biosurfactant and ion-channel forming properties of two lipopeptides from the syringomycin group is available.³⁴ This includes a cmc measurement of 0.8 mg ml^{-1} for both syringomycin and syringopeptin from a *Pseudomonas syringae* strain. Calcium ion channel formation was observed at nanomolar concentrations showing the cytotoxicity of these lipopeptides to plant cells.³⁴

Surfactin is a highly surface-active biosurfactant reducing the surface tension of water to 27 mN m^{-1} at a concentration as low as $20 \text{ } \mu\text{M}$.^{10b} The cmc at room temperature is $7.5 \text{ } \mu\text{M}$.

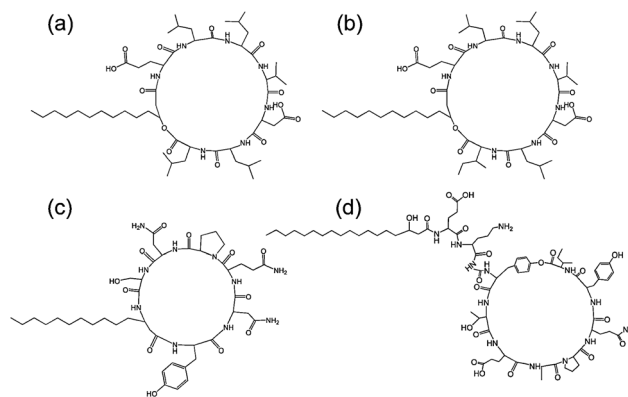


Fig. 7 Structure of some lipopeptides produced by *B. subtilis*. (a) Surfactin (b) lichenysin A, (c) iturin A, (d) fengycin/plipastatin A. Redrawn from ref. 5b. As detailed in ref. 5b, the expressed lipopeptides actually comprise a mixture of lipid chain lengths.





Fig. 8 Molecular structures of lipopeptides produced by *Pseudomonas* species. (a) Amphisin³⁵ (b) syringomycin,³⁶ (c) tolaasin I.³⁷

The temperature dependence of the cmc has been examined, along with the thermodynamic parameters of micellization, *via* isothermal titration calorimetry.³⁸ The cmc of surfactin is very low compared to that of many surfactants such as non-ionic surfactants of the C_nEO_m class where EO denotes ethylene oxide and the subscripts are the number of repeats. This is shown in Fig. 9 which plots the water/phospholipid bilayer partition coefficient against cmc.³⁸ The partition coefficient, K , was analysed as part of a study on membrane permeabilization. There is also a relationship between the surfactant-to-lipid mole ratio at the onset of solubilisation and $K \times \text{cmc}$.³⁸

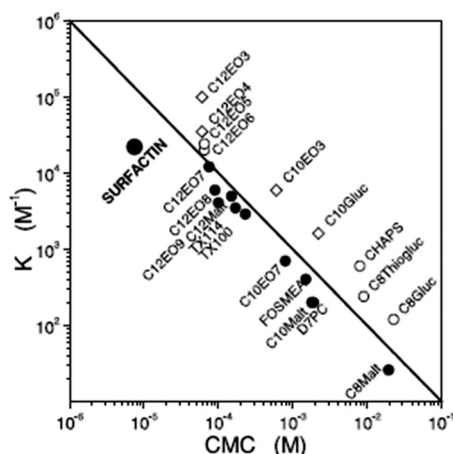
The aggregation of surfactin (Fig. 7a) in bulk solution and at air/water and hydrophobic solid/water interfaces was investigated using neutron scattering techniques.³⁹ Small-angle neutron scattering indicated the presence of small (association number $p = 20$) spherical micelles in bulk aqueous solution whilst at the

interface (either air/water or silane/water) surfactin was found to adopt a globular conformation.³⁹ Neutron reflectometry was also used to probe the interaction between surfactin and solid-supported phospholipid bilayers – below the cmc of surfactin a mixed bilayer was reported.⁴⁰ Later, as part of a study also including other *Bacillus subtilis* lipopeptides, the self-assembly of surfactin in aqueous solution was investigated *via* cryogenic transmission electron microscopy (cryo-TEM) and small-angle X-ray scattering (SAXS), and the secondary structure was probed using circular dichroism (CD) and FTIR spectroscopic methods.³⁰ Cryo-TEM and SAXS revealed spherical micelles with a radius of 2.0 nm, consistent with the findings of Shen *et al.*³⁹ but contradictory to the report of Ishigami *et al.*⁴¹ who reported large rod-shaped micelles, albeit in a different buffer (NaHCO_3). CD and FTIR were consistent with random coil and turn structures for surfactin (again contrary to Ishigami *et al.* who reported β -sheet secondary structure⁴¹). Similar findings in regard to self-assembled nanostructure and secondary structure were noted for plipastatin (Fig. 7d). Unexpectedly, and in complete contrast to surfactin and plipastatin, mycosubtilin shows a distinct self-assembly motif – extended nanotapes based on bilayers of lipopeptide molecules.³⁰ This difference may be due to branching in the lipid chains of mycosubtilin and/or due to conformational differences in the cyclic peptide headgroup, although plipastatin also includes backbone and side-chain aromatic units.

