Review

Short self-assembling peptides as building blocks for modern nanodevices

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Short, self-assembling peptides form a variety of stable nanostructures used for the rational design of functional devices. Peptides serve as organic templates for conjugating biorecognition elements, and assembling ordered nanoparticle arrays and hybrid supramolecular structures. We are witnessing the emergence of a new phase of bionanotechnology, particularly towards electronic, photonic and plasmonic applications. Recent advances include self-assembly of photoluminescent semiconducting nanowires and peptide-conjugated systems for sensing, catalysis and energy storage. Concurrently, methods and tools have been developed to control and manipulate the self-assembled nanostructures. Furthermore, there is growing knowledge on nanostructure properties such as piezoelectricity, dipolar electric field and stability. This review focuses on the emerging role of short, linear self-assembling peptides as simple and versatile building blocks for nanodevices.

Self-assembling biomolecules in nanotechnology

Development of tools and techniques with high precision and resolution for imaging, production, characterization and manipulation of materials has ushered in the modern era of nanotechnology. 'Top-down' approaches that are limited by properties of the bulk starting material are being replaced by 'bottom-up' nanofabrication [1,2]. However, fabrication of nanostructures still requires tedious manipulation and implementation procedures that can be time-consuming and limited to small-scale production [3–6]. Self-organization provides molecular nanotechnology with a powerful alternative to both top-down miniaturization and bottom-up nanofabrication methods. It is directed at self-fabrication by controlled assembly of ordered, integrated and connected operational systems by hierarchical growth, as seen in the integrated biological processes of living systems [3]. Functional devices such as sensors, optical and electronic devices that involve controlled energy, light or charge transfer, form the core of molecular and supramolecular technologies [3].

The use of self-assembling biomolecules to create nanoscale-ordered templates and components for functional devices is an emerging area of bionanotechnology [2]. Although biomolecules such as DNA have good recognition capabilities, mechanical rigidity and amenability to high-precision processing, they are unstable under specific chemical conditions required for certain industrial procedures such as metallization [4]. Peptides are particularly attractive as molecular building blocks because their structural folding and stability have already been studied in detail [7–9]. Self-assembling peptides have unique assembly characteristics that can be readily tuned by changing the amino acid sequence and conjugating chemical groups [2,10]. Their assembly mechanisms are governed by noncovalent intermolecular interactions such as electrostatic, hydrophobic, van der Waals, hydrogen bonds and aromatic π-stacking [7]. Self-assembling peptides can adopt diverse 3D architectures such as vesicles, micelles, monolayers, bilayers, fibers, tubes, ribbons and tapes [11]. Furthermore, short peptides can be easily produced by standard chemical synthesis, avoiding the overall complexities of synthesizing large proteins [12]. They also provide necessary control over self-assembly based on physicochemical parameters such as pH, ionic strength, solvent, light and temperature [7,11]. Their biocompatibility makes them ideal candidates for stabilizing labile components such as enzymes used in biosensors and bionanodevices. In this review, we focus on the recent advances in using short self-assembling linear peptides as building blocks for modern nanodevices.

Self-assembling peptides derived from natural systems

Diphenylalanine (FF) – the shortest self-assembling peptide

An extensively studied short, self-assembling peptide is the diphenylalanine (FF), a fragment of the Alzheimer's β-amyloid protein. This dipeptide can self-assemble into highly ordered nanotubes/microtubes [13–15] (Figure 1a,b), microcrystals [16], vertically aligned nanowires [17] and nanoforests [18]. Diphenylalanine nanotubes are of particular interest because metals can be deposited within and outside the hollow cores of the nanotube to form electromagnetic coaxial nanowires [19].

A breakthrough study has used stiff, hollow FF nanotubes in solution as templates for casting metal nanowires [14]. This has paved the way for extensive research on the use of FF self-assemblies for nanotechnological applications. For example, FF nanotubes have been deposited on the surface of screen-printed graphite and gold electrodes to improve their sensitivity for biosensing [20,21]. Very recently, an FF nanoforest-based biosensor has been

