Designing peptide based nanomaterials

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This *tutorial review* looks at the design rules that allow peptides to be exploited as building blocks for the assembly of nanomaterials. These design rules are either derived by copying nature (α-helix, β-sheet) or may exploit entirely new designs based on peptide derivatives (peptide amphiphiles, π-stacking systems). We will examine the features that can be introduced to allow self-assembly to be controlled and directed by application of an externally applied stimulus, such as pH, light or enzyme action. Lastly the applications of designed self-assembly peptide systems in biotechnology (3D cell culture, biosensing) and technology (nanoelectronics, templating) will be examined.

1. Introduction

Traditionally objects and devices have been made through a "top-down" approach where an item is carved, or moulded out of a larger bulk material. Nanomaterials are at the limit of our ability to etch, requiring specialised techniques such as e-beam and atomic force microscopy based lithography. A "bottom-up" approach provides an alternative route to the development of nanoscale objects and devices. An obvious source of inspiration for the design of nanoscale materials from the bottom up is provided by Nature. Evolution has produced a range of self-assembling nanoscale systems based on lipids, nucleic acids and amino acids. In these systems, disordered mixtures of molecular building blocks spontaneously arrange themselves to form highly organised structures with well defined properties. These perform a vast array of functions, with lipids primarily acting as barriers, polynucleic acids as information storage and retrieval mechanisms and amino acid based polymers (peptides and proteins) acting as the machinery of life. In this review we concentrate on the design of self assembly systems based on the latter type.

There are 20 natural amino acids (Fig. 1) that are utilised in the synthesis of peptides and proteins in biological cells. All natural amino acids, except glycine (G), are chiral and in nature only exist in the L-form. They have the same basic structure and vary only in the R-group at the central carbon (Cα) position of the molecule. Peptides adopt specific configurations depending on which R-groups are near one another in a peptide chain. As a consequence there is a relationship between amino acid sequence and structure. In peptide based nanomaterials, the structure formed by a single peptide can interact with another complementary peptide *via* non-covalent interactions: ionic, hydrophobic, hydrogen bonding and π-stacking. When a large number of these building blocks are contained supramolecular structures can be formed. It is easy to see why rational design of these structures is challenging. Even a short peptide of 5 amino acids has a possible 20^5 or 3.2 million possible sequences just using natural amino acids. The number of possible sequences (and structures) that can be formed are nearly endless. However, of the enormous number of possible peptide sequences, only comparatively few are encountered in biological systems. The

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Andrew Smith obtained his PhD in Biochemistry from the University of Sussex studying a coiled-coil based peptide fibre. He then spent 3 years as a Wellcome Trust Postdoctoral Fellow at the University of Leeds studying amyloid and virus assembly. He is currently an EPSRC Postdoctoral Research Associate in Rein Ulijn's group working on the molecular structure of self-assembled peptide hydrogels. His work on the design and characterisation of self-assembling peptides and proteins and has covered a range of peptide structures.

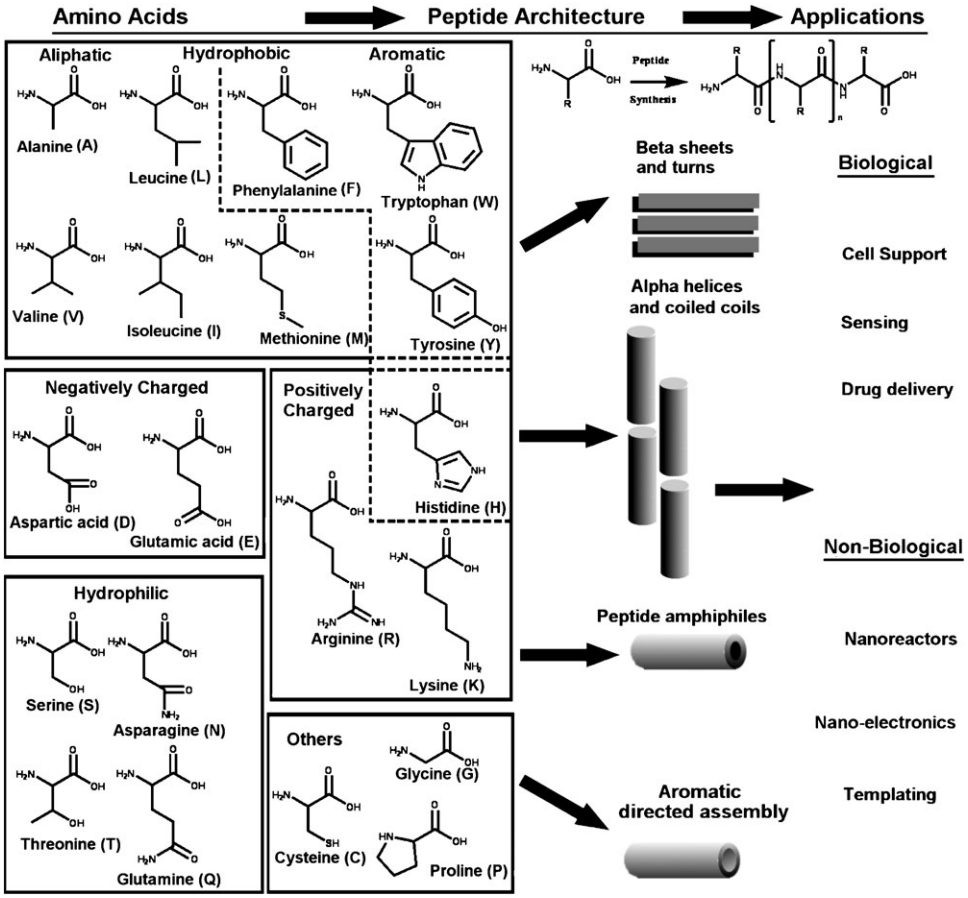

Fig. 1 Amino acid structures are shown with their common name and the one letter abbreviations that are commonly used in peptide sequences. The four main classes of possible architecture that peptides can adopt are shown, with potential applications for these architectures.

challenge is to find the rules that link peptide sequence to structure. Researchers have made significant progress in this area and it now becomes possible to design structures that have specific properties to fulfil a specific purpose.

This tutorial review looks at the design rules that allow peptides to be exploited as building blocks for the assembly of nanomaterials. These design rules are either derived by copying nature (α-helix, β-sheet) or may exploit entirely new designs based on peptide derivatives (peptide amphiphiles, π-stacking systems). Additionally cyclic peptides that utilise alternating D- and L-forms of amino acids have been exploited in the formation of peptide nanotube structures. The first of these was demonstrated in 1993 by Gadhiri[1] and there have been extensive variations on this structure, many of which have been covered in detail in other reviews.[2] We will examine the design features that can be introduced to allow self-assembly to be controlled and directed by application of an externally applied stimulus, such as pH, light or enzyme action. Lastly the applications of designed self-assembly peptide systems in biotechnology (3D cell culture, biosensing) and technology (nanoelectronics, templating) will be examined.