The critical micelle concentration of iturin A (Fig. 7c) was determined by fluorescence assays using the intrinsic fluorescence of the D-tyrosyl residue (which is common to all iturins^{10a}) leading to a value of 25–40 μM in water.^{10a,42} The distribution of conformers was found to be influenced by the cmc. The interaction of the lipopeptide with model (egg phosphatidylcholine) vesicles was investigated: above the cmc, fluorescence revealed self-association of iturin A inside the lipid membranes, an effect not observed below the cmc.⁴² The micelle size and aggregation number have been estimated.²⁵ Far above the cmc, TEM images suggest the presence of vesicles and an interdigitated bilayer structure was proposed for the vesicle walls.⁴³ Iturin A has been shown to form potassium ion-conducting channels in lipid bilayers.^{10a,44}

Surfactin is able to permeabilize lipid membranes,^{38,45} and it forms ion channels in planar lipid bilayer membranes.⁴⁶ The interaction of surfactin with POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) vesicles has been investigated *via* isothermal titration calorimetry and this has been correlated to vesicle lysis probed by calcein fluorescence.⁴⁷ Detergent-like permeabilization effects were observed on increasing concentration even below the cmc (in the range 7.5 μM to 10 μM depending on buffer^{38,45}) although complete solubilisation and the formation of mixed micelles occurs at the cmc.^{45,47} The study by Carillo *et al.* suggests that membranes are permeabilized by dimers of surfactin.⁴⁵

Surfactin has a wide range of demonstrated activities, including antifungal and antibiotic properties.^{9b,10b,c,d} For the latter, a pore-formation mechanism was proposed based on permeabilization studies (using carboxyfluorescein as a fluorescent probe) using model lipid membranes.⁴⁵ A range of other



very useful activities have been reported^{9b,10c,d,48} including antiviral and antitumor properties and effectiveness as an anti-mycoplasma treatment. Surfactin has been shown to suppress inflammatory responses through inhibition of phospholipase A₂.⁴⁹ It is able to suppress biofilm formation by bacteria such as *Salmonella enterica* and this was examined in PVC plates as well as urethral catheters, pointing to relevance to applications in healthcare.⁵⁰ Surfactin C can enhance plasminogen activation, and enhance fibrinolysis *in vivo*.⁵¹ This thrombolytic activity is relevant to conditions involving blood clotting including myocardial and pulmonary disorders. Linear analogues of surfactin have been shown to have reduced haemolytic activity compared to native cyclic variants. They were also observed to protect against lytic (Triton) surfactant-induced haemolysis.⁵² This was correlated to micellization, *i.e.* there is evidence for a relationship between self-assembly and bioactivity from this study. Two mechanisms for this were proposed – (i) the formation of mixed micelles of the lytic surfactant and the protective surfactin derivative, (ii) differences in the insertion of the two surfactants into the cell membrane.⁵²

It has been reported that lichenysin (Fig. 7a) has a much lower cmc than surfactin (22 μ M vs. 220 μ M in 5 mM Tris buffer) and is also a better chelator of Mg²⁺ and Ca²⁺ ions.^{27b} As noted above, the cmc of surfactin is very low in water and other buffers,^{38,45} and in fact the value 220 μ M for surfactin seems too large compared to those reported previously (also in Tris buffer).

Immune response lipopeptides

A series of lipopeptides that act as Toll-like receptor (TLR) agonists have been developed, with important applications in the treatment of infectious diseases. TLRs are transmembrane proteins with a key role in the immune system and as such are important therapeutic targets to treat infections.⁵³ A list of TLR targeting immuno-stimulatory agents being developed in animal models to treat various viral and bacterial infections is available.⁵⁴ The toll-like receptor domains have been studied by X-ray crystallography and, upon binding to Pam_nCSK₄ lipopeptides (*vide infra*) reveal remarkable horse-shoe structures resembling partial β -barrels (Fig. 10).⁵⁵ The lipopeptide is bound to the outer surface of the complex.

Pam₃Cys lipopeptides (Fig. 11) are synthetic versions of the N-terminal sequence of the principal lipoprotein of *E. coli*, also known as Braun's lipoprotein,⁵⁷ that is abundant in the cell wall of Gram-negative bacteria.⁵⁸ Pam₃Cys-based lipopeptides are able to stimulate cytotoxic T lymphocyte (CTL) responses against influenza virus-infected cells (when linked to influenza nucleoprotein viral peptides).⁵⁹ A related lipopeptide (Pam₃CysSerSer linked to a foot-and-mouth disease virus protein sequence) is able to stimulate antibodies against foot-and-mouth disease.⁶⁰ Pam₃CysSerSer is able to mediate attachment to the cell membrane, internalization into the cytoplasm and to activate macrophages to secrete cytokines.⁵⁹ As shown in Fig. 10, Pam₃CSK₄ binding leads to an m-shaped heterodimer of the TLR1 and TLR2 ectodomains, not observed for Pam₂CSK₄.⁵⁶



Fig. 10 Two views of X-ray crystal structure for the TLR1/TLR2 complex bound by Pam₃CSK₄ (pdb file 2Z7X).⁵⁶ The right-hand image shows the location of the bound lipopeptide molecule (represented as yellow spheres).

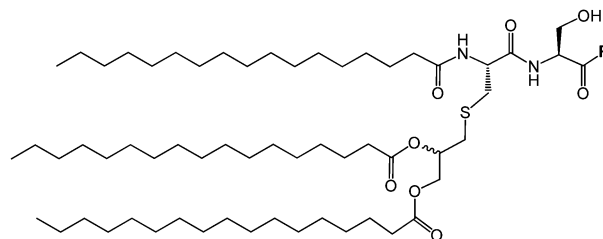


Fig. 11 Scheme of generic Pam₃Cys-based lipopeptide structure where R indicates a peptide sequence.