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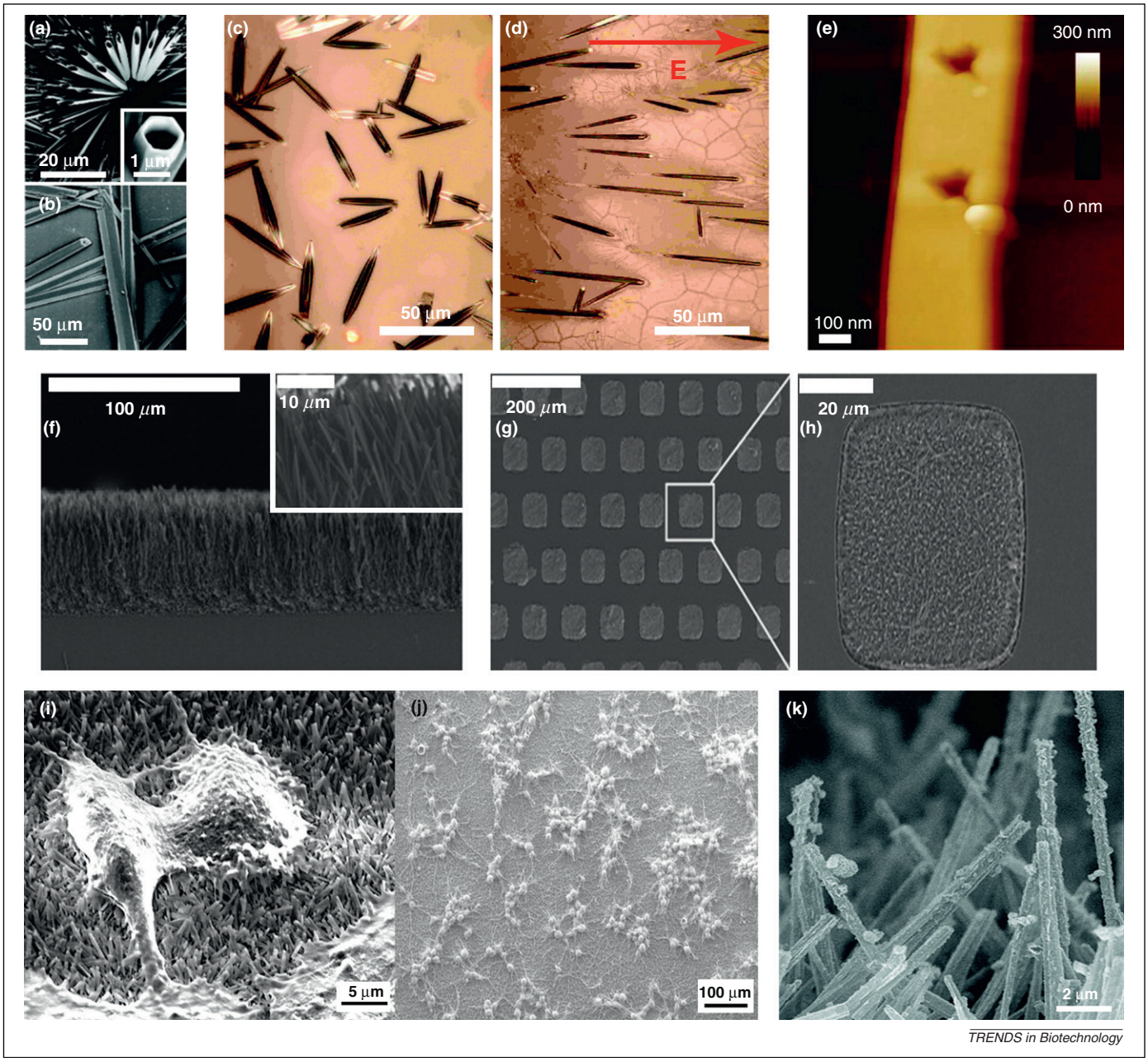

Figure 1. Supramolecular structures formed by the self-assembly of FF and their applications in functional nanodevices. **(a, b)** Field Emission Scanning Electron Microscopy (FESEM) images of hexagonal nanotubes and microtubes formed by FF. Optical microscope images of the hexagonal tubular structures formed without **(c)** and aligned with **(d)** the horizontal external electric field. **(e)** Nanothermal Atomic Force Microscopy (AFM) imprinting of FF nanotubes **(f)**. Side view of vertically aligned peptide nanotubes formed by vapor deposition. **(g, h)** Scanning Electron Microscopy (SEM) images of a silicon substrate patterned with arrays of FF peptide nanotubes fabricated by physical vapor deposition. Low vacuum SEM image of **(i)** Hela and **(j)** PC12 cells grown onto a peptide-nanowire-modified gold surface after 36 h of culturing to form a combined sensing/culture platform. **(k)** SEM image showing the morphology of hybrid FF/cobalt oxide nanowires used for energy storage. Adapted with permission from [15,25,30,31], Copyright American Chemical Society and [29], Copyright Elsevier.

developed and found to have 17-fold higher sensitivity than the uncoated screen-printed control electrodes. Furthermore, the electrode modified with FF nanoforests exhibits greater sensitivity to electrodes modified with carbon nanotubes (CNTs) or combined coating of CNTs and peptide nanostructures [22]. The improvement in sensitivity is attributed to a remarkable increase in functional surface area of the electrode. Horizontal alignment of modified and nonmodified FF nanotubes is achieved using strong magnetic fields [12,23]. In addition, FF nanotubes are patterned using inkjet technology [24], machined by thermomechanical lithography via atomic force microscopy (Figure 1e) [25], manipulated and immobilized using dielectrophoresis [26], as well as arranged on surfaces with controllable wettability by low-energy electron irradiation [27]. The FF nanotubes are even used as an etching mask material in a process named reactive-ion etching for the fabrication of silicon nanowires that can be used in different applications [28]. This new method using the peptide nanotubes significantly reduces the fabrication time, cost and the use of aggressive chemicals reagents.