2. The building blocks—amino acids

A tutorial review on designed peptide nanomaterials requires first of all a discussion of the building blocks. Sequences of amino acids provide the primary structure, normally written as one letter codes (Fig. 1). Depending on the properties of the R-group, amino acids are categorised as hydrophobic, hydrophilic, charged, or ''other''. The hydrophobic residues can be split into two groups the aliphatic residues, A, I, L, M, V and the aromatic residues F, W and Y (Fig. 1). The aliphatic residues provide a general hydrophobic environment. Aromatic residues can become involved in π–π stacking where p-orbitals in π-conjugated system overlap. These interactions are increasingly recognised as important in protein and peptide folding. The hydrophilic, uncharged residues can be involved in hydrogen bonding interactions, either *via* OH (S, T) or CONH (N, Q) groups. These can be involved in hydrogen bonding interactions. The charged residues can be positively charged, H, K, R, and have pK_a values of 6.5, 10 and 12, respectively, or negatively charged, D, E, and both have similar pK_a of 4.4 (Fig. 1). Charged residues can be exploited to create specific charge–charge interactions that may help form assemblies (exploiting oppositely charged groups) or to prevent assembly (using equal charges). The ''other'' category contains several specialised amino acids that either offer structural modifications such as bends in peptide chains, or sites for chemical modification. The presence of two hydrogen atoms in glycine (G) removes steric hindrances normally imposed by the R-group, as a result glycine residues introduce a higher degree of flexibility than other amino acids.

By contrast, proline introduces structural rigidity due to the locked conformation caused by the side chain being covalently linked to the amino terminus. Cysteine (C) offers a unique chemical reactivity in the amino acid side chains so as a result is often used as a target for chemical modification and inter-peptide crosslinking. Additionally it can also be used to bind to gold surfaces. Repeat sequences of histidine (H) are useful for binding metal ions, for example Ni^{2+}. While tyrosine (Y), serine (S), and threonine (T) are suitable targets for chemical or enzymatic modification.

3. Strategies for making materials by self assembly

There are two general categories into which designed self assembling peptide systems fall, natural and non-natural. The first category utilises the basic conformational units of naturally existing proteins, β-sheets and turns, α-helices and coiled coils. Through the examination of protein sequences it has been possible to derive simple rules that promote the formation of one of the basic conformational units. Using these rules it is possible to create an arrangement of the peptides through careful selection of amino acids and consequently interaction types. The second category either covalently links amino acids to other molecules; either an alkyl chain to form a peptide amphiphile, or to an aromatic group to create π–π interactions between the aromatic groups.

3.1 β-Sheets and β-hairpins

The parallel and anti-parallel structures of β-sheets were identifed by Pauling and Corey[3] and others in the early 1950's when the possible structures that proteins could adopt were identified. Zhang first demonstrated the use of these structures in the design of soft materials in the early 1990s.[4] β-Sheets consist of multiple peptide chains that have an extended backbone arrangement that permits hydrogen bonding between the backbone amides and carbonyls. Each chain is referred to as a strand, the hydrogen bonded strands are referred to as a sheet. β-Sheets can be orientated so that all their C-termini are at one end of a structure, described as a parallel structure, or so that the N and C termini alternate, described as an anti-parallel structure. This has an important impact on the orientation of hydrogen bonds between sheets and side chain orientations and interactions.

β-Sheets are well known for their ability to assemble into long fibrous structures, as is seen in amyloid diseases like Alzheimer's and Parkinson's diseases.[5] There is a basic motif present in most β-sheets which consists of alternating hydrophobic, hydrophilic residues (Fig. 2A). As a consequence of this alternating pattern, when assembled into a sheet, this gives rise to a hydrophobic and hydrophilic face. Two sheets may come together to bury this hydrophobic face from the surrounding water. Early papers showed it was possible to create a fibrous system using β-sheets by creating a pattern of hydrophobic amino acids and complementary charges between peptides (*e.g.* $(RADA)_4$).[4]

The main challenge of designing a self-assembling fibrous system is to control the assembly to form uniform reproducible structures. A number of different hierarchical structures can be formed from β-sheet peptides, which have been classed as tapes, ribbons, fibrils and fibres, all of which vary in the number of sheets that pack together to form the final structure (Fig. 2A).[6] For a given sequence the formation of these higher order structures is controlled by the peptide concentration, and salt concentration. Increasing the peptide concentration allows for higher level assemblies to form. Studies into the systematic variation of peptide sequence by altering the hydrophobic residues (**FK**F**E**), (**IK**I**E**) and (**VK**V**E**) and the number of these amino acid repeats in a peptide between 2 and 4 have helped to identify key features that control the assembly of these systems. Sequences with more hydrophobic residues demonstrate lower critical concentrations. Additionally, peptide length may also alter the critical concentration with 16 amino acid peptides giving rise to higher critical concentrations compared to 8mers, due to a competition between enthalpy and entropy.[7] It is now known that much shorter peptides of as little as 2 amino acids can also form β-sheet structures when further stabilised by aromatic interactions, as discussed in section 3.4. As charge–charge interactions are involved in the assembly of these systems salt concentration can affect the assembly of these systems by masking the charges involved in interactions between multiple sheets. The EAK16-II (AEAEAKAKAEAEAKAK) peptide has been used in a study of the effect of salt to mask the charges in the peptide. At low concentration salt promotes assembly of fibres by preventing random interactions and only permitting the desired interactions as these are stronger, however, this effect is reduced as the peptide concentration is raised.[8]

An interesting feature of hydrogels based on β-sheets and a number of other self assembly systems is that after mechanical stress like sonication the gross morphology of the material is disrupted, but the supramolecular structure is not. This has been illustrated with the $RADA_4$ peptide of Zhang's group where before and after sonication the circular dichroism spectrum is virtually identical indicating that the molecular structure is still intact, but, by atomic force microscopy (AFM) long fibres of micron lengths are broken down to short 20–100 nm long fibres. After sonication, over a period of 2 h the short fibres reassemble into long micron length fibres (Fig. 2B). It is proposed that the reassembly is due to exposed hydrophobic ends interacting with one another to allow the short fibres to fuse together to form longer fibres again.[9]

An alternative to β-sheets are β-hairpins, these consist of two short β-sheet sequences linked by a turn sequence (Fig. 2C). With β-sheets containing repeating amino acid sequences it is possible for the peptides to line up out of register so that the end amino acids do not interact with another peptide, but by using a β-hairpin ensures that the peptides stack in register with one another. One designed system by Schneider and Pochan, uses a turn sequence –V^{D}PPT– that permanently forces a type II′ turn and prevents a *cis*-prolyl bond that could create heterogeneity in the unfolded population of peptides. The structure is forced due to the presence of the non-natural D-form of proline. This turn sequence is flanked by two 8 amino acid sequences with a high β-sheet propensity. Similar turns could be formed from natural amino acids. At low pH the system is unfolded but at high pH the peptide forms the β-hairpin, these then associate to

