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REVIEW ARTICLE

Constructing biomaterials using self-assembling peptide building blocks

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Abstract Molecular self-assembly is ubiquitous in nature and has recently emerged as a new bottom-up approach in constructing biomaterials. Synthetic peptides assemble through specific molecular recognition and form diverse nanostructures. The resulting versatile peptide self-assemblies may be used in a wide range of biological and medical applications. Examples of two self-assembling peptide systems are presented and techniques for self-assembly control are discussed.

Keywords peptide, biomaterial, self-assembly

1 Introduction

Molecular self-assembly is ever-present in nature and has recently emerged as a new approach in the disciplines of nanotechnology, materials science, chemical synthesis, and engineering [1]. The process of self-assembly is based on spontaneous organization of molecules through smart recognition. This process is often governed by non-covalent interactions, such as electrostatic, hydrophobic, metal-ligand interactions and van der Waals forces, hydrogen bond formation and aromatic π-stacking [2,3]. Although these interactions are rather weak individually, when sufficient in number they can govern the structural conformation and generate stable assemblies. It is necessary to develop an understanding of the forces governing the molecular assembly, accurate design concerning recognition of complementarity and structural compatibility. This will enable the correct observance of key elements used in smart recognition that will result in successful engineering of supramolecular architectures with well-defined structures and adjustable properties to cover a wide range of possible applications [2].

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Peptides have recently emerged as promising building blocks for nano-biomaterials using the so called ‘bottom up’ approach. They are a particularly attractive class of molecules for their secondary structural alteration and nanoscale folding and stability, as well as having several favorable properties with regards to biocompatibility, immunogenicity, biodegradability and non-toxic waste production. Peptides can introduce structural smartness into nanostructures due to their responsiveness and sensitivity to surrounding conditions/parameters (i.e., pH, temperature, electronic or photonic band gaps, and the presence of chelating metals) [4]. Moreover, the ability to incorporate non-natural amino acids or nonpeptidic moieties is also a valuable feature for the introduction of multifunctional peptide assemblies, which may significantly broaden the range of applications [5–8].

Advancement in synthetic tools has facilitated unprecedented control over composition, structure, and organization of peptide-based artificial materials. During the past decade, several self-assembling peptide systems have been discovered, with applications ranging from modeling of protein folding and protein conformational diseases, to molecular materials that produce peptide nanofibers, peptide scaffolds, peptide surfactants and peptide ink. These processes have been created by using well-defined strategies or simply by serendipitous discovery [1,9]. Furthermore, controllable assembly or transition of nanostructures has been obtained by introducing novel designs of peptide sequences. These self-assembly systems are important advancements in molecular engineering for a variety of technological innovations. In this review, we will focus on two peptide self-assembly systems: amphiphilic peptides and ionic complementary peptides, as well as highlighting some recent progress in the field of controllable assembly. Undoubtedly, these achievements and future development will allow the combination of biological design concepts in materials science and fulfill their desired functions [10].

2 Amphiphilic peptides

Amphiphilicity is one of the most important features in molecular self-assembly. Molecules containing both hydrophilic and hydrophobic elements have the propensity for aggregating with hydrophilic domains exposed to aqueous environments and hydrophobic parts accumulated inside the core of the assembly. An important class of amphiphilic peptides is comprised of a peptidic hydrophilic head and alkyl/lipid chains as the hydrophobic tail. Several examples of this class have been published for their bioactive applications [11].

In a work reported by Stupp and coworkers [12], an amphiphilic peptide was designed and studied for application in tissue engineering and biomineralization. In their peptide amphiphile an alkyl tail with 16 carbon atoms was coupled to a peptidic head. In the peptidic region four consecutive cysteine residues, one phosphoserine residue, and the peptide sequences Arg-Gly-Asp (RGD) were incorporated as functional groups (Fig. 1). The Disulfide bonds between the cysteine residues in adjacent peptides induced robust and reversible interactions. The phosphoserine and RGD sequences were respectively designed to promote nucleation of hydroxyapatite and adhesion of cells on the self-assembly surface. This peptide amphiphile has shown pH-dependent and reversible self-assembly; the fibrillar assemblies were able to direct mineralization of hydroxyapatite on their surface to form a composite material. In another work, the amphiphilic peptide self-assembly was developed as peptide scaffolds for nerve cell growth [13]. Laminin is an extracellular martrix protein which is known to direct neurite growth. A peptide amphiphile included a laminin epitope, and isoleucine-lysine-valine-alanine-valine (IKVAV), has shown to have the ability to promote the differentiation of neural progenitor cells into neurons in rats, while inhibiting astrocyte differentiation [13].