A scale-up strategy for the production of large, self-assembled arrays of FF nanotubes has been explored by vapor deposition methods [13]. The length and density of

the nanotubes can be fine-tuned by controlling the supply of monomers from the gas phase. The potential applications of these arrays in developing ultracapacitors for energy storage, highly hydrophobic self-cleaning surfaces, and microfluidic chips have also been illustrated (Figure 1f–h) [13,29]. Very recently, an array of FF nanofibers grown on a gold microelectrode formed the basis of a combined cell culture and biosensing platform [30]. To improve the low conductivity of the peptide nanostructures, the nanowires were modified with a conductive polymer that enabled detection of dopamine at physiological concentrations. The same type of structure was used for growth of two different cell lines, PC12 and HeLa cells (Figure 1i,j) [30].

Vertically aligned FF/cobalt oxide composite nanowires are synthesized via high temperature peptide self-assembly by treating amorphous FF film with aniline vapor (Figure 1k) [31]. The feasibility of these hybrid FF nanowires as energy storage material has been demonstrated by using them as negative electrodes for Li-ion batteries and examining their charge/discharge behavior. Such hybrid nanowires containing metal oxides can also be used in gas sensing and catalysis [31]. The same group used $FePO_4$ mineralized peptide nanofibers of Fmoc–FF to make suitable cathode materials for rechargeable Li-ion batteries [32]. FF nanowires with high stability are self-assembled in the reaction zone of a microfluidic system and hybridized to Pd nanoparticles for facilitating heterogeneous catalytic reactions [33]. Significantly higher product yields are obtained for Suzuki coupling and microchemical reactions carried out in microfluidic reactors with built-in peptide/Pd nanowires; compared to plain reactors without nanowires [33].

Evidence of a dipolar electric field and existence of opposite charges on the two ends of FF nanotubes have been discovered (Figure 1c,d) [15]. Moreover, self-assembled FF nanotubes are found to demonstrate strong and robust shear piezoelectric activity [34]. The shear deformations observed are significantly greater than for collagen fibrils and comparable to standard piezoelectric crystals such as $LiNbO_3$. Thus, bio-organic peptide nanotubes are promising candidates for 'green' nanopiezoelectrics that could be the building blocks for future biosensors compatible with human tissues [34].

Very recently, vapor-phase self-assembly of linear FF peptides has been used to synthesize semiconducting, blue-luminescent and single-crystalline cyclo-FF nanowires [35]. Photoluminescent peptide nanotubes have also been made by hybridization to lanthanide complexes and used for the detection of a neurotoxic organophosphate, paraoxon [36]. Exposure to paraoxon inhibits cascaded energy transfer via photosensitizers from the FF nanotubes to the lanthanide ions, resulting in photoluminescence quenching. Besides FF nanotubes, self-assembled F-moc–FF hydrogel has also been shown as a versatile platform for enzyme-based optical biosensors [37]. Physical entrapment of functional enzyme bioreceptors (glucose oxidase) and fluorescent reporters (quantum dots) within the gel matrix has been achieved by simply mixing an aqueous solution containing quantum dots and enzymes with the peptide monomer. The resultant photoluminescent hydrogel has been used for detection of analytes such as glucose and toxic phenolic compounds. The advantages of such a platform include efficient diffusion of target analytes through the hydrogel matrix, high encapsulation efficiency, and most importantly, simple fabrication via self-assembly that provides more options for mass production [37].

Possible mechanisms for FF self-assembly have been proposed and molecular dynamics simulations as well as crystallographic work have been conducted to understand the structure and process towards self-organization [38–40]. However, the understanding is far from complete and more work needs to be done to determine the exact mechanism by which the aromatic residues interact and organize during self-assembly. In fact, new studies have emerged that shed fresh light on FF nanostructures. For instance, the significant mechanical, thermal and chemical stability reported on FF nanotubes and nanowires increases their potential for use in functional nanodevices [19,41,42]. However, in the case of the peptide nanotubes, the characterization was done on dried samples after solvent evaporation. Very recent experiments have raised fresh questions as to the stability of FF nanotubes in solution [43]. These experiments have demonstrated that when FF nanotubes are dried, they subsequently dissolve in many common solvents such as water and phosphate-buffered saline. This could be a limitation for their use in biosensor applications involving submersion of the nanotubes in a solvent, such as a biological field effect transistor [43]. More interestingly, the FF nanotubes grown under saturated water vapor or by diluting stock solution of the peptide with water, and nanowires grown in the presence of aniline vapor, show different stabilities in liquids. Although the nanotubes dissolve very rapidly in liquids [43], the nanowires are more stable [42]. A difference in stability is also noticed when these two nanostructures are tested in the ion-reaction etching chamber. Although the nanowires are rapidly destroyed, the nanotubes are able to withstand this process for a longer period of time [28]. By contrast, another group has reported that the nanowires are more resistant to thermal, chemical and proteolytic attacks compared to the nanotubes [42]. Although methods have been proposed to overcome the existing problems, such studies illustrate the need for detailed investigation and characterization under different conditions to define the limits and clarify the challenges to be resolved before using a self-assembled biological nanomaterial in different applications.