A similar crystal structure is reported for Pam₂CSK₄ bound to the TLR2/TLR6 heterodimer.⁵⁵

The Pam₂Cys homologue of Pam₃Cys lipopeptides has been shown to correspond to the lipid moiety of macrophage-activating lipopeptide 2 from mycoplasma.⁶¹ The presence of only two lipid chains in Pam₂Cys lipopeptides along with a free amino group leads to improved water solubility. MALP-2, where MALP stands for macrophage-activating lipopeptide, was developed based on this lipopeptide with an attached GNNDES-NISFKEK sequence (Fig. 12).^{61a,62} This lipopeptide is potent in stimulating macrophages or monocytes at pM concentrations.⁶² MALP-2 activates epithelial cells through TLR6 and TLR2, but not TLR1 and TLR2.⁶³ Indeed, TLR2 and TLR6 respond exclusively to lipopeptides possessing a diacylglycerol group.⁶⁴ Pam₃CSK₄ is recognised by TLR1 and TLR6 and does not require TLR2.^{63,64b} In addition, the *R* stereoisomer of MALP-2 and Pam₂CSK₄ and Pam₃CSK₄ are more active than the respective *S* stereoisomer. Together, these results indicate the important role of the chirality of the central carbon in the diacylglycerol group in TLR recognition.^{64b} The role of specific functional groups at the C terminus in the TLR2 activity of Pam₂Cys was examined which showed that a variant containing a 1,3-dihydroxypropyl moiety showed higher activity than the 1,2-dihydroxypropyl derivative.^{3b} Pam₂Cys has been incorporated in other compounds, for instance a PEGylated analogue was prepared to examine the effect on influenza immunity when administered without antigen.⁶⁵ Attachment of synthetic branched peptides rich in terminal arginine or glutamic acid residues to Pam₂Cys leads to enhanced protein immunogenicity



due to binding of charged protein (BSA as model agonist) to the oppositely charged branched lipopeptide derivative.⁶⁶ The immune response was studied using influenza infected mice.

Immun contraceptive vaccines have been developed, incorporating a peptide sequence EHWSYGLRPG from luteinizing hormone-releasing hormone (LHRH).⁵⁸ Lipopeptides incorporating LHRH are able to stimulate antibodies against this “self” hormone, leading to sterilization in a female mouse model. Lipopeptides were synthesized based around Pam₃Cys and Pam₂Cys templates incorporating the LHRH sequence which incorporates one or more epitopes for B cells (denoted [B]) as well as the CD4⁺ T cell epitope GALNNRFQIKGVELKS (denoted [T]) from the L chain of influenza virus hemagglutinin.⁵⁸ Lipopeptides with different architectures were examined, branched lipopeptides with [T] and [B] attached peripherally, such as [T]-Lys-Pam₂Cys-Ser-Ser-[B] induced a higher antibody titer than that observed for linear lipopeptides with [T]-[B] at the C terminus. In general, Pam₂Cys-containing lipopeptides were stated to be better immunogens than Pam₃Cys ones.⁵⁸ It has recently been shown that monoacyl PamCys lipopeptides may be conveniently prepared *via* direct thiol-ene coupling, enabling to the preparation of self-adjuvating antigenic lipopeptides with TLR2 agonist activity.⁶⁷ A truncated sequence from a cytomegalovirus (CMV) protein was used a model epitope as it is able to stimulate CD8⁺ cytotoxic T-cells.

Lipopeptide-based vaccines have been developed to treat several diseases including HIV,⁶⁸ hepatitis B⁶⁹ and HPV (human papilloma virus)⁷⁰ infections (HPV is also implicated in several types of cancer including cervical cancer).

We recently investigated the self-assembly properties of three PamnCSK4 lipopeptides ($n = 1-3$) (Fig. 13).⁷¹ A remarkable dependence of morphology on the number of attached hexadecyl lipid chains was found, with spherical micelle structures for mono- and di-lipidated structures, but flexible wormlike micelles for the homologue containing three lipid chains. Images from cryogenic transmission electron microscopy (cryo-TEM) are shown in Fig. 14. The self-assembled structure was also confirmed by *in situ* small-angle X-ray scattering (SAXS) which enables the dimensions of the nanostructures to be determined. To investigate the thermal stability of the secondary structures, and the possible role of lipid chain melting, we performed temperature-dependent CD measurements. Neither PamCSK4 nor Pam₂CSK4 show any significant change in the disordered

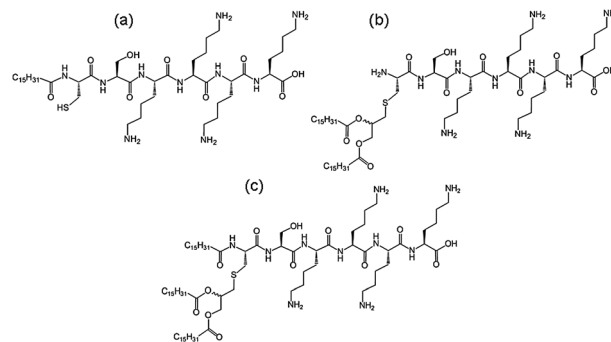


Fig. 13 Structure of Pam_nCSK₄ lipopeptides. (a) PamCSK₄, (b) Pam₂CSK₄, (c) Pam₃CSK₄.⁷¹



Fig. 14 Cryo-TEM images obtained for 2 wt% solutions. (a) PamCSK, (b) Pam₂CSK4, (c) Pam₃CSK4. Enlarged images are shown at top in (a) and (b) and to the right in (c).⁷¹

conformation in the temperature range 20–60 °C which covers palmitoyl lipid chain melting temperatures, *i.e.* the lipid chain melting transition previously observed for palmitoyl lipopeptides.⁷² In contrast, Pam₃CSK4 exhibits a discontinuity in spectra between 30 °C and 40 °C, from a β -sheet conformation at lower temperature to disordered at high temperature. This transition is partially reversible. We associate this transition with the lipid chain melting transition within the tri-palmitoyl chain lipopeptide. The transition from the lipid gel to sol state (at around 40 °C) has previously been shown to change the nanostructure of self-assemblies (twisted ribbons or tapes) of gemini C₁₆ surfactants.⁷³ We suggested that the distinct modes of assembly may have an important influence on the bioactivity of this class of lipopeptide. Interestingly, SAXS on more concentrated samples (5 wt% lipopeptide) indicates that Pam₂CSK4 and Pam₃CSK4 form complex cubic structures.⁷⁴ In the same study, a lipolanthionine peptide (2*R*,6*R*)-Pam₃LanHdaSer(Lys)₄-NH₂ [Lan: lanthionine, Hda: hexadecanoic acid] was found to form a lamellar phase (this lipopeptide in contrast to the Pam_{*n*}CSK4 compounds exhibits an inhibitory effect on activation of human macrophages).⁷⁴ We are currently investigating the lyotropic polymorphism of Pam_{*n*}CSK4 compounds in more detail.