A class of cationic amphiphilic peptides is known for their antimicrobial ability. The antimicrobial peptides are recognized as possible pharmaceuticals for treatment of antibiotic resistant bacterial infections [14]. In a recent study, Liu et al. [15] designed a novel class of self-assembling peptides that may function as antimicrobial agents. Three functional moieties were engineered into this amphiphilic peptide (CG_3R_6TAT): the cell penetrating peptide TAT, six positively charged arginine residues (R_6) (for improving electrostatic interaction with negatively charged membranes and promoting membrane translocation [16]), and a membrane permeability promoting cholesterol [17] (C) as the hydrophobic tail. The three glycine residue moiety was a linkage to connect the functional blocks. *In vitro* results have shown strong antimicrobial properties against a wide range of bacteria, yeasts and fungi; an *in vivo* test showed that the peptide nanoparticles are capable of crossing the blood brain barrier. The results comparing inhibitory efficiency between CG_3R_6TAT and its partial functional counterparts: G3TAT, G3R6, G3R12, G3R6TAT supported the necessity of the incorporation model for promoting antimicrobial activity.

Zhang and coworkers have designed a class of amphiphilic peptides, comprising solely of amino acids, using natural lipids as a guide [18–20]. These peptide monomers generally contain 7–8 amino acids and have a hydrophilic head, composed of 1–2 charged aspartic acids (D) or lysines (K), and a tail of six consecutive hydrophobic amino acids. The length of each peptide is around 2 nm to mimic phospholipids. The hydrophobicity of peptides is tunable by using alanine (A), valine (V), or leucine (L) [19,20]. Peptides of this sort with various lengths were also designed by adding one amino acid at a time [18]. These peptides self-assemble in water to form open-ended nanotubes and nanovesicles with an average diameter of 30–50 nm (Fig. 2). The self-assemblies formed by the peptides consisting of alanines (A) and valines (V) were more homogeneous and stable in structure than those comprised of leucines (L), isoleucine (I) and glycines (G). Referring to the molecular models of peptide packing behavior of forming tubular and vesicle structures (as shown in Fig. 2), it seems that steric obstacles and hydrophobic and hydrophilic ratios played a vital role. The large side chains of L and I might prevent the peptides from packing tightly, while G does not have side chains to help stabilize the self-assemblies through hydrophobic interactions. These peptides have been further reported for their ability to solubilize and stabilize several membrane proteins by encapsulation of the membrane proteins to protect them from undesirable aggregation [21–23].

3 Ionic complementary peptides

Ionic complementary peptides are a unique class of self-assembling peptides. The first member EAK-II was derived from a segment of a left-handed Z-DNA binding protein in yeast [24]. These peptides are featured by the repetition of alternating hydrophobic residues and negatively or positively charged residues. This unique amino acid sequence contributes to the formation of two distinct surfaces following the peptide self-assembly: one hydrophobic and the other hydrophilic. Types I, II, III and IV of these peptides are classified based on the charge distribution on the hydrophilic side. Charge distributions for these peptides are as follows: Type I, $-+$; Type II, $--++$; Type III, $---+++$; Type IV, $----++++$. The structure features of "side to side" and ionic complementarity allow the peptides to form stable β-sheet structures (as shown in Fig. 3).