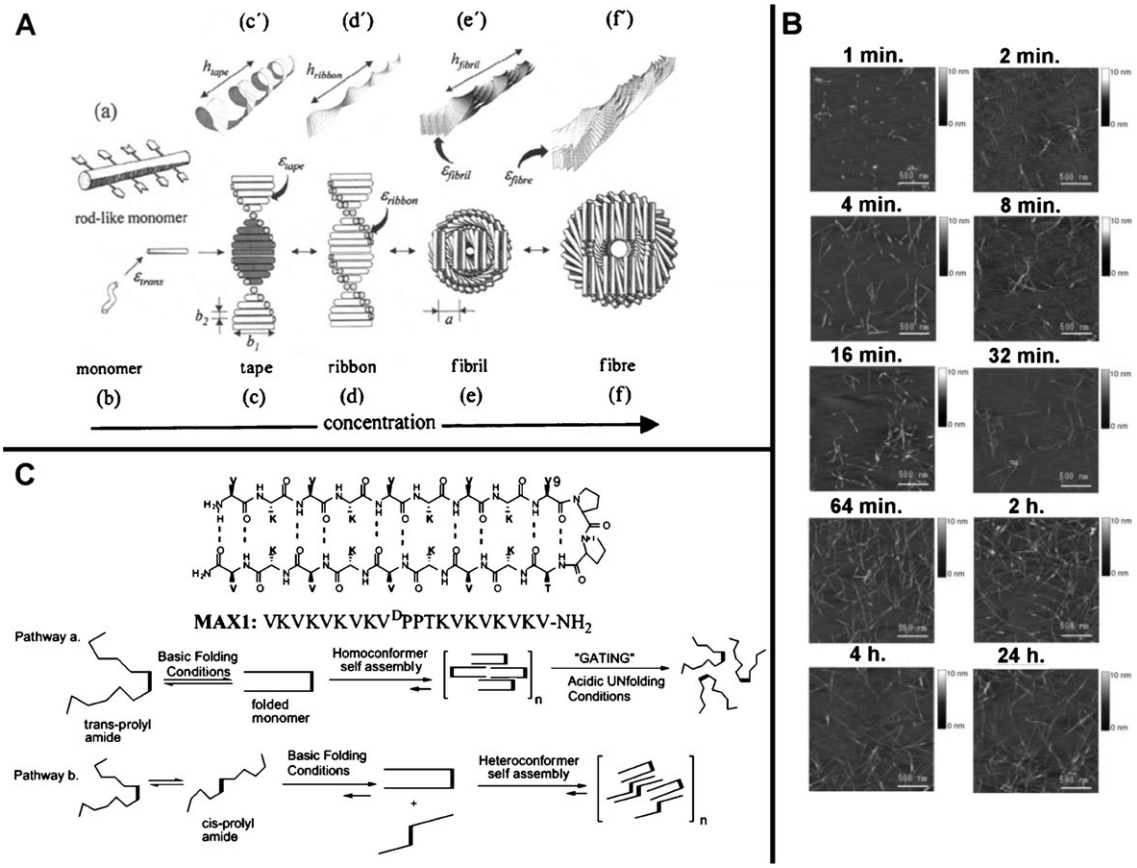

Fig. 2 β-Sheet based fibres can form a series of hierarchical structures (A) in a concentration dependent manner. These structures range from the basic peptide structure (a) as a monomer (b) which assembles into a tape (c & c′), two of which can bury hydrophobic residues by forming a ribbon (d & d′). Ribbons can further assemble by lying face to face to form fibrils (e & e′) and additionally side by side to form fibres (f & f′). β-Sheet based fibres have been shown to have dynamic assemblies, that after disruption by sonication can reassemble to long fibres over a matter of a couple of hours as shown by the AFM time course in (B). The design of a β-hairpin is shown in (C), the inclusion of a –V^{D}PPT– turn forces the *trans*-arrangement of the turn so that folding follows pathway (a), if this was not the case then pathway (b) would be followed and a heterogeneous self-assembly structure would form. [Figure adapted from ref. 6 (Copyright 2001 National Academy of Sciences), ref. 9 (Copyright 2005 National Academy of Sciences), reprinted with permission from ref. 10, Copyright 2002 American Chemical Society.]

form a fibrous hydrogel structure.[10] With similar properties to β-sheet systems the addition of the turn makes the peptide more amenable to control as will be discussed later.

In summary the design of β-sheets is well understood with a number of designed systems that have been extensively studied. They can form a hierarchical array of structures and have the potential to reform their macroscopic properties after disturbance.

3.2 Alpha helices/coiled coils

An alternative to using β-sheets as a basis for a fibrous structures are α-helices which for the purposes of self-assembly are used as components of coiled-coils as described first by Pauling, Corey and Crick.[11,12] These present a motif where long helices can inherently form fibrous structures. There are seven residues (a heptad), labelled *a* through *g*, to every two turns of a helix. This heptad can be visualised as a helical wheel (Fig. 3Ciii). The amide of the $i + 4$ amino acid hydrogen bonds to the carbonyl of the -*i*th residue in the helix. Coiled coils consist of at least two helices that come together to bury a hydrophobic interface. The design rules for forming coiled coils are well understood having been elucidated from naturally occurring systems. The first and fourth (*a* and *d*) residues are hydrophobic. As such, aliphatic side chains (L, I, V) create a stripe down one face of the helix which is buried through the interactions with another helix to form the coiled coil (also known as leucine zippers). Additionally a hydrophilic residue can be incorporated in the hydrophobic core to create specificity. For example asparagine has been used as this can form a hydrogen bond to an asparagine in a mirrored position in the facing helix of a dimeric coiled coil. The fifth and seventh residues (*e* and *g*) are generally charged residues that complement the charges on the facing helix and can help determine partners in heterodimeric systems. The oligomeric state of the coiled-coil is determined by the residues in the hydrophobic interface. It is possible to make more complicated interfaces involving multiple helices interacting with a number of neighbours. Examples of the use of coiled-coiled systems include stimuli-responsive hydrogels based on proteins with terminal leucine zipper peptide domains.[13]

An extensively studied design of a fibrous coiled-coil based system has been demonstrated by the Woolfson group using ''sticky end'' assembly. A heterodimeric parallel coiled coil,