Self-assembling peptide from the fiber protein of adenovirus

A pioneering study has used a genetically modified variant of the self-assembling N-terminal and middle region (NM) of yeast prion protein Sup35p to form amyloid fiber templates for metal nanowires [4]. Replacing a lysine residue in the NM region with cysteine allows colloidal gold particles to be covalently linked to the peptide. In addition, selective metal deposition produces wires roughly 100 nm in diameter that demonstrate the conductive properties of a solid metal wire, such as low resistance and ohmic behavior [4].

Similarly, a self-assembling octapeptide, NSGAITIG, found in the fiber protein of adenovirus, has been exploited to fabricate conductive nanowires [44]. Cysteine residues

introduced at the position of N and S yield three modified peptides with metal binding affinity, namely, CSGAITIG, NCGAITIG and CNGAITIG. Modified peptides with amidated C termini also form fibrils, and effectively bind gold, silver and platinum nanoparticles. In addition, the serine residues enhance metal-binding capability of these peptides through hydroxyl group (electron donor) interactions with metal ions [44].

To facilitate controlled positioning and integration of these modified peptides with nano-assemblies and microsystems, precise 3D patterning of amyloid fibrils from a CNGAITIG peptide has been carried out [45]. This technique utilizes femtosecond laser technology, thiol chemistry and biotin-avidin conjugation on a polymer matrix. Peptide fibrils assemble into micron-sized bridges on a functionalized 3D polymer matrix. Thus, it can be envisioned that peptides functionalized with metal/semiconductor-binding sequences will enable the direct self-assembly of nanoscale electronic circuits [45].

Recently, the same modified linear octapeptides have been used as biorecognition elements for electrochemical detection of copper ions in solution [46]. The self-assembled nanofibers were immobilized on gold electrodes due to the strong interaction between the cysteine groups present on the nanofiber structure and the gold microelectrode. The developed biosensor exhibited good stability and the possibility of reuse after applying an electrochemical regeneration of the sensor to a copper-free state. Moreover, the system has multiplexing potential because the amino acid sequence can be modified to detect other metals by complexation between metal and amino acid [46]. However, for multiplexing, it is necessary to examine interference of other metal ions and how it affects performance. Moreover, the amino acid modification should be done so as not to affect the self-assembling capacity of the peptide [46].

Modified peptide from yeast protein for an enzyme biosensor

The self-assembling, ionic-complementary peptide EAK16-II (AEAEAKAKAEAEAKAK) was discovered during a study of the yeast protein, Zuotin [47]. This peptide is used for surface modification of both hydrophilic (mica) as well as hydrophobic surfaces (highly oriented pyrolytic graphite; HOPG) [48]. The density of coated nanofibers on both surfaces is controlled by adjusting peptide concentration and contact time of the peptide solution with the surface. Besides improving the water wettability of hydrophobic surfaces such as graphite, the peptide has outwardly oriented charged residues (K and E) that could be exploited for binding or immobilization of enzymes, analytes and biomolecules [48]. This attribute has been exploited using EFK16-II (FEFEFKFKFEFEFKFK), a modification of EAK16-II [49,50]. The EFK16-II nanofiber-modified HOPG electrode has been used to detect glucose through covalent immobilization of glucose oxidase by succinimide activation (Figure 2). Succinimide activation 1 h before enzyme addition results in crosslinking of the peptides, reducing the amount of enzyme immobilized on the surface [49]. This problem has been overcome by an improved methodology involving simultaneous addition of 1-ethyl-3(3-dimethylaminopropyl) carbodiimide (EDC), sulfo-N-hydroxysuccinimide (sulfo-NHS) and enzyme. The peptide-modified biosensor is also thought to provide a more biocompatible environment for the enzyme, thus imparting good stability. However, it should be noted that the peptide-modified electrode shows significant attenuation of cathodic and anodic currents relative to the unmodified electrode when used at higher scan rates of 100 mV/s [49]. Thus, conductivity of the peptide interface needs to be improved before higher scan rates can be used.