Chen and coworkers have studied the mechanisms of self-assembly of EAK16s systematically [25–28]. EAK16-II can form stable β-sheets in aqueous solution spontaneously. The β-sheets can bear a broad range of

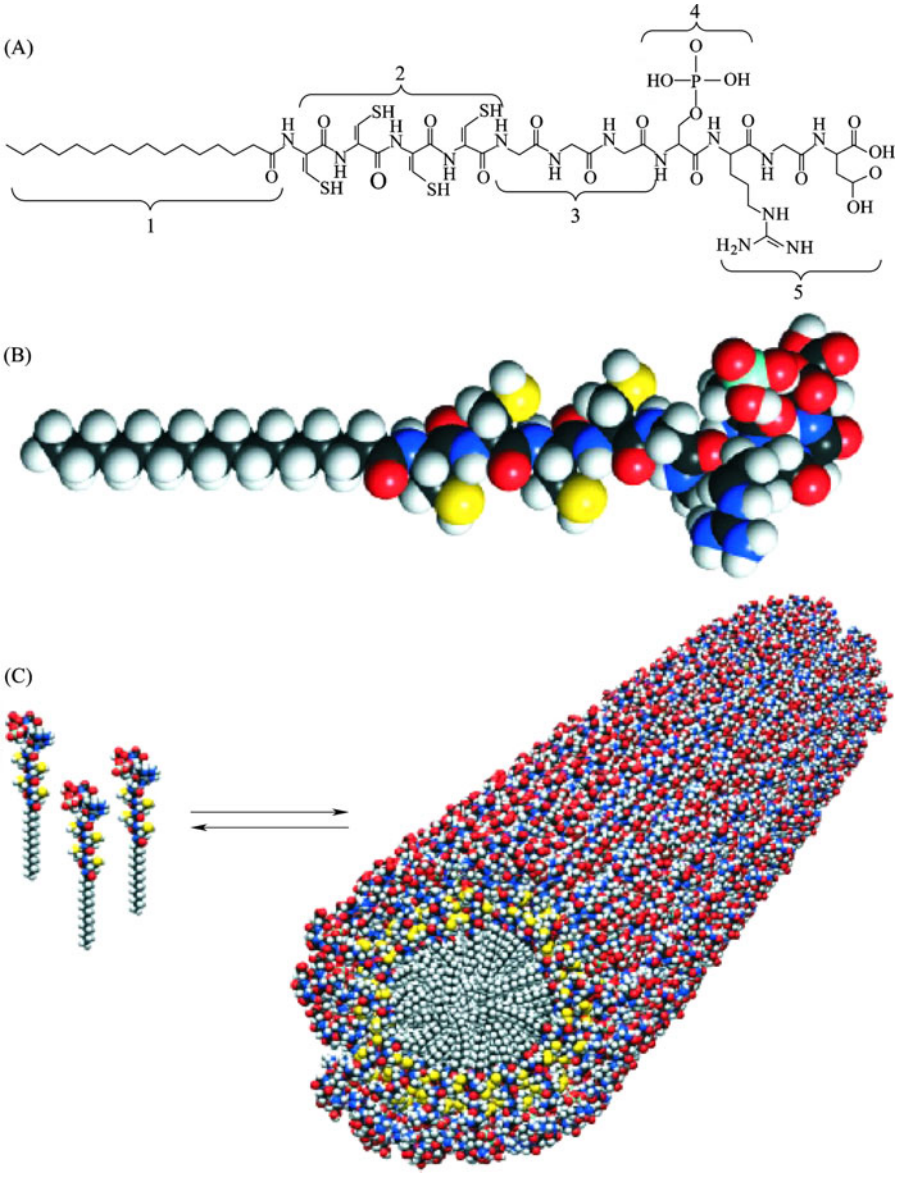

Fig. 1 **(A)** Chemical structure of the peptide amphiphile, highlighting five key structural features. Region 1 is a long alkyl tail that conveys a hydrophobic characteristic to the molecule and when combined with the peptide region, makes the molecule amphiphilic; Region 2 is composed of four consecutive cysteine residues that when oxidized may form disulfide bonds to polymerize the self-assembled structure; Region 3 is a flexible linker region of three glycine residues to provide the hydrophilic head group flexibility from the more rigid cross-linked region; Region 4 is a single phosphorylated serine residue that is designed to interact strongly with calcium ions and help direct mineralization of hydroxyapatite; Region 5 displays the cell adhesion ligand RGD. **(B)** Molecular model of the PA showing the overall conical shape of the molecule going from the narrow hydrophobic tail to the bulkier peptide region. Color scheme: C, black; H, white; O, red; N, blue; P, cyan; S, yellow. **(C)** Schematic showing the self-assembly of PA molecules into a cylindrical micelle. (From Ref. [11], reprinted with permission from AAAS)