The French National Agency for AIDS research (ANRS) has developed a series of HIV-lipopeptides as part of a strategy to

Table 1 Structures of HIV-lipopeptides developed by the ANRS study team. NEF, GAG and ENV are HIV-1 proteins. From Pialoux *et al.*^{68a}

Lipo-peptide	Long peptide	Sequence	M_w	No. AA
L-N1	N1 (a.a. NEF66–97)	VGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGLK(Pam)	3862.8	32(+1)
L-N2	N2 (a.a. NEF 117–147)	TQGYFPDWQNYTPGPGVRYPLTFGWCYKLVPK(Pam)	4017.8	31(+1)
L-N3	N3 (a.a. NEF182–205)	EWRFDRLAFHHVARELHPEYFKNK(Pam)	3451.1	24(+1)
L-G1	G1 (a.a. GAG183–214)	DLNTMLNTVGGHQAAMQMLKETINEEAAEWDRK(Pam)	3983.7	32(+1)
L-G2	G2 (a.a. GAG253–284)	NPPIPVGEIYKRWILGLNKIVRMYSPTSILDK(Pam)	4063.1	32(+1)
L-E	E (a.a. ENV303–335)	TRPNNNTRKSIHIGPGRAFYATGEIIGDIRQAHK(Pam)	4027.7	33(+1)

**Fig. 15** Structure of theradigm lipopeptide.⁶⁹

create vaccines based on an induced CTL response.⁶⁸ A list of typical structures is provided in Table 1. This builds on the use of simian immunodeficiency virus (SIV) as a model for vaccination using related lipopeptides (based on peptide sequences from the SIV NEF or GAG proteins) that induce a CTL response.⁷⁵ To our knowledge, the self-assembly properties of these compounds has yet to be investigated.

A lipopeptide (termed “Theradigm”) used to create a vaccine against chronic hepatitis B virus (HBV) comprises a peptide sequence from a CTL linked to a T-helper peptide epitope TetTox and two palmitoyl lipid chains (Fig. 15).⁶⁹ CTLs are able to recognise small 8–10 residue antigenic peptides. To our knowledge, the self-assembly behaviour of this lipopeptide has not yet been examined.

A lipopeptide with activity against HPV is C₁₆-KSSTLGIVCPI which contains the TLGIVCPI peptide sequence associated with the E7 gene of HPV-16 as well as a hydrophilic KSS linker.⁷⁰ The lipopeptide was shown to have activity in generating cellular immune responses in women with cervical cancer. A lipopeptide-based vaccine treatment for hepatitis C has also been trialled based on peptide sequences conjugated to the Pam₂Cys lipopeptide.⁷⁶

Lipopeptides for applications in cosmetics

We recently showed that the commercially available lipopeptide Matrixyl™ (C₁₆-KTTSK) undergoes self-assembly in aqueous solution. This lipopeptide is used in skincare applications as an antiwrinkle activity is suggested.^{4,77} The peptide headgroup KTTSK is a sequence taken from a study on minimal Pro-collagen peptide sequences able to stimulate collagen and fibronectin production in fibroblasts.⁷⁸

At neutral pH and below the lipid chain melting temperature, highly extended nanotape structures are observed, as shown in Fig. 16.

We showed that the bilayer nanotape self-assembled structure of C₁₆-KTTSK is stable in the pH range 3–7 at room temperature, although there was evidence for twisting of the tapes at pH 4,

but not pH 3 or pH 7.⁷⁹ However, reduction of pH (to pH 2)⁷⁹ or increase of temperature (above the lipid chain melting temperature, around 35 °C dependent on concentration)⁸⁰ leads to the formation of spherical micelles. This is accompanied by a loss of β -sheet secondary structure, as revealed by circular dichroism spectroscopy and X-ray diffraction.⁷⁹ Although very low pH is not relevant to the application of the compound in skincare (the pH of skin is around 4–6 although diseased skin can have lower pH), the lipid chain melting temperature is close to core body temperature although again the bilayer structure is expected to be stable near the surface of the skin in the stratum corneum.

This lipopeptide is able to stimulate collagen production by fibroblasts (dermal and corneal) in a dose-dependent manner close to the measured in DMEM media $cac = 0.0055$ wt%, as shown by the results from a Sirius red assay for type I collagen shown in Fig. 17.⁸¹ Since C₁₆-KTTSK causes a slight decrease in cell number following initial cell seeding, the collagen production per cell increases more than the total collagen (Fig. 17). This study was the first systematic *in vitro* study on the collagen-stimulating properties of C₁₆-KTTSK in the peer-reviewed literature.

We also examined the self-assembly in water of related lipopeptides of interest in skincare applications, C₁₆-GHK and C₁₆-KT as well as a commercial sample of C₁₆-KTTSK (with higher polydispersity than the 95%+ purity grade sample used in our earlier studies).⁸² The KT peptide is a fragment of KTTSK while GHK is a copper-chelating peptide with a collagen-stimulating

**Fig. 16** Fibrillar superstructure of peptide amphiphile Matrixyl.⁷² (a) Confocal optical microscopy image (fluorescent labelling with rhodamine B, 0.014 wt% Matrixyl in water), (b) apple green birefringence observed by polarized optical microscopy upon staining with Congo red, (c) differential optical microscopy image (1 mg ml^{−1} = 0.1 wt%).