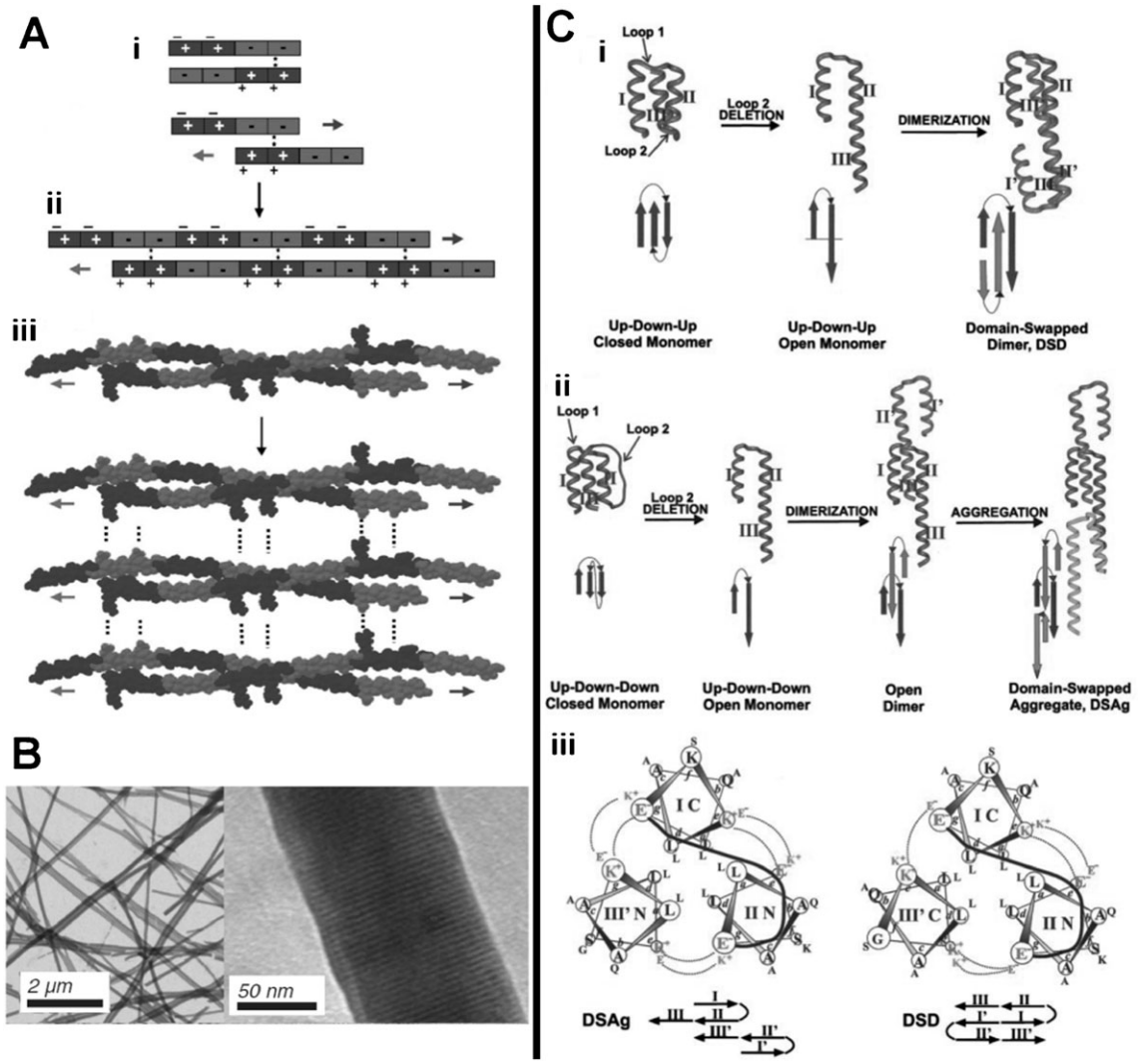

Fig. 3 The design concept for the self-assembly fibre (SAF) system is illustrated in (A), a normal coiled-coil would have a blunt arrangement with complementary charges and a paired up asparagine residue (*) in the core (i). To create a fibrous structure a sticky-ended system was created by circular mutation of the blunt arrangement allowing propagation of the coiled-coil structure (ii). Features on the outside of the coiled-coil repeat and these permit further assembly of the structure in a lateral direction to create large bundles of coiled-coils (iii). These fibres are illustrated by TEM images in (B), these fibres have micron scale lengths, and additionally have regularly arranged nanometer scale patterns on the individual fibres. Use of a domain swapped structure to create a self-assembly structure is illustrated in (C). Rearrangement of a three helix bundle to form a dimer is shown in (i) and a fibre in (b) in ribbon form and cartoon form. [Figure adapted from ref. 15 (Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission), ref. 16 (Copyright 2001 National Academy of Sciences), ref. 18 (Copyright 2001 National Academy of Sciences).]

SAF-p1 and SAF-p2, was designed to have a staggered hydrophobic interface, unlike all natural coiled-coils that form blunt assemblies (Fig. 3A). Due to the staggered nature of the system a peptide overhang or ''sticky end'' is available to form a coiled-coil interface with another peptide and in so doing propagate the structure along the long axis of the coiled-coil.

The initial designs gave rise to a fibrous system, however, they formed larger fibres than anticipated due to unintended interactions between the coiled-coils causing lateral as well as longitudinal assembly.[14] Peptide SAF-p2 was modified by incorporation of arginine residues to allow salt bridges to form with aspartic acid residues at the interface between coiled-coils. This approach resulted in fibers with increased stability.[15] When observed by transmission electron microscopy (TEM), striations perpendicular to the fibre axis were observed, indicating a high degree of order in this system (Fig. 3B).[16] Further studies demonstrated the labelling of the peptides with fluorophores and revealed that assembly under near equilibrium conditions take place in a polar fashion by the addition of specifically labelled molecules to only one end of the fibres.[17] This was the first example of a designed polar assembly system.

Another method to create fibrous structures involves non-covalently exchanging one structural domain of one peptide with that of another (Fig. 3C). In this example the domain swapped peptides are from a designed three helix bundle, which requires a specific orientation of the third helix in the bundle to create a compact structure. The structure can be

engineered so that the third helix is not incorporated in the coiled-coil and forms a ''sticky end'' and as a consequence the three helix bundle is not formed. To reform the three helix bundle a helix from another peptide needs to be inserted, which allows formation of dimeric structures or fibres. In one design, a second molecule can insert the extended helix into the structure of the first molecule to create a three helix bundle, in so doing it also accepts the extended helix of the partnered molecule to satisfy the formation of its three helix bundle (Fig. 3Ci). In an alternate design a second molecule can interact with the first molecules ''sticky end'' to create a three helix bundle, however, it still has a free helix left that interacts with a third and so on to create a chain of molecules or a fibre (Fig. 3Cii). This method presents a simple way of converting a compact folding unit into a extended fibrous structure.[18]

In summary, coiled coils provide an opportunity to prepare peptide based nanofibrous structures based on a number of well established design rules derived from natural systems. ''Sticky-ends'' and domain swapping allows for high levels of control over supramolecular behaviour whereas rational incorporation of specific amino acids gives rise to predictable structural changes.

3.3 Peptide amphiphiles

Peptide amphiphiles (PA) consist of oligo-peptides that are modified with a hydrophobic alkyl tail to form molecules with distinctly hydrophobic and hydrophilic ends, similar to lipids. Extensive work has been done on a class of PA that consist of a linear hydrophobic tail coupled to a short peptide sequence. A peptide of interest can be added to the end of the short peptide sequence to incorporate functionality that is presented to the aqueous bulk. When these molecules are in water they generally assemble into high aspect ratio rods with a hydrophobic core consisting of the 12–16 carbon alkyl tails and the peptide presented radially from the core where the peptide can adopt a β-sheet conformation (Fig. 4). By varying the charges used in the peptide portion of the molecule and whether the alkyl chain is placed on the N or C terminus of the peptide the molecules can be made to assemble under a range of conditions. It has been shown that more stable rods can be created by mixing oppositely charge PA molecules to promote closer interactions and hence potential hydrogen bonding between peptide moieties in a β-sheet configuration.[19]

One key feature of PA self-assembly is the formation of β-sheet regions in the core of the peptide, closest to the alkyl chain, this has been carefully examined using a series of *N*-methylated PA molecules. Preventing hydrogen bonding in the first four amino acids after the alkyl tail prevents the formation of a good mechanically resistant gel and spherical micelles are observed by TEM. Preventing hydrogen bonding of the outer amino acids appears to have little affect on the formation of a gel and thus the molecular structure. These results indicate that the peptides hydrogen bond in a twisted or helical ribbon with the outer residues able to adopt a variety of configurations as only the inner amino acids are close enough together to hydrogen bond to one another.[20] These results clearly indicate that the formation of rods by peptide amphiphiles is driven by a combination of both the hydrophobic

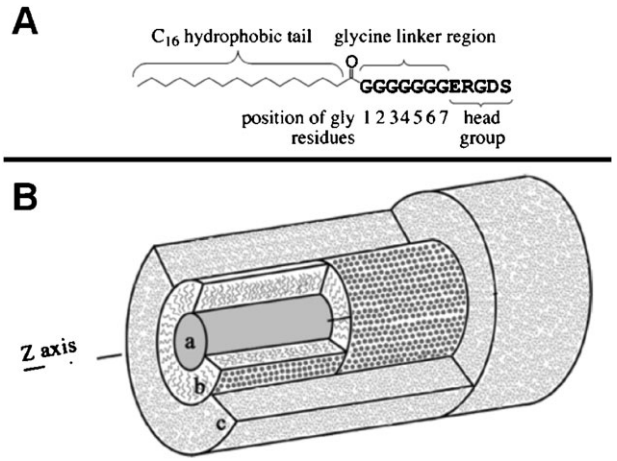

Fig. 4 The basic overall chemical structure of a peptide amphiphile is shown in (A), the alkyl tail acts as a hydrophobic core, the first four glycine residues are required for hydrogen bonding the structure together and the outer residues in the peptide are free to adopt a random structure (B). [Figure reprinted with permission from ref. 20 (Copyright 2006 American Chemical Society).]

interaction between the alkyl tails and hydrogen bonding of the peptides together. The rigidity of the rods formed can also be altered by the addition of a phospholipid which can only interact *via* the alkyl tail and not hydrogen bond to the peptide portion of a PA. Low percentages of phospholipid allow the rheological properties of the gel to be altered with a slight increase in the mechanical properties, possibly due to a more optimal geometry of interactions between PA molecules for hydrogen bonding. While higher percentages result in a disruption of the β-sheet hydrogen bonding network and hence the gel.[21]

In summary, while the design principles of PAs are known, the assembly and mechanical properties of PAs are tuneable by either chemical modification or co-assembly of complementary building blocks. Due to the flexibility in design of the peptide section that is presented, these materials have found a number of exciting biotechnological applications (See section 5).