physicochemical conditions, and were even impervious to denaturing agents. The critical assembly concentration, CAC, of 0.1 mg/mL for EAK16-II was determined. The β-sheets were stacked in an antiparallel fashion to form fibrillar assemblies at concentrations above CAC and form protofibrils below CAC. Compared to EAK16-II, EAK16-IV formed globular assemblies, resulting from the formation of β-hairpins by intramolecular electrostatic interactions. Changing the pH neutralized charged residues, which trigged a transition of EAK 16-IV assemblies from a globular structure to a fibrillar structure [29]. Another construct of EAK16-IIGGH bound copper ions, which was affected by the type of anions, as reflected in the nanofiber length and β-sheet content [30]. The potential of EAK16-II self-assemblies as carriers of hydrophobic molecules was also reported [31,32]. The effect of hydrophobicity and charge distribution on the formation of peptide-drug complexes was further

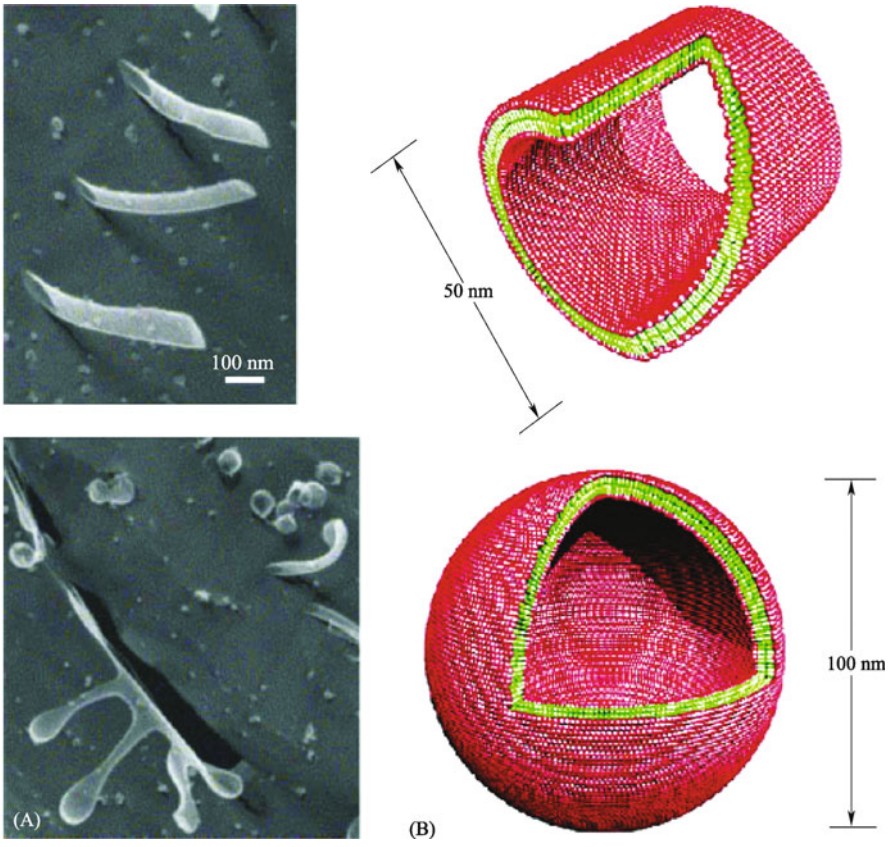

Fig. 2 **(A)** TEM images of nanotubes and nanovesicles from a surfactant-like peptide — V_6D. The sample was flash frozen in liquid propane (–180°C) to preserve the structure formed in solution. **(B)** Molecular modeling of cut-away structures of a nanotube and a nanovesicle formed from surfactant-like peptides. Color code: red, polar heads; green, nonpolar tails. The modeled dimension is 50–100 nm in diameter. (Reprinted from Ref. [8], Copyright 2010, with permission from Elsevier)