Fig. 17 Collagen assay results showing the stimulation of human fibroblasts by C₁₆-KTTKS.⁸¹ (a) and (b) Human corneal fibroblasts, (c) and (d) human dermal fibroblasts. Left, total amount of collagen deposited by cells and right, total amount of collagen produced per cell ($n = 3$, $*P \leq 0.005$, $**P \leq 0.01$ and $***P \leq 0.001$).

activity, involved in wound healing.⁸³ All three lipopeptides showed similar cac values (although the transition is not as sharp as that observed for higher purity lipopeptides) and also had the common feature in SAXS of lamellar reflections indicating bilayer-based aggregates. However, TEM showed that C₁₆-GHK and C₁₆-KT form small crystallite structures whereas C₁₆-KTTKS forms extended nanotapes. This latter material exhibits other “amyloid-like” aggregate features, including the uptake of the dye Congo red and a cross- β fibre X-ray diffraction pattern.⁸²

Summary and future directions

This review has considered the bioactivity and self-assembly of three classes of lipopeptide (i) bacterially-expressed cyclic lipopeptides with major applications in practice as antibacterial and antifungal agents, (ii) linear toll-like receptor agonist lipopeptides with activity in immune therapies and (iii) several linear bio-derived lipopeptides with applications in skincare, in particular one with a collagen-stimulating activity (trade-name Matrixyl™). In classes (ii) and (iii) recent work using cryo-TEM and SAXS has established the self-assembled nanostructure which is micelles or bilayer tapes, depending on the nature of the lipopeptide (in particular the number of lipid chains for the Pam_nCSK4 lipopeptides) as well as the self-assembly conditions (temperature, pH *etc.*). There are some studies on self-assembly of class (i) bacterially expressed lipopeptides with a cyclic peptide headgroup as cited above, but in many cases this has not yet been examined. Clearly, this is a fascinating direction for future research.

One key question concerns the relationship, if any, between self-assembly and bioactivity. This is a complicated question for a number of reasons. Firstly, self-assembly is often concentration-dependent and decoupling the effect of concentration on bioactivity from the self-assembly process may be difficult. This may be circumvented by, for example, cross-linking self-assembled

structures to trap a particular thermodynamic state. This itself may not be straightforward as the cross-linking process will influence the self-assembled structure and potentially have an impact on bioactivity. Second, it is not in general straightforward to probe interactions between the substrate and molecules and/or aggregate without perturbing the molecule-aggregate equilibrium, *i.e.* the self-assembly process, which is expected to be a closed process. There are likely to be cases where self-assembled structures themselves impart bioactivity – this is implied in much work on synthetic lipopeptides where lipopeptide assemblies present bioactive motifs at high density.⁸⁴ This is also expected to be a significant effect for Matrixyl™ where the extended fibrils may provide a scaffold to enhance the collagen-stimulating activity of the peptide and/or provide a “filler” for anti-wrinkle skincare applications. It also seems likely to be the case that the aggregation of bacterially-expressed lipopeptides is important in stimulating or overcoming a host immune response. In other cases there may be no direct relationship between self-assembly and bioactivity (this will certainly be the case where bioactivity is observed at very low concentration below any cmc or cac). There may just be an indirect relationship in the sense that amphiphilicity is important to bioactivity (*e.g.* compatibility with the cell membrane) and the fact that this amphiphilicity in turn leads to self-assembly at high concentration. In cases such as the TLR agonist lipopeptides, crystal structures suggest binding sites for unaggregated molecules and the role of aggregate structures (if any) is not clear. The presence of possible oligomeric species on or off the aggregation pathway is a further complicating factor.

Moving forward, an interesting question is whether any general rules of self-assembly can be established for the large class of bacterially expressed lipopeptide, relating properties of the molecule (charge, shape) to aggregation propensity, self-assembled structure *etc.* In contrast to lipids, where models such as the surfactant packing parameter or interfacial geometry can be used to give guidance on the formation of self-assembled structures for a given molecule at a particular concentration,⁸⁵ such models have yet to be developed for lipopeptides. This in part may reflect the complexity of intermolecular interactions involving both lipid chains and peptide headgroups. In the latter case, electrostatic, aromatic π -stacking and hydrogen bonding interactions are of competing importance depending on the sequence and this is counterbalanced by hydrophobic interactions of the lipid chains.