3.4 Aromatic short peptide derivatives

Interest in the area of aromatic short peptide derivatives has come from two different directions. It has been demonstrated that the core sequence of some amyloid peptides is di-phenylalanine, which on its own can self-assemble to form stable peptide nanotubes.[22] It was proposed that FF nanotubes form by a combination of hydrogen bonding and π-stacking of the phenyl rings to interact with one another.[22] This has been further illustrated by evaporating away the solvent used to leave nanoforests of needles[23] (Fig. 5A).

Secondly, it was demonstrated that the chemical coupling of a variety of aromatic groups to short peptides may aid in self assembly *via* π–π stacking. For example, it has been shown that the addition of an aromatic group, such as carbobenzyloxy, naphthalene, or fluorenylmethoxycarbonyl (Fmoc) to the N-terminus of some peptides allows them to form stable hydrogels.[24] These gels are self supporting[24,25] displaying rheological behaviour that is characteristic of

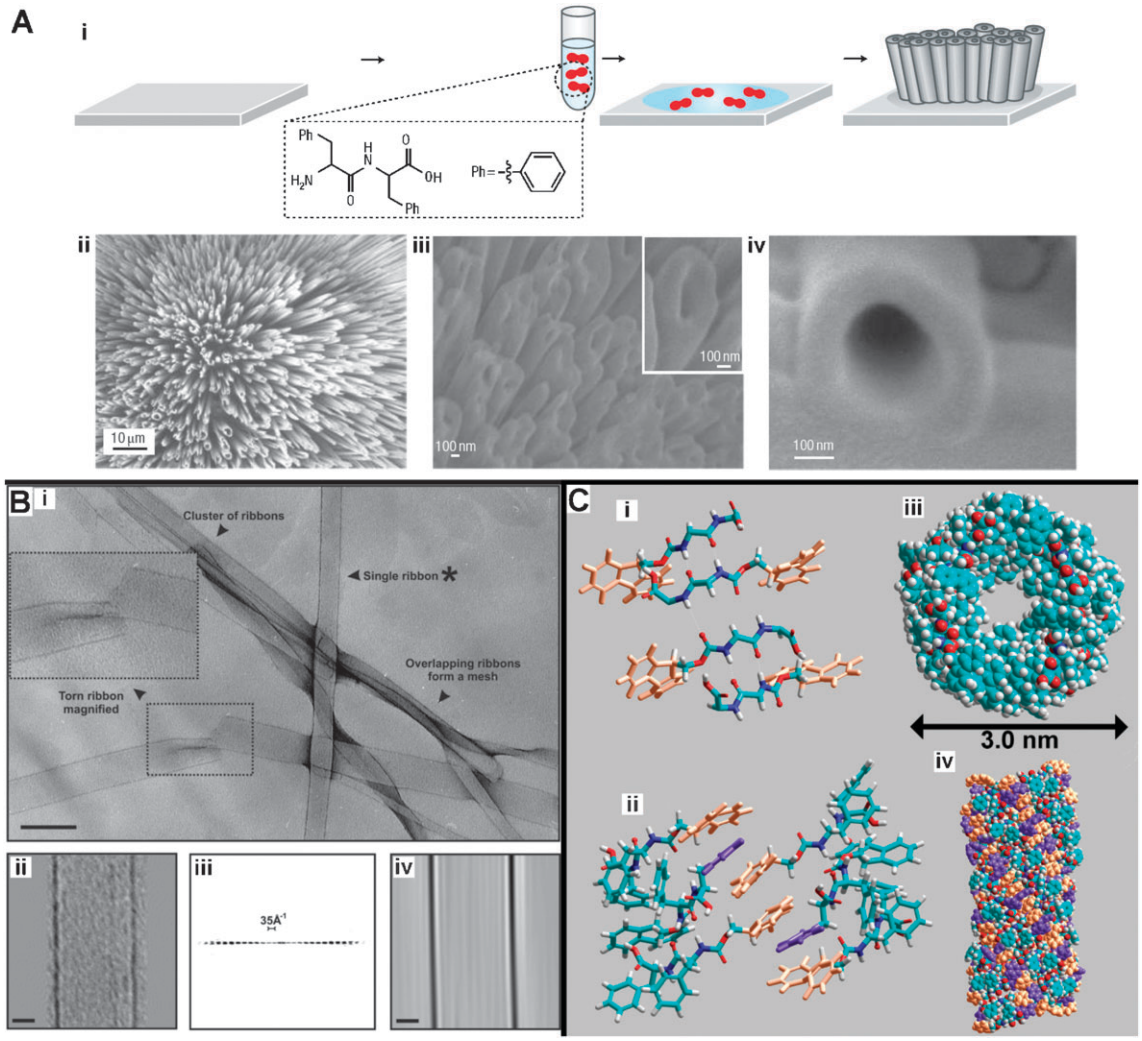

Fig. 5 The formation of ''nanoforests'' of diphenylalanine nanotubes uses an organic solution of diphenylalanine applied to a siliconised glass surface. SEM and cold field-emission gun high resolution scanning electron microscope (CFEG-HRSEM) images of the resulting vertical structures show a uniform dense arrangement of needles (A). Electron microscopy images of Fmoc-FF hydrogels reveal a flat fibrous structure made up of fibrils that have a width of 35 Å as identified by overlaying multiple images (ii) and determining a fast Fourier transform of the image to identify the repeat distance across the fibre (iii) (B) consistent with a cylindrical model that has been proposed (C). The peptide organises into anti-parallel β-sheets (i) which then come together through π–π interactions between the Fmoc groups (in orange) (ii) like a zipper to create a cylindrical structure (iii & iv). [Figure adapted from ref. 23 (Reprinted by permission from Macmillan Publishers Ltd: Nature Nanotechnology, Copyright 2006), 26 (Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission).]

solid like gel materials.[26] The morphology and dimensions of the nanostructures that were obtained depended both on the route of self assembly (involving pH changes,[24–26] solvent polarity changes[22,23] or enzyme action[27]) and the chemical nature of the building blocks,[25] highlighting the versatility of using aromatic short peptide derivatives in self-assembly. Recently a novel molecular architecture has been proposed for one member of this class of self-assembling peptides, Fmoc-FF. The proposed model accounts for the observed β-sheet signals for the peptide portion of the molecule and the fluorescence signal from the aromatic portion of the molecule. According to this model, the peptides are arranged as an anti-parallel arrangement of β-sheets with the Fmoc groups acting like a zipper to bring neighbouring sheets together, and because of the twist introduced by the β-sheet structure the neighbouring sheets are rotated in relation to one another creating a cylindrical structure (Fig. 5B & C).[26]

Self-assembly systems based on aromatic short peptide derivatives are still being investigated and progress towards understanding their structures and design rules is well underway. The approach allows for the use of a much shorter peptide sequence compared to those used in other categories of peptide self assembly. A number of biological and technological applications have been described, as discussed in sections 5 and 6.