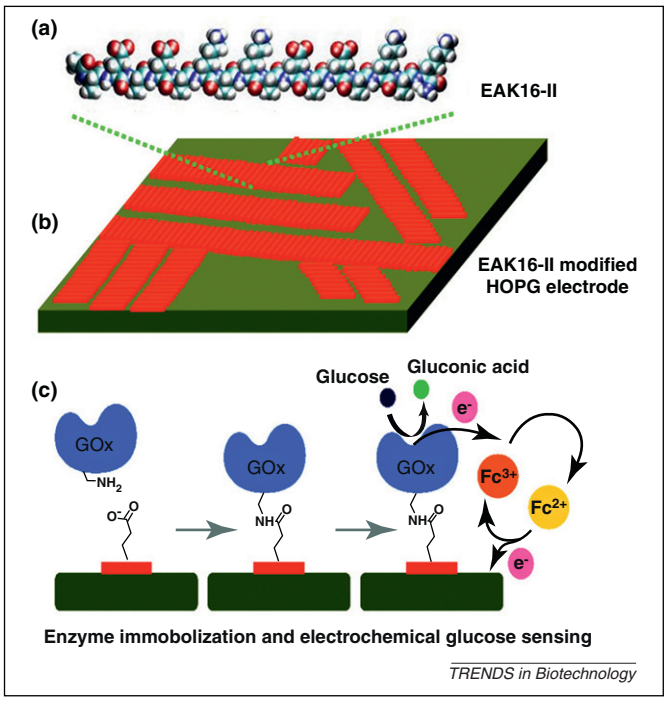

Figure 2. Schematic depiction of an enzyme-based biosensor for glucose detection; constructed by using modified form of ionic-complementary peptide EAK16-II from yeast protein. **(a)** Schematic representation of EAK16-II from yeast protein, Zuotin. **(b)** Diagram of the peptide-modified highly ordered pyrolytic graphite electrode. **(c)** Illustration of glucose detection by enzyme glucose oxidase conjugated to the peptide-modified electrode. Adapted with permission from [49], Copyright American Chemical Society.

Chemically modified peptides and peptide conjugates

A peptide nanotube based biosensor has been developed for label-free detection of viruses, multiple pathogens and lead ions [51–53]. Self-assembled peptide nanotubes are made using the monomer bis(Na-amidoglycylglycine)-1,7-heptane dicarboxylate. These have been used as templates for immobilizing antibodies for pathogen detection and physisorption of Pb-specific peptide for lead detection [51]. Excellent sensitivity has been reported for lead ion detection (as low as 0.01 nM Pb), which is 10 000 times lower than that reported by earlier peptide- or DNA-based sensors using optical probes, and specificity is comparable to that of enzyme biosensors. The main advantages of such a sensing platform include compact design, inexpensive fabrication and electrochemical transduction for simplified circuit integration [51]. By avoiding pre-immobilization of the nanotube on the electrode, a reusable system has been fabricated for pathogen detection, where bacteria nanotube complexes can be washed out easily by gentle rinsing with water [52]. This modified biochip design is based on an AC field impedimetric transduction mechanism and circulating nonconductive

peptide nanotubes for detecting pathogens. Antibody-conjugated peptide nanotubes in solution agglutinate cells via specific biorecognition and the bacteria–nanotube complexes sediment quickly onto the surface of the transducer. The presence of insulating cells increases impedance at high frequency compared to those without agglutinated pathogens [52].

An elegant, one-pot approach simultaneously combines peptide self-assembly and peptide-based nucleation of discrete metal nanoparticles to provide a platform for design and large-scale production of a range of relatively complex nanoparticle superstructures [54]. The water-soluble AG3 peptide, that is, PEP_{Au} or AYSSGAPPMPPF, isolated through phage-display methods [55] has been used. This peptide was chosen for recognition and binding to specific inorganic compounds due to its high affinity for gold and silver surfaces. In this approach, the peptide is modified by addition of an organic part to facilitate the self-assembly process. Succinimide-activated dodecanoic acid is conjugated to the N terminus of PEP_{Au} to make a self-assembling peptide amphiphile. In the presence of chloroauric acid and HEPES buffer (reducing agent), highly ordered left-handed gold nanoparticle double helices are synthesized (Figure 3) [54]. A variety of other nanoparticle superstructures is produced by changing the organic moiety and its length, as well as modification of the peptide by addition of amino acids [56,57]. Furthermore, surface chemistry of the nanoparticles is tuned by adding citrate and ATP to the one-pot synthesis solution, thereby allowing tailoring of particle size and interhelical distance [58].

The AG4 self-assembling peptide (NPSSLFRYLPSD) (discovered through phage display methods [55]) has been used in the synthesis of multifunctional organic–inorganic hybrid superstructures for electronic applications [59]. Hybrid spheres containing peptides and gold nanoparticles are simultaneously synthesized in water. The peptides act as reducing agents and sphere size is precisely controlled by changing the operating temperature [59]. The role of peptides as directing agents for the synthesis, growth, and assembly of nanostructured inorganic materials has been comprehensively reviewed [2].