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Notes and references

- # Notes and references
- (a) D. W. P. M. Löwik, E. H. P. Leunissen, M. van den Heuvel, M. B. Hansen and J. C. M. van Hest, *Chem. Soc. Rev.*, 2010, **39**, 3394–3412; (b) D. W. P. M. Löwik and J. C. M. van Hest, *Chem. Soc. Rev.*, 2004, **33**, 234–245; (c) H. G. Cui, M. J. Webber and S. I. Stupp, *Pept. Sci.*, 2010, **94**, 1–18; (d) J. B. Matson, R. H. Zha and S. I. Stupp, *Curr. Opin. Solid State Mater. Sci.*, 2011, **15**, 225–235; (e) J. Boekhoven and S. I. Stupp, *Adv. Mater.*, 2014, **26**, 1642–1659; (f) I. W. Hamley, *Soft Matter*, 2011, **7**, 4122–4138; (g) A. Dehsorkhi, V. Castelletto and I. W. Hamley, *J. Pept. Sci.*, 2014, **20**, 453–467.
 - (a) V. G. Fowler, Jr., H. W. Boucher, G. R. Corey, E. Abrutyn, A. W. Karchmer, M. E. Rupp, D. P. Levine, H. F. Chambers, F. P. Tally, G. A. Vigliani, C. H. Cabell, A. S. Link, I. DeMeyer, S. G. Filler, M. Zervos, P. Cook, J. Parsonnet, J. M. Bernstein, C. S. Price, G. N. Forrest, G. Faetkenheuer, M. Gareca, S. J. Rehm, H. R. Brodt, A. Tice, S. E. Cosgrove and S. A. E. Bacteremia, *N. Engl. J. Med.*, 2006, **355**, 653–665; (b) T. Owen, R. Pynn, J. S. Martinez and A. Butler, *Langmuir*, 2005, **21**, 12109–12114; (c) J. Hill, I. Parr, M. Morytko, J. Siedlecki, X. Y. Yu, J. Silverman, D. Keith, J. Finn, D. Christensen, T. Lazarova, A. D. Watson and Y. Zhang, *Lipopeptides as antibacterial agents*, U. S. Pat., US6911525B2, 2005.
 - (a) T. H. Wright, A. E. S. Brooks, A. J. Didsbury, G. M. Williams, P. W. R. Harris, P. R. Dunbar and M. A. Brimble, *Angew. Chem., Int. Ed.*, 2013, **52**, 10616–10619; (b) W. G. Zeng, E. Eriksson, B. Chua, L. Grollo and D. C. Jackson, *Amino Acids*, 2010, **39**, 471–480.
 - (a) K. Lintner and O. Peschard, *Int. J. Cosmet. Sci.*, 2000, **22**, 207–218; (b) L. R. Robinson, N. C. Fitzgerald, D. G. Doughty, N. C. Dawes, C. A. Berge and D. L. Bissett, *Int. J. Cosmet. Sci.*, 2005, **27**, 155–160.
 - (a) J. M. Bonmatin, O. Laprevote and F. Peypoux, *Comb. Chem. High Throughput Screening*, 2003, **6**, 541–556; (b) P. Jacques, in *Surfactin and other lipopeptides from Bacillus spp.*, ed. G. Soberon-Chavez, Springer, Berlin, 2011.
 - (a) M. Ongena and P. Jacques, *Trends Microbiol.*, 2008, **16**, 115–125; (b) J. M. Raaijmakers, I. de Bruijn, O. Nybroe and M. Ongena, *FEMS Microbiol. Rev.*, 2010, **34**, 1037–1062; (c) P. Jauregi, F. Coutte, L. Catiau, D. Lecouturier and P. Jacques, *Sep. Purif. Technol.*, 2013, **104**, 175–182.
 - T. A. Hill, N. E. Shepherd, F. Diness and D. P. Fairlie, *Angew. Chem., Int. Ed.*, 2014, **53**, 13020–13041.
 - H. Saito, S. Lund-Katz and M. C. Phillips, *Prog. Lipid Res.*, 2004, **43**, 350–380.
 - (a) E. Z. Ron and E. Rosenberg, *Environ. Microbiol.*, 2001, **3**, 229–236; (b) P. Singh and S. S. Cameotra, *Trends Biotechnol.*, 2004, **22**, 142–146.
 - (a) R. Maget-Dana and F. Peypoux, *Toxicology*, 1994, **87**, 151–174; (b) F. Peypoux, J. M. Bonmatin and J. Wallach, *Appl. Microbiol. Biotechnol.*, 1999, **51**, 553–563; (c) G. Seydlova and J. Svobodova, *Cent. Eur. J. Med.*, 2008, **3**, 123–133; (d) I. M. Banat, A. Franzetti, I. Gandolfi, G. Bestetti, M. G. Martinotti, L. Fracchia, T. J. Smyth and R. Marchant, *Appl. Microbiol. Biotechnol.*, 2010, **87**, 427–444; (e) J. M. Raaijmakers, I. de Bruijn and M. J. D. de Kock, *Mol. Plant-Microbe Interact.*, 2006, **19**, 699–710.
 - C. Heinis, T. Rutherford, S. Freund and G. Winter, *Nat. Chem. Biol.*, 2009, **5**, 502–507.