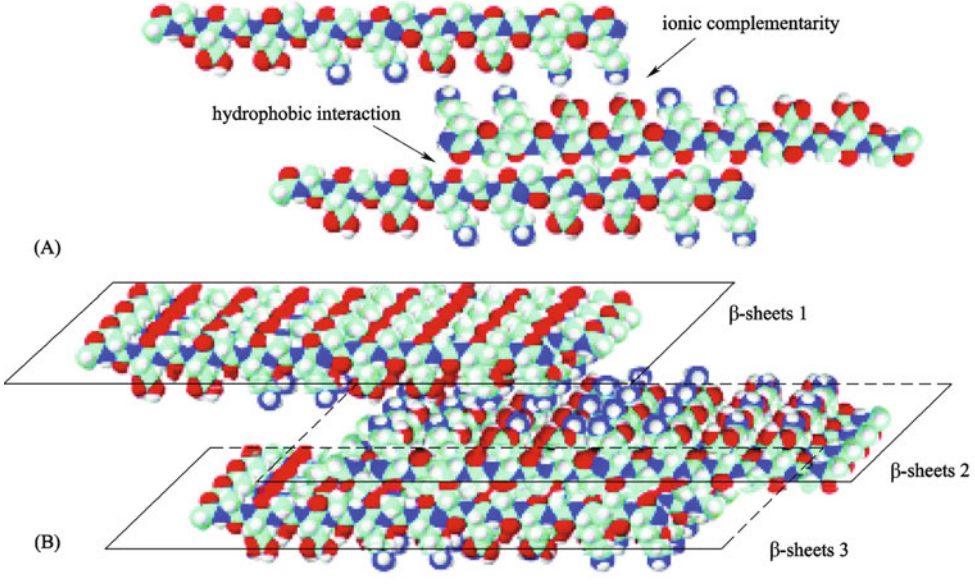

Fig. 3 **(A)** A scheme of EAK16-II self-assembly through hydrophobic interaction and ionic-complementarity. In addition to the hydrogen bonding, hydrophobic and electrostatic interactions help to make stable β-sheets. **(B)** A proposed model of EAK16-II self-assembly into β-sheet-based aggregates. (Reprinted from Ref. [3], Copyright 2010, with permission from Elsevier)

evaluated among EAK16-II, EAK16-IV and EFK16-II [33], which provided some bases for designing a peptide-based drug delivery system.

Other similar types of ionic peptides, RAD16s, as well as EAK16s, can further associate to form macroscopic membraneous matrices [34,35]. The matrices also supported mammalian cell attachment, which might be due to the similarity of sequences to cell adhesion motif RGD. In a recent study the release kinetics of RAD16-I peptide hydrogel for protein was investigated showing potential applications for sustained release of therapeutic proteins [36]. This type of peptide has been further modified by adding two functionalized peptides segments, RGD containing sequence GPRGDSGYRGDS and VEGF agonist motif G_4KLTWQELYQLKYKGI, respectively. Both of these new peptides displayed significant endothelial cell growth [37]. These examples show that peptide scaffolds are promising for 3D cell culture, tissue engineering and regenerative medicine applications.