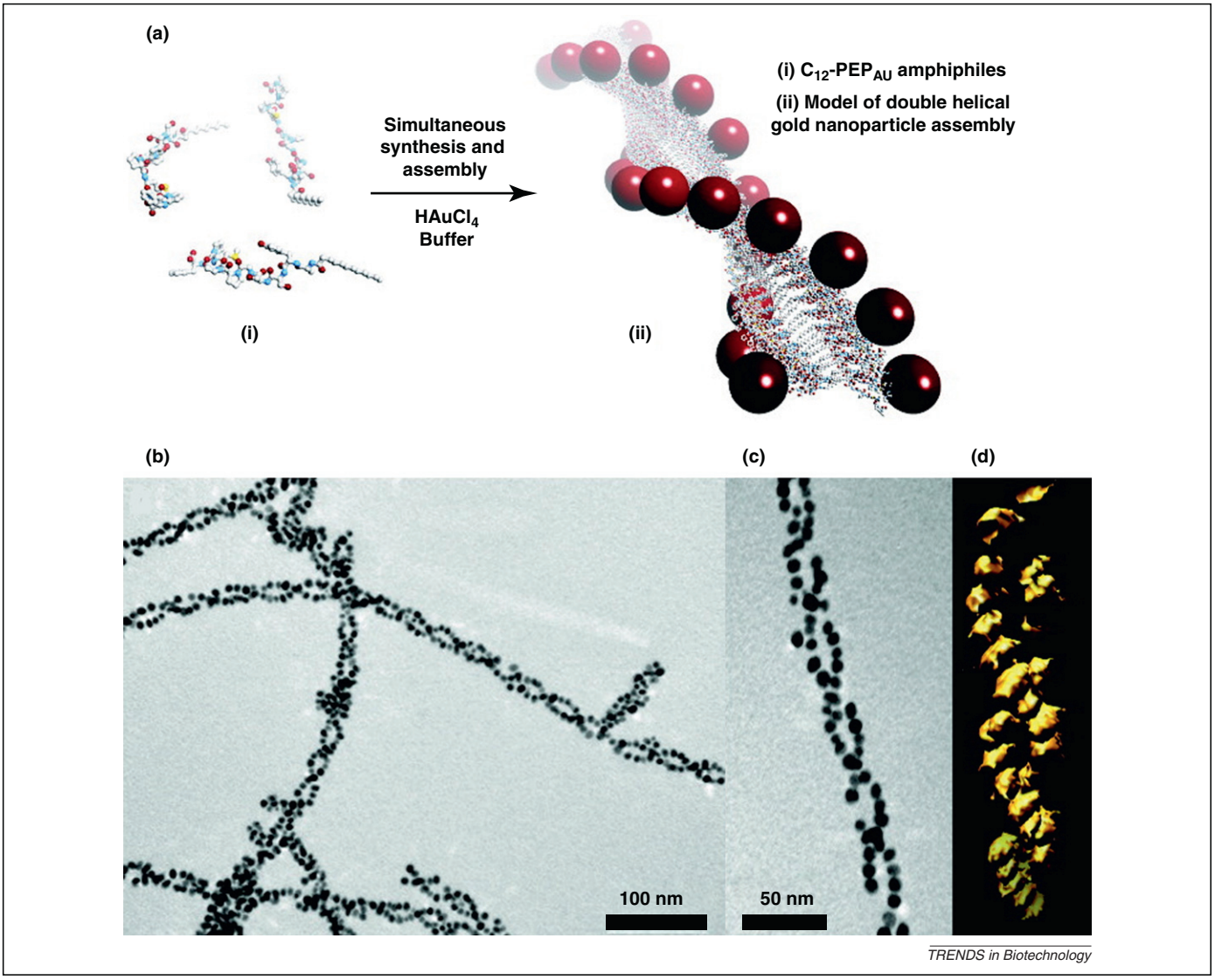

Figure 3. Simultaneous peptide self-assembly and peptide-based nucleation of discrete gold nanoparticles to form highly ordered double helices **(a)**. Schematic depiction of the formation of gold nanoparticle double helices facilitated by the self-assembling peptide **(b, c)**. Transmission electron microscopy images of left-handed gold nanoparticle double helices **(d)**. Tomographic 3D reconstruction image of the double helices. Adapted with permission from [54], Copyright American Chemical Society.

Hybrid systems through conjugation of fluorophores

Amyloid fibrils formed from self-assembling peptides have been used as templates for the development of light-harvesting nanomaterials [60–63]. A major challenge in the design and fabrication of artificial light-harvesting systems is control of relative distance, orientation and interfacial area between the electron donor and acceptor species. Improved charge transport has been reported by incorporation of amyloid fibrils in the active layer of organic solar cells [60]. The fibrils serve as a template to orient and enhance the interfacial area between donor and acceptor polymers. Novel hybrid systems have been developed by conjugating a self-assembling fragment of transthyretin (i.e. $TTR_{105–115}$ with the sequence YTIAALLSPYS) to a cargo species such as a fluorophore [61]. Peptide self-assembly is used to drive nanoscale organization of the cargo species to elicit interesting optical effects that can be exploited in advanced optoelectronic devices. For instance, a binary system is created by conjugating donor and acceptor fluorophores to the $TTR_{105–115}$ peptide fragment [62]. Co-assembly of two independent luminescent moieties in the same peptide scaffold enables formation of nanoscale linear arrays of donor and acceptor groups. More importantly, the fluorophores do not adversely affect self-assembly of the peptide. Upon illumination, excitation of the donor by an incident photon is followed by resonance energy transfer to acceptor sites where the energy is reconverted to light in the form of an emitted photon. By tuning the molar ratio of the precursors, the average distance between donor and acceptor species can be controlled. Furthermore, by using a higher molar ratio of donor-conjugated peptides and a donor species with increased lifetime compared to the acceptor, light energy can be captured over a larger surface area and transported to discrete spatial acceptor sites, thus mimicking a natural light-harvesting system [62].