 - (a) V. Miao, M. F. Coeffet-LeGal, P. Brian, R. Brost, J. Penn, A. Whiting, S. Martin, R. Ford, I. Parr, M. Bouchard, C. J. Silva, S. K. Wrigley and R. H. Baltz, *Microbiology*, 2005, **151**, 1507–1523; (b) J. N. Steenbergen, J. Alder, G. M. Thorne and F. P. Tally, *J. Antimicrob. Chemother.*, 2005, **55**, 283–288.
 - M. Debono, B. J. Abbott, R. M. Molloy, D. S. Fukuda, A. H. Hunt, V. M. Daupert, F. T. Counter, J. L. Ott, C. B. Carrell, L. C. Howard, L. D. Boeck and R. L. Hamill, *J. Antibiot.*, 1988, **41**, 1093–1105.
 - (a) J. K. Muraih and M. Palmer, *Biochim. Biophys. Acta, Biomembr.*, 2012, **1818**, 1642–1647; (b) J. Qiu and L. E. Kirsch, *J. Pharm. Sci.*, 2014, **103**, 853–861.
 - J. A. Silverman, N. G. Perlmutter and H. M. Shapiro, *Antimicrob. Agents Chemother.*, 2003, **47**, 2538–2544.
 - T. H. Zhang, J. K. Muraih, B. MacCormick, J. Silverman and M. Palmer, *Biochim. Biophys. Acta, Biomembr.*, 2014, **1838**, 2425–2430.
 - (a) J. H. Lakey and M. Ptak, *Biochemistry*, 1988, **27**, 4639–4645; (b) D. Jung, A. Rozek, M. Okon and R. E. W. Hancock, *Chem. Biol.*, 2004, **11**, 949–957; (c) D. Jung, J. P. Powers, S. K. Straus and R. E. W. Hancock, *Chem. Phys. Lipids*, 2008, **154**, 120–128.
 - I. W. Hamley, S. Kirkham, M. Reza and J. Ruokolainen, 2015, unpublished work.
 - D. W. Denning, *Lancet*, 2003, **362**, 1142–1151.
 - M. Debono and R. S. Gordee, *Annu. Rev. Microbiol.*, 1994, **48**, 471–497.
 - (a) N. Milton, K. P. Moder, J. L. Sabatowski and S. A. Sweetana, *US Pat.*, US06960564, 2005; (b) N. Milton, K. P. Moder, J. L. Sabatowski and S. A. Sweetana, *US Pat.*, US07709444, 2010.
 - M. Mazzola, I. de Bruijn, M. F. Cohen and J. M. Raaijmakers, *Appl. Environ. Microbiol.*, 2009, **75**, 6804–6811.
 - (a) T. R. Neu, T. Hartner and K. Poralla, *Appl. Microbiol. Biotechnol.*, 1990, **32**, 518–520; (b) H. S. Saini, B. E. Barragan-Huerta, A. Lebron-Paler, J. E. Pemberton, R. R. Vazquez, A. M. Burns, M. T. Marron, C. J. Seliga, A. A. L. Gunatilaka and R. M. Maier, *J. Nat. Prod.*, 2008, **71**, 1011–1015.
 - V. Groupe, L. H. Pugh, D. Weiss and M. Kochi, *Proc. Soc. Exp. Biol. Med.*, 1951, **78**, 354–358.
 - S. Lang, *Curr. Opin. Colloid Interface Sci.*, 2002, **7**, 12–20.
 - (a) <http://www.lipidmaps.org/data/LMSDRecord.php?Mode=View&ViewType=ChemDraw&LMID=LMPK14000008>, in 2015; (b) A. Kwa, S. K. Kosiakou, V. H. Tam and M. E. Falagas, *Expert Rev. Anti-Infect. Ther.*, 2007, **5**, 811–821.
 - (a) M. M. Yakimov, K. N. Timmis, V. Wray and H. L. Fredrickson, *Appl. Environ. Microbiol.*, 1995, **61**, 1706–1713; (b) I. Grangemard, J. Wallach, R. Maget-Dana and F. Peypoux, *Appl. Biochem. Biotechnol.*, 2001, **90**, 199–210.
 - N. Naruse, O. Tenmyo, S. Kobaru, H. Kamei, T. Miyaki, M. Konishi and T. Oki, *J. Antibiot.*, 1990, **43**, 267–280.
 - D. Landman, C. Georgescu, D. A. Martin and J. Quale, *Clin. Microbiol. Rev.*, 2008, **21**, 449–465.
 - I. W. Hamley, A. Dehsorkhi, P. Jauregi, J. Seitsonen, J. Ruokolainen, F. Coutte, G. Chataigné and P. Jacques, *Soft Matter*, 2013, **9**, 9572–9578.
 - M. Ongena, P. Jacques, Y. Toure, J. Destain, A. Jabrane and P. Thonart, *Appl. Microbiol. Biotechnol.*, 2005, **69**, 29–38.
 - D. Julkowska, M. Obuchowski, I. B. Holland and S. J. Séror, *J. Bacteriol.*, 2005, **187**, 65–76.
 - W. Li, H. Rokni-Zadeh, M. De Vleeschouwer, M. G. K. Ghequire, D. Sinnave, G. L. Xie, J. Rozenski, A. Madder, J. C. Martins and R. De Mot, *PLoS One*, 2013, **8**, e62946.
 - M. L. Hutchison and D. C. Gross, *Mol. Plant-Microbe Interact.*, 1997, **10**, 347–354.
 - D. Sorensen, T. H. Nielsen, C