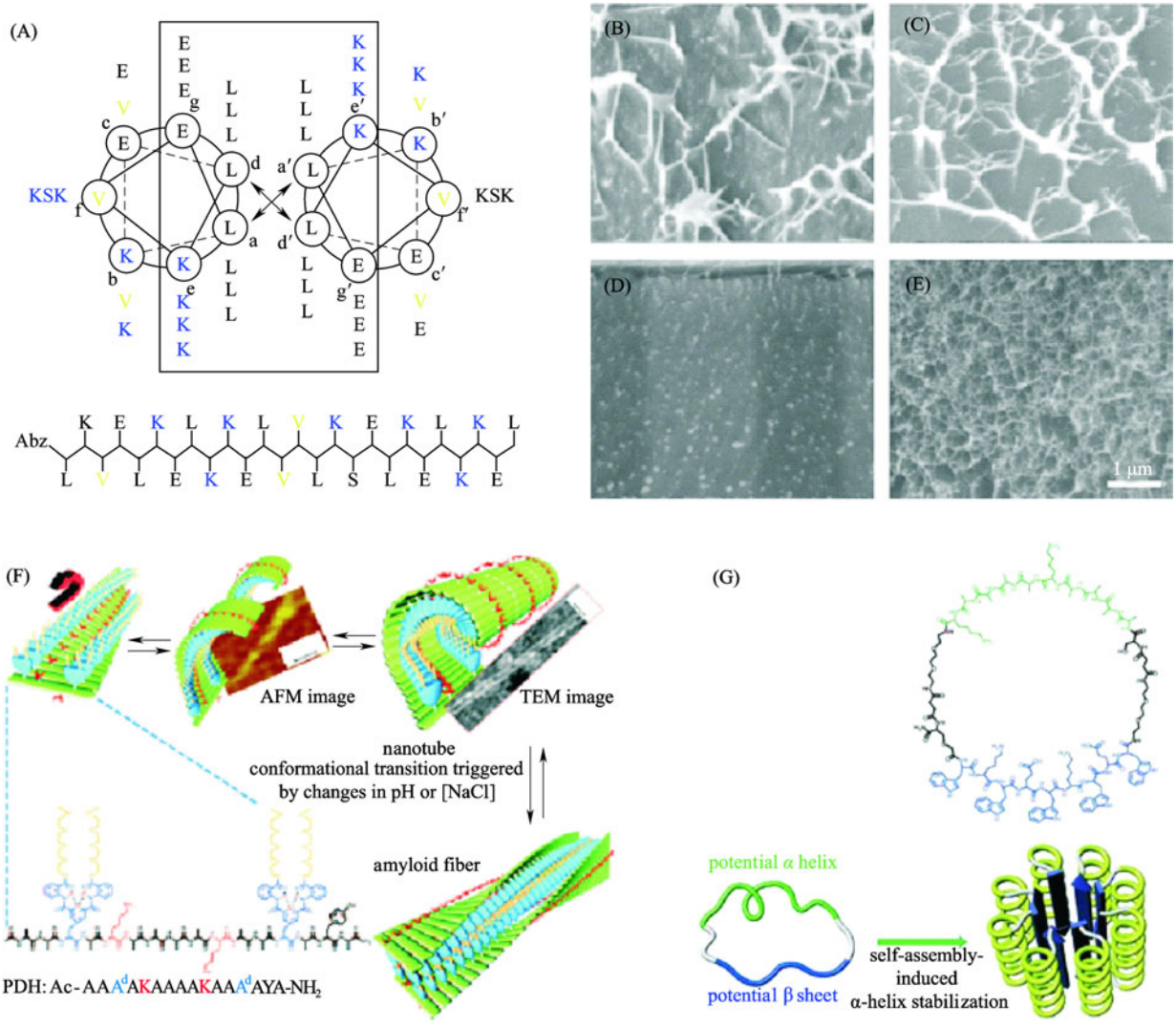

Fig. 4 **(A)** Helical wheel and sequence of model peptide VW19. Frame: positions inducing the α-helical coiled coil structure. Blue: positions to destabilize the helical structure at acidic pH. Yellow: positions favoring a β-sheet conformation. (Reprinted with permission from Ref. [38], Copyright 2010, American Chemical Society) **(B)(C)(D)(E)** Network formation as observed by cryogenic scanning electron microscopy for hSAFQQQ (B), hSAFAAA (C) and hSAFAAQ (D), all assembled on ice for 15 min; and for hSAFAAA assembled on ice for 3 min and then at room temperature for 12 min (E). (Reprinted by permission from Macmillan Publishers Ltd.: [Nature Materials] [39], Copyright 2010) **(F)** Schematic representation of nanotube and amyloid fiber formed by self-assembly of PDH. AFM image: a portion of the AFM image showing suprastructural undulations indicative of rolled-tape nanotube precursors (scale bar: 100 nm). TEM image: high-resolution image of a single nanotube (scale bar: 10 nm). A^d= dendron-substituted alanine. (Reproduced with permission from Ref. [40], Copyright Wiley-VCH Verlag GmbH & Co. KGaA) **(G)** Chemical structures of macrocyclic peptides (Segments: α helix, green; β sheet, blue; linker, black) and the partially stabilized helical structure, which is further stabilized and multivalently presented on the surface of a nanostructure upon self-assembly of the β-sheet segment (Segments: α helix, green; β sheet, blue; linker, gray). (Reproduced with permission from Ref. [41], Copyright Wiley-VCH Verlag GmbH & Co. KGaA)

4 Other peptides contribute to controllable self-assembly

Pagel et al. reported a designer coiled coil malleable peptide VW19 that underwent three secondary structures-random coil, α-helix or β-sheet formation facilitated by changing pH or peptide concentrations [38]. The peptide contained a positively charged domain at the b, e, and f positions of the primary heptad repeat structure (Fig. 4(A)). Unfolding of the α-helix was trigged by protonation of the domain. The alternating hydrophobic and hydrophilic residues helped to form a β-sheet ribbon.

Unlike Pagel's designer coiled coil, peptides with different intermoleclular forces have been designed and used in making heat responsive hydrogels [39]. These coiled coil peptides adopted AAA or QQQ at positions b, c, and f to connect neighboring α-helix pairs via either hydrophobic interaction or hydrogen bonds. The results showed the hydrogen bonded hydrogel network melt on heating, whereas the hydrophobic ones got thicker when warmed. A hybrid peptide AAQ did not form any network (Fig. 4(B)–(E)).