Strong chromophores are precisely ordered along the inner and outer compartment walls of a paracrystalline nanotube formed by the self-assembling amyloid-β 16–22 peptide, that is, Ac-KLVFFAE-NH2 [63]. Light-harvesting ability of this scaffold has been demonstrated by Forster resonance energy transfer from the donor molecule, rhodamine 110, to the acceptor, Alexa 555. The utilization of amyloid self-assembly to form nanoscale-ordered supramolecular arrays with functional pigments is the first step towards a self-assembling scaffold for new bio-inspired nanoscale antennas and photosynthetic devices [63].

***De novo* designed peptides**

Hybrid peptide–amphiphiles (PAs) with hydrophobic alkyl chains

A novel technique named sonication-assisted solution embossing (SASE) achieves simultaneous self-assembly, alignment, and patterning of PA nanofibers over large areas (Figure 4a) [64]. This soft lithographic technique consists of PA self-assembly by solvent evaporation, under the influence of ultrasonic agitation and spatial confinement within the topography of a polydimethylsiloxane (PDMS) stamp. This technique has also been used to guide the nanofibers around sharp corners (45–135°) and is not limited to uniaxial alignment of parallel nanofibers (Figure 4b). The versatility of this method could be employed in aligning other self-assembling supramolecular systems comprising small molecules in solution [64]. The influence of factors such as ultrasonication, channel width, and nanofiber persistence length on the degree of nanofiber alignment has also been evaluated [65]. Histidine-rich PA nanofibers with Fe^{2+} and Fe^{3+} binding sites as templates have recently been used to grow magnetic nanocrystals (Figure 4c,d) [66]. The PA–magnetite assemblies resemble the linear arrangement of magnetite crystals along a filamentous structure found in bacterial magnetosomes [66]. Such arrays of magnetic nanocrystals have potential applications in designing electromagnetic circuits for nanodevices.

Designed peptides with charged residues

Electrostatic interactions between nanoparticles and self-assembling peptide templates with positively charged residues are very effective for precise nanoscale assembly of small negatively charged nanoparticles. For example, sheets of gold nanoparticles have been prepared using a self-assembled template from a *de novo* designed peptide, $(VK)_4$-VPPT-$(KV)_4$ [67]. This peptide assembles into β-sheets with a laminated morphology. Complementary electrostatic interactions between positively charged lysine residues (regularly arranged across the width of the fibril) and negatively charged gold nanoparticles (intercalated within fibril laminates) results in linear nanoparticle arrays [67]. 1D gold nanoparticle arrays with precise axial separation based on electrostatic interactions with positively charged histidine patches are new, promising candidates for constructing nanoscale optoelectronic devices [68].

Amphiphilic peptide with a thyminyl moiety

Recently, a nucleobase pairing strategy was used to achieve an ordered nanopattern arrangement of gold nanoparticles on β-sheet peptide templates (Figure 4e–g) [69]. A β-sheet-forming peptide with the sequence Ac-$(DL)_2$-$[DK(Thy)_x(Ac)_{1-x}]$-$(DL)_5$-PEG_{70} was used to form a self-assembled monolayer template with a linearly striped pattern (Figure 4f). Hydrogen bonding of adenine-bound gold nanoparticles to thymine-containing peptide template resulted in an ordered nanopattern arrangement (Figure 4g). Desired 2D patterns can be achieved by modifying the amino acid and the position of thymine in the peptide [69].

Multidomain self-assembling peptides as coatings

A series of self-assembling multidomain peptides have been designed as coatings for individually suspending and stabilizing single walled carbon nanotubes (SWCNTs) in water, while simultaneously preserving their strong near-IR luminescence [70]. One of the engineered peptides acted as a good surfactant for the nanotubes and enabled SWCNT emission around four times higher than in common biocompatible coating agents such as Pluronic F127, ssDNA and bovine serum albumin (BSA). This study has demonstrated that biocompatible, self-assembling peptides are promising coatings that could enable development of SWCNT-based optical sensing applications in biological environments. Furthermore, peptide coatings could enable chemical linkage of agents designed for specialized sensing or biological targeting [70].