4. Responsiveness—assembly on demand

A major challenge in molecular self-assembly is to control the self-assembly process and avoid defects in the assembled structures. Many single and multi-component systems suffer

from the problem that as soon as the material is placed into water or buffer, self-assembly is initiated, *i.e.* nucleation and growth are poorly controlled. As a result, there is significant interest in developing systems that assemble and disassemble on cue. There are many ways to drive or direct self-assembly and here we illustrate a few of the possible methods of triggering self-assembly or dis-assembly on demand.

4.1 Ionic strength/pH

Possibly the simplest method to control assembly in a self-assembly system is application of a pH switch. These systems are often also controllable by ionic strength as raising the ionic strength of a solution will mask the effect of charged amino acids and hence remove the pH trigger. A pH switch can be introduced by either complimentary or opposed charges in the peptide design. A number of designed peptides are inherently pH sensitive due to the use of charge interactions in the self assembly system itself, such as the β-sheet based peptides discussed earlier.[8,10]

One system intended to be reversible is based on a coiled-coil that undergoes a pH sensitive transition. The design is that of a standard trimeric coiled-coil, with one alteration, the *d* residue of alternate heptads is replaced with a histidine residue. At pH values above that of the pK_a of histidine the peptide adopts the coiled-coil conformation and forms a long fibrous structure, but below the pK_a the histidine is protonated and disrupts the core of the trimeric coiled-coil and the peptide adopts a random coil configuration as determined by circular dichroism spectroscopy (Fig. 6A).[28]

4.2 Enzymes

A challenge in molecular self-assembly of peptides is to control the self-assembly process under physiological conditions. Enzymes have recently emerged as tools to achieve this by converting non-assembling precursors into self-assembling building blocks under constant conditions of pH, ionic strength, temperature. Designing peptide based systems that are responsive to enzymes can make them useful in biological

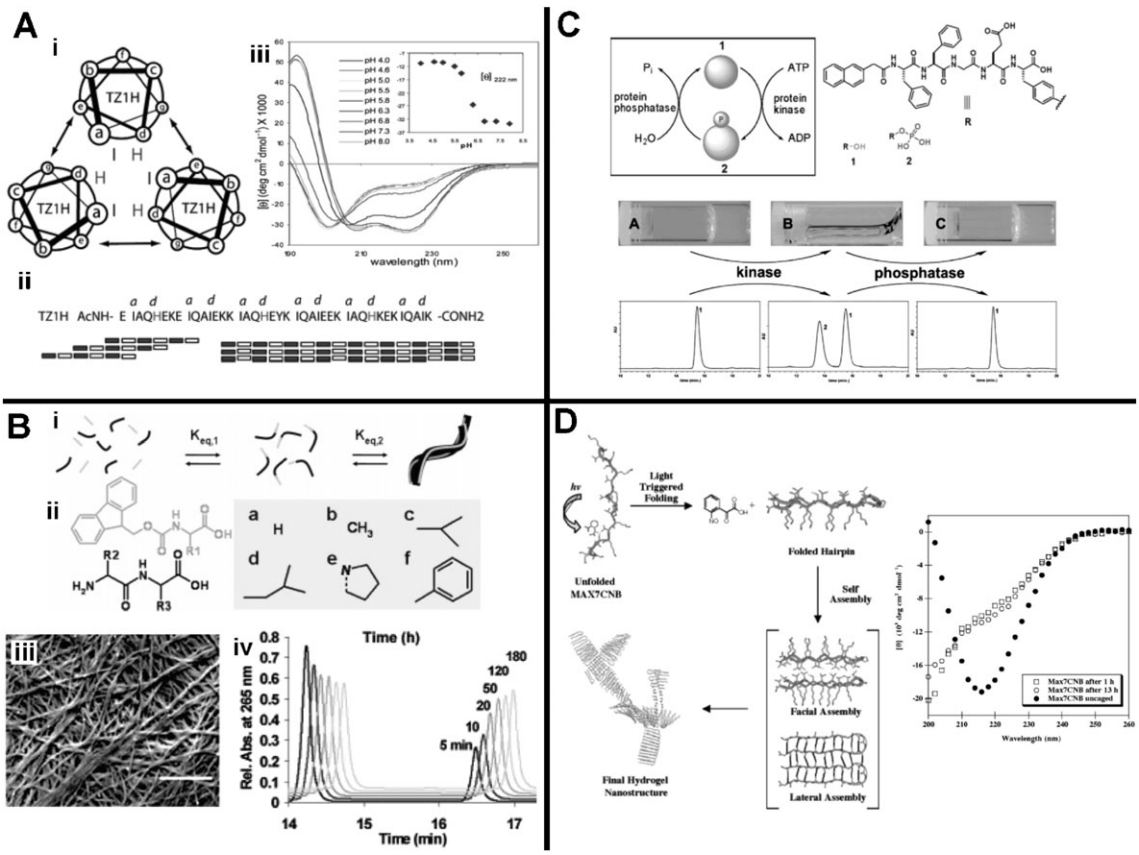

Fig. 6 An example of a pH-responsive switch is the three helix bundle with a buried histidine in the core (i), above pH 6.5 the peptides assemble in a staggered arrangement to create a fibrous structure as illustrated in the cartoon by the black (histidine-) and white (non-histidine-) containing heptads (ii), below this pH the structure is disrupted by the positive charge on the histidines as seen by the circular dichroism spectra (iii) (A). Illustrated in (B) is a schematic of the process by which a protease can be used to form Fmoc-peptides from a Fmoc-amino acid and a dipeptide (ii) due to the second self-association equilibrium on the first enzymatic equilibrium (i). SEM images confirm the gel is formed and HPLC traces over a time course show the formation of a Fmoc-tripeptide (iv right hand peak). Reversible modification of the subunits of a gel by a phosphatase/kinase reaction where the addition of a phosphate group to the tyrosine of the molecule causes the gel to disassemble, addition of a phosphatase removes the phosphate group and triggers gelation again (C). A known self-assembling β-hairpin design was modified with a light activated molecule that prevents assemble while it is attached to the peptide, shining light of the correct wavelength causes the molecule to be released and the β-hairpin to fold and assemble to a fibrous structure (D). (Figure adapted from ref. 27, 28, 30 reprinted with permission, Copyright 2006 American Chemical Society, and ref. 31 reprinted with permission, Copyright 2005 American Chemical Society.)

situations, for example taking advantage of an early enzyme in blood clotting may help prevent blood loss if a liquid can be switched to a gel.

Enzyme-assisted self assembly can be achieved either by catalysing the synthesis of a self-assembly molecule, or by removing a blocking group from a molecule to allow assembly. A vast array of natural enzymes are known, most catalyse a defined reaction but the degree of specificity to their substrate varies from highly specific to non-specific. This range of specificities means they can be utilised to react with non-natural molecules as well as their natural substrates.