In another work, a peptide, Ac-AAA^{d}AKAAAAKAA-A^{d}AYA-NH_2, was designed for controllable interconversion of nanostructures [40]. At neutral pH, the β-sheets formed by the peptide were prevented from packing with each other by electrostatic repulsion. Instead of forming fibrillar nanostructures, β-sheets might roll over to sequester a hydrophobic dendron (d) branch in the interior and form nanotubes. A change in screening or pH was adopted to modulate charge repulsion, thus controlling the interconversion of the nanostructures (Fig. 4(F)).

The α-helix is an essential secondary structure in bioactive proteins. One of the main challenges in biotechnology involves the issue of stabilizing α-helix forming peptide segments. Lim et al. conjugated one α-helix forming segment and one β-sheet forming segment within a cyclic structure to stabilize an α-helical peptide [41]. They hypothesized that the cyclic structure would decrease conformational entropy of the unfolded state [42], while the formation of a β-ribbon would further constrain the conformation (Fig. 4(G)). The CD spectrum indicated the coexistence of α-helical and β-sheet conformations. The α-helical structure of the Rev peptide, which correlated with specific binding to RRE RNA [43], was maintained by this approach.

5 Outlook

The examples illustrated herein show that peptide-based self-assemblies are powerful and can serve as versatile building blocks for constructing functional biomaterials on different length scales. The well ordered structures, which are formed by peptide building blocks, incorporate biological recognition epitopes or functional groups and can have a promising future in 3D cell culture, tissue engineering, controlled cell differentiation, as antimicrobial agents, and in drug/protein delivery applications. The designed synthetic self-assembling peptides allow unprecedented control over composition, structure, and aggregation of the peptide materials. These discoveries provide new opportunities for studying complex biological phenomena, such as protein folding and crystal structure of membrane proteins. In the near future, functional peptide materials and controllable self-assemblies are likely to become the main trends, accelerating the progress in the biomaterials science and nanobiotechnology fields.

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