- 51 T. Kikuchi and K. Hasumi, *Biochim. Biophys. Acta*, 2002, **1596**, 234–245.
- 52 S. Dufour, M. Deleu, K. Nott, B. Wathelet, P. Thonart and M. Paquot, *Biochim. Biophys. Acta, Gen. Subj.*, 2005, **1726**, 87–95.
- 53 N. J. Gay and M. Gangloff, *Annu. Rev. Biochem.*, 2007, **76**, 141–165.
- 54 E. J. Mifsud, A. C. L. Tan and D. C. Jackson, *Front. Immunol.*, 2014, **5**, 1–10.
- 55 J. Y. Kang, X. Nan, M. S. Jin, S. J. Youn, Y. H. Ryu, S. Mah, S. H. Han, H. Lee, S. G. Paik and J. O. Lee, *Immunity*, 2009, **31**, 873–884.
- 56 M. S. Jin, S. E. Kim, J. Y. Heo, M. E. Lee, H. M. Kim, S. G. Paik, H. Y. Lee and J. O. Lee, *Cell*, 2007, **130**, 1071–1082.
- 57 V. Braun, *Biochim. Biophys. Acta*, 1975, **415**, 335–377.
- 58 W. G. Zeng, S. Ghosh, Y. F. Lau, L. E. Brown and D. C. Jackson, *J. Immunol.*, 2002, **169**, 4905–4912.
- 59 K. Deres, H. Schild, K. H. Wiesmuller, G. Jung and H. G. Rammensee, *Nature*, 1989, **342**, 561–564.
- 60 K. H. Wiesmuller, G. Jung and G. Hess, *Vaccine*, 1989, **7**, 29–33.
- 61 (a) P. F. Muhlrardt, M. Kiess, H. Meyer, R. Sussmuth and G. Jung, *J. Exp. Med.*, 1997, **185**, 1951–1958; (b) P. F. Muhlrardt, M. Kiess, H. Meyer, R. Sussmuth and G. Jung, *Infect. Immun.*, 1998, **66**, 4804–4810.
- 62 M. Morr, O. Takeuchi, S. Akira, M. M. Simon and P. F. Muhlrardt, *Eur. J. Immunol.*, 2002, **32**, 3337–3347.
- 63 O. Takeuchi, T. Kawai, P. F. Muhlrardt, M. Morr, J. D. Radolf, A. Zychlinsky, K. Takeda and S. Akira, *Int. Immunol.*, 2001, **13**, 933–940.
- 64 (a) O. Takeuchi, S. Sato, T. Horiuchi, K. Hoshino, K. Takeda, Z. Y. Dong, R. L. Modlin and S. Akira, *J. Immunol.*, 2002, **169**, 10–14; (b) K. O. Omuetti, J. M. Beyer, C. M. Johnson, E. A. Lyle and R. I. Tapping, *J. Biol. Chem.*, 2005, **280**, 36616–36625.
- 65 A. C. L. Tan, E. J. Mifsud, W. G. Zeng, K. Edenborough, J. McVernon, L. E. Brown and D. C. Jackson, *Mol. Pharmaceutics*, 2012, **9**, 2710–2718.
- 66 B. Y. Chua, D. Pejowski, S. J. Turner, W. G. Zeng and D. C. Jackson, *J. Immunol.*, 2011, **187**, 1692–1701.
- 67 T. H. Wright, A. E. S. Brooks, A. J. Didsbury, G. M. Williams, P. W. R. Harris, P. R. Dunbar and M. A. Brimble, *Angew. Chem., Int. Ed.*, 2013, **52**, 10616–10619.
- 68 (a) G. Pialoux, H. Gahery-Segard, S. Sermet, H. Poncelet, S. Fournier, L. Gerard, A. Tartar, H. Gras-Masse, J. P. Levy, J. G. Guillet and A. V. S. Team, *AIDS*, 2001, **15**, 1239–1249; (b) C. Durier, O. Launay, V. Meiffredy, Y. Saidi, D. Salmon, Y. Levy, J. G. Guillet, G. Pialoux and J. P. Aboulker, *AIDS*, 2006, **20**, 1039–1049.
- 69 A. Vitiello, G. Ishioka, H. M. Grey, R. Rose, P. Farness, R. Lafond, L. L. Yuan, F. V. Chisari, J. Furze, R. Bartholomeuz and R. W. Chesnut, *J. Clin. Invest.*, 1995, **95**, 341–349.
- 70 M. A. Steller, K. J. Gorski, M. Murakami, R. W. Daniel, K. V. Shah, E. Celis, A. Sette, E. L. Trimble, R. C. Park and F. M. Marincola, *Clin. Cancer Res.*, 1998, **4**, 2103–2109.
- 71 I. W. Hamley, S. Kirkham, A. Dehsorkhi, V. Castelletto, M. Reza and J. Ruokolainen, *Chem. Commun.*, 2014, **50**, 15948–15951.
- 72 V. Castelletto, I. W. Hamley, J. Perez, L. Abezgauz and D. Danino, *Chem. Commun.*, 2010, **46**, 9185–9187.
- 73 A. Brizard, C. Aime, T. Labrot, I. Huc, D. Berthier, F. Artzner, B. Desbat and R. Oda, *J. Am. Chem. Soc.*, 2007, **129**, 3754–3762.
- 74 A. B. Schromm, J. Howe, A. J. Ulmer, K. H. Wiesmuller, T. Seyberth, G. Jung, M. Rossle, M. H. J. Koch, T. Gutsmann and K. Brandenburg, *J. Biol. Chem.*, 2007, **282**, 11030–11037.
- 75 I. Bourgault, F. Chirat, A. Tartar, J. P. Levy, J. G. Guillet and A. Venet, *J. Immunol.*, 1994, **152**, 2530–2537.
- 76 E. M. Y. Eriksson and D. C. Jackson, *Curr. Protein Pept. Sci.*, 2007, **8**, 412–417.
- 77 K. Lintner, *US Pat.*, US2004/0132667, 2003.
- 78 K. Katayama, J. Armendarizborunda, R. Raghow, A. H. Kang and J. M. Seyer, *J. Biol. Chem.*, 1993, **268**, 9941–9944.
- 79 A. Dehsorkhi, V. Castelletto, I. W. Hamley, J. Adamcik and R. Mezzenga, *Soft Matter*, 2013, **9**, 6033–6036.
- 80 J. F. Miravet, B. Escuder, M. D. Segarra-Maset, M. Tena-Solsona, I. W. Hamley, A. Dehsorkhi and V. Castelletto, *Soft Matter*, 2013, **9**, 3558–3564.
- 81 R. R. Jones, V. Castelletto, C. J. Connon and I. W. Hamley, *Mol. Pharmaceutics*, 2013, **10**, 1063–1069.
- 82 V. Castelletto, I. W. Hamley, C. Whitehouse, P. Matts, R. Osborne and E. S. Baker, *Langmuir*, 2013, **29**, 9149–9155.
- 83 (a) F. X. Maquart, L. Pickart, M. Laurent, P. Gillery, J. C. Monboisse and J. P. Borel, *FEBS Lett.*, 1988, **238**, 343–346; (b) F. X. Maquart, G. Bellon, B. Chaqour, J. Wegrowski, L. M. Patt, R. E. Trachy, J. C. Monboisse, F. Chastang, P. Birembaut, P. Gillery and J. P. Borel, *J. Clin. Invest.*, 1993, **92**, 2368–2376.
- 84 J. B. Matson and S. I. Stupp, *Chem. Commun.*, 2012, **48**, 26–33.
- 85 I. W. Hamley, *Introduction to Soft Matter*, Wiley, revised edn, 2007.