One example of an enzyme assisted self assembly system is *via* reversed hydrolysis. In this case, a protease was used to drive the self-assembly of peptide hydrogelators *via* coupling of non-assembling precursors. A range of Fmoc-amino acids and FF or LL were used to synthesise Fmoc-tripeptide derivatives (Fig. 6Bi).[27] This method relies on an enzymatic step that is thermodynamically 'up-hill', which is driven thermodynamically by the self-assembly process (Fig. 6Bii & iii). It is thought that the fully reversible nature of this method will help prevent formation of kinetic aggregates and favour the thermodynamically stable assembly, thereby paving the way to a self-assembled structure with fewer defects.

An example of an irreversible system that utilises an enzyme as a trigger to a conformational transition is that of the triggering of an *O*- to *N*-acyl migration through peptide cleavage. This system was designed to allow study of the initial steps of self assembly, specifically in this case amyloid fragments, peptides that assemble into β-sheet structures. In this system a β-sheet-forming peptide sequence is attached through the side chain of a serine, threonine or cysteine and a short capping peptide is attached to the amino group. This capping peptide is designed to be cleaved off by a specific protease. Once this occurs a chemical rearrangement happens that repositions the desired peptide chain onto the amino group leaving the normal serine, threonine or cysteine side chain present. Due to this chemical rearrangement after the action of the protease the process is irreversible.[29]

A reversible system using two enzymes that catalyse opposite reactions (phosphorylation/dephosphorylation) was demonstrated to control the gel–sol transition of a peptide in a highly controlled manner. The peptide used is a naphthalene linked to FFGEY, this peptide is a gel in water (Fig. 6C), in the presence of adenosine triphosphate, kinase adds a phosphate group to the tyrosine residue, resulting in a transition to a solution. The further addition of a phosphatase results in the removal of the phosphate and another transition to a gel.[30] The use of enzymes in self-assembly adds a level of control over the self assembly process and is therefore expected to play a key role in the future development of more complex

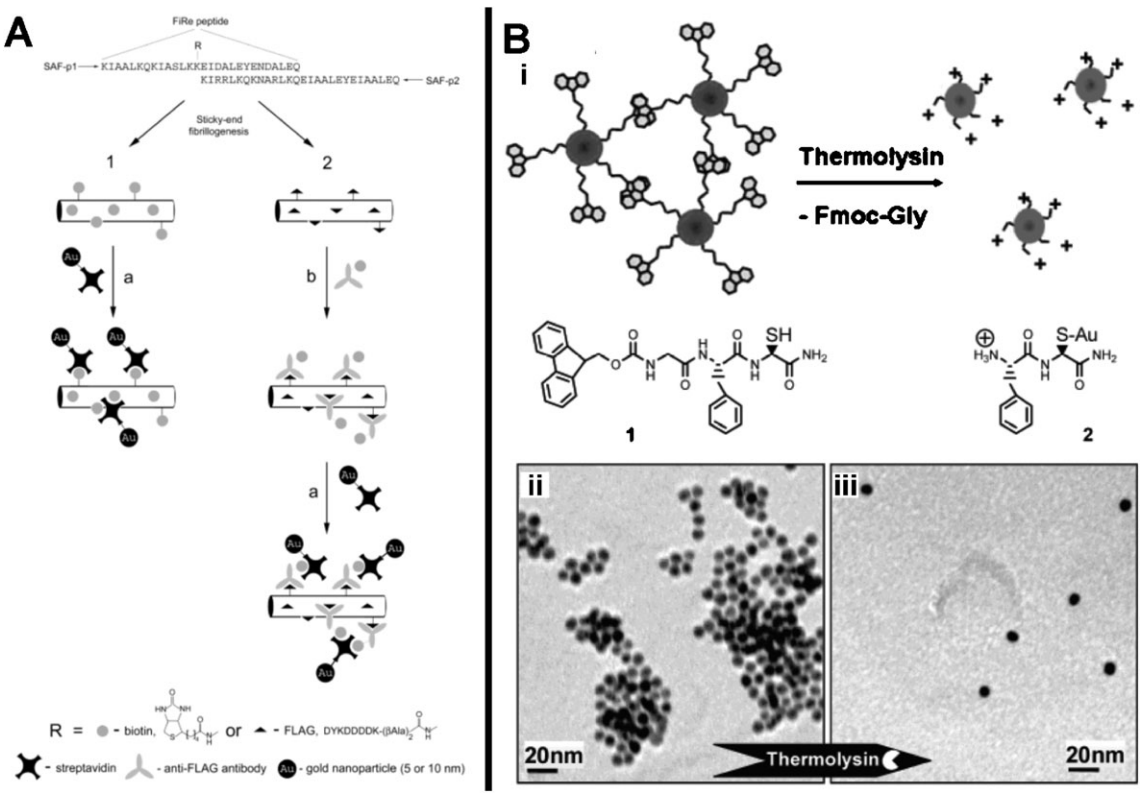

Fig. 7 Schematic of the modification of the coiled-coil SAF system by the addition of biotin or FLAG peptides to an exposed lysine residue. The biotin was then used to bind streptavidin labelled with gold particles or in the case of the FLAG peptide, antibody labelled with biotin that the bound streptavidin labelled with gold (A). Schematic of gold particles coated with a Fmoc peptide containing a protease-sensitive sequence. With the peptide intact the gold particles cluster, after cleavage with the protease, thermolysin in this case, the gold particles should disperse (a). Electron microscopy results show this to be the case with clusters of particles prior to cleavage (b) and a dispersion afterwards (c). (Figure adapted from ref. 34 reprinted with permission, Copyright 2004 American Chemical Society, reprinted with permission from ref. 35, Copyright 2007 American Chemical Society.)

structures with fewer defects. Applications have already been demonstrated in wound care and biosensing, as discussed in sections 5 and 6.

4.3 Light

An alternate method for controlling self-assembly is to utilise an external stimulus, such as light, that does not perturb the solution but interacts with the material. This has the advantage of not having localised changes in the environment which can occur for example, when attempting pH changes. A light trigger has been built into the β-hairpin system developed by Schneider and Pochan. A cysteine residue was introduced into the hydrophobic face of the peptide as a reactive site for modification and shown to still fold to the expected β-hairpin, a photocage, α-carboxy-2-nitrobenzyl, was then attached to this cysteine. The presence of the cage prevents folding of the peptide to a β-hairpin and therefore self-assembly, but exposure to UV light causes decaging which allows the peptide to fold to the β-hairpin structure and hence self assemble (Fig. 6D).[31]

5. Biological applications

One major area of interest for designed systems, is that of 3D cell culture. There is an interest in growing cells in a more "natural" environment. To do this an artificial extracellular matrix (ECM) is needed. In their natural environment (*i.e.* within tissues), cells grow through a matrix of proteins and glycoproteins, the most common being collagen. There have been a number of peptide based fibrous networks that have been developed that allow cells to grow in a 3D environment similar to the ECM.[25,32]

In the context of controlling cell behaviour in a manner that mimics the ECM, designed self-assembly systems were modified with bioactive molecules. In an example based on peptide amphiphiles, a heparin-binding peptide motif was incorporated into the PA structure. Heparin is a highly sulfated glycosaminoglycan, that binds angiogenic growth factors that are of relevance in new blood vessel formation. It is proposed that the heparin molecules bind *via* electrostatic interactions to the surface of the PA fibres as visualised by attaching gold nanoparticles to the heparin. This combined system has been shown to promote the formation of blood vessels.[33]

An alternative approach to decorating self-assembled fibres with biomolecules was demonstrated in coiled-coil systems. Here peptides and proteins of interest were attached to the designed peptides through the ε-amino group of a lysine in a solvent-exposed position (Fig. 7A). The system was assembled with only one of the two peptides involved in the assembly modified with a marker. One molecule added was biotin, which is shown to be able to recruit streptavadin labelled with gold particles to the surfaces of the fibre formed by the peptides, showing a protein–ligand interaction (Fig. 7A1). The second system demonstrated uses the so-called FLAG peptide, a designed peptide tag which can be bound by an anti-FLAG antibody, this demonstrates the systems applicability to antigen–antibody interactions (Fig. 7A2).[34]

Self-assembly systems can also be utilised in biosensing by using the sensed molecule as the trigger for self-assembly or

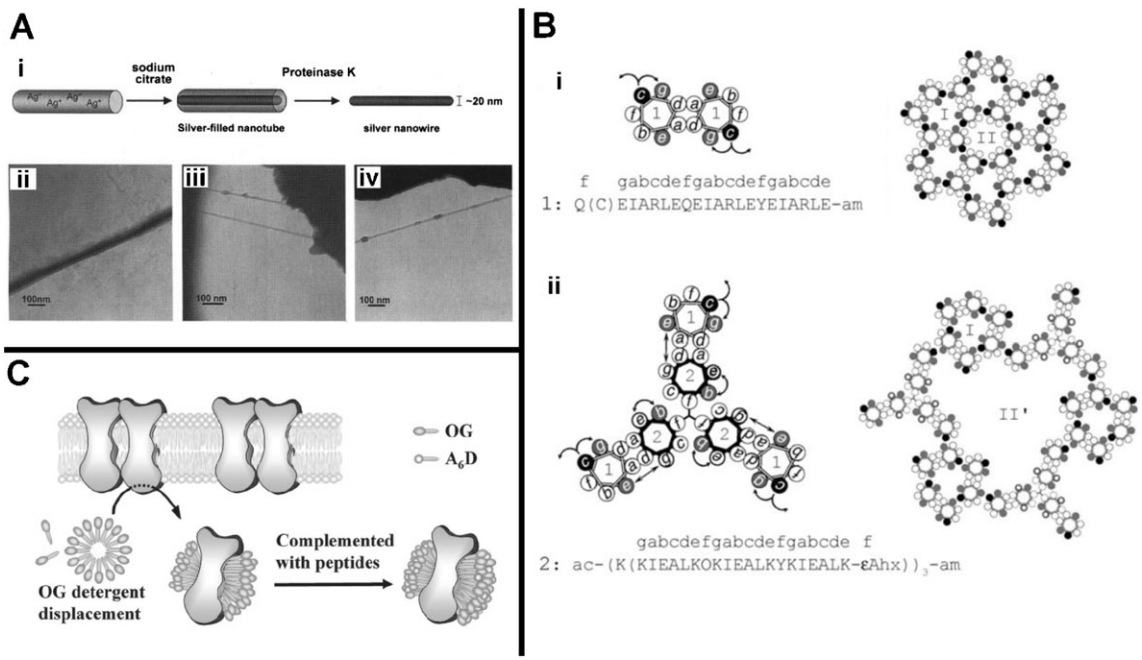

Fig. 8 Creating a silver nanowire by using a diphenylalanine tube as a template for the formation of the wire (A). Schematic representation of the supradendrimer design: SD-1 consists of a coiled-coil dimer that trimerises to form a branching cell through charge interactions; SD-2 consists of three helices linked through an ac-KK-am hub containing an ε-aminohexanoic acid (εAhx) as a spacer. Inclusion of SD-2 in the self-assembled structure creates a larger cell. SD-2 molecules are highlight with black circle (B). A proposed model for the action of amphiphilic peptides like A_6D; normally rhodopsin would sit in the lipid bilayer of cells. The protein is solubilised from the membrane with a detergent and then stabilised by the addition of the amphiphilic peptide (C). (Figure adapted from ref. 22 (Reprinted with permission from AAAS), 36 (Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission), 37 (Copyright 2006 National Academy of Sciences).)

disassembly and monitoring the change in solution properties. A recent example demonstrated the use of gold nanoparticles that were linked to a short peptide with a Fmoc group, a large aromatic group, attached to it (Fig. 7Bi). The peptide sequence was designed to be initially cleaved by thermolysin. In the absence of the enzyme the gold nanoparticles clustered together due to interactions between the aromatic group (Fig. 7Bii), in the presence of the enzyme the peptide is cleaved removing the aromatic group from the particle and revealing a charged amine, which results in dispersion *via* electrostatic repulsion (Fig. 7Biii). Dispersion of gold nanoparticles results in a blue to red colour shift that is visible to the naked eye. A variant of the system used a different peptide sequence specific to an enzyme directly linked to prostate cancer. The system is able to detect concentrations of the enzyme with higher sensitivity compared to existing methods.[35]

6. Non-biological applications

An exciting potential application of self-assembled peptide systems is in nano-electronics. Nanoscale fibres or tubes may be used as templates for the formation of metal nano-wires. The latter approach has been demonstrated with an aromatic system by the Gazit group. Here a diphenylalanine tube was used to template a silver nanowire inside the tube, the peptide was then removed using proteinase K to give a fine nanowire 20 nm in diameter (Fig. 8A).[22]

Another application is to exploit cavities in self-assembled structures as nanoreactors. This has been demonstrated through the use of coiled-coil interactions, where multiple reactors are formed that, by careful positioning of amino acid side chains, allow for directed crystal growth. By exploiting interfaces around the coiled-coil a complex structure could be formed to allow for formation of multiple nanoreactors in a single self-assembled structure (Fig. 8Bi). Addition of a second complementary peptide with a covalently triplicated starburst structure was used to expand the original self-assembly structure to form an open ring systems (Fig. 8Bii). This system was shown to be able to form silver nanoparticles of a size consistent with the cavities observed by TEM.[36]

An application that has both a biological and non-biological connotation is the solubilisation of membrane-bound proteins into an aqueous solution. One method proposed by Zhang is to use amphiphilic peptides with a structure similar to lipids, a tail section of hydrophobic amino acids with one or two charged amino acids as a head group. Sequences based on this design, for example A_6D have been shown to be able to stabilise the G protein-coupled receptor bovine rhodopsin in combination with traditional lipids (Fig. 8C).[37] Other peptides with similar designs have been shown to solubilize a range of normally membrane-bound proteins and complexes, and it has been proposed that this process could be used to create films of light harvesting complex I to turn light into electrical energy.[38]

Conclusions

In conclusion, peptides offer a versatile method for creating nanomaterials, firstly by using amino acid sequences to create structures based on an understanding of natural motifs. Secondly, amino acids can be used purely as chemical moieties with specific properties to be exploited and the peptide structure is secondary to the formation of a supramolecular structure. Both these approaches have been successful and the understanding of how assembly occurs and is controlled have expanded over the last few years. As biologists discover and come to understand new peptide folding motifs, new structures may become possible. In the future more emphasis is likely to go into the control of self-assembly, as has been seen in the last few years. There are a number of ways of controlling assemblies, with varying degrees of complexity from pH switches to light activated molecules, to enzymatic control. The use of enzymes in the control of self assembly is likely to increase as enzymes represent a dynamic element that can be exploited to create highly selective enzyme-responsive materials with applications in drug delivery and biosensing.[39] Enzymes may also be exploited in the assisted assembly of complex and highly ordered nanostructures that may be difficult to achieve *via* un-assisted self-assembly that is prone to formation of kinetically trapped aggregates (misfolding).[27,40] The range of applications for peptide-based nanomaterials is steadily expanding with applications emerging as replacement extracellular matrix for regenerative medicine, highly sensitive sensors for diagnostics, and increasingly complex templates for nanotechnology applications.

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