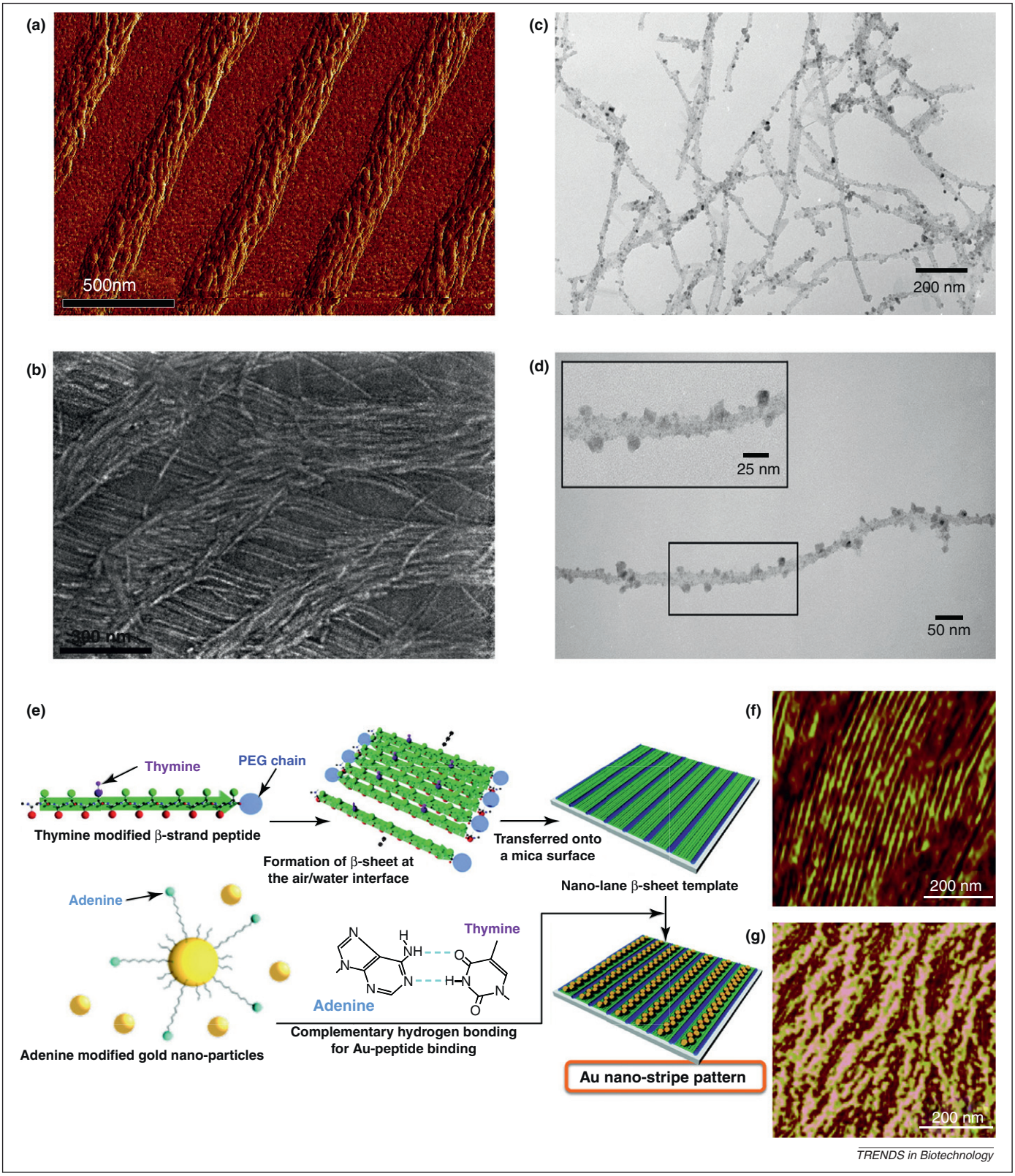

Figure 4. *De novo* designed peptides as organic templates for ordered nanopattern arrangement of nanoparticles/nanocrystals. **(a)** AFM image of supramolecular nanofibers of a peptide amphiphile aligned by soft lithography. **(b)** SEM images of nanofibers of a peptide amphiphile aligned in capillaries defined by electron-beam lithography. **(c, d)** Transmission Electron Microscopy (TEM) micrograph of magnetite nanocrystals on fibers formed from peptide amphiphiles. **(e)** Schematic illustration of nucleobase-pairing strategy for fabricating a unique 2D assembly pattern of gold nanoparticles on a β-sheet monolayer peptide template. **(f)** Template of thyminyl-modified β-sheet peptide. **(g)** Adenine-bound gold nanoparticles assembled on the peptide template through complementary base pairing. Adapted with permission from [64,66,69], Copyright American Chemical Society.

Short self-assembling peptide surfactants

A recently invented class of short, self-assembling peptide surfactants effectively stabilize transmembrane proteins such as glycerol-3-phosphate dehydrogenase [71], the photosystem-I protein complex [72,73], and the G-protein-coupled receptor (GPCR) bovine rhodopsin [74]. Very recently, these peptide surfactants were used to produce milligram quantities of GPCRs from *Escherichia coli* cell-free systems [75]. The GPCRs produced included the human formyl peptide receptor, human trace amine-associated receptor, and two olfactory receptors [75]. Proper protein folding in the presence of the peptide surfactants was confirmed using circular dichroism and one of the olfactory receptors was found to bind its known ligand heptanal. These studies suggest that peptide surfactants may serve as good candidates for the production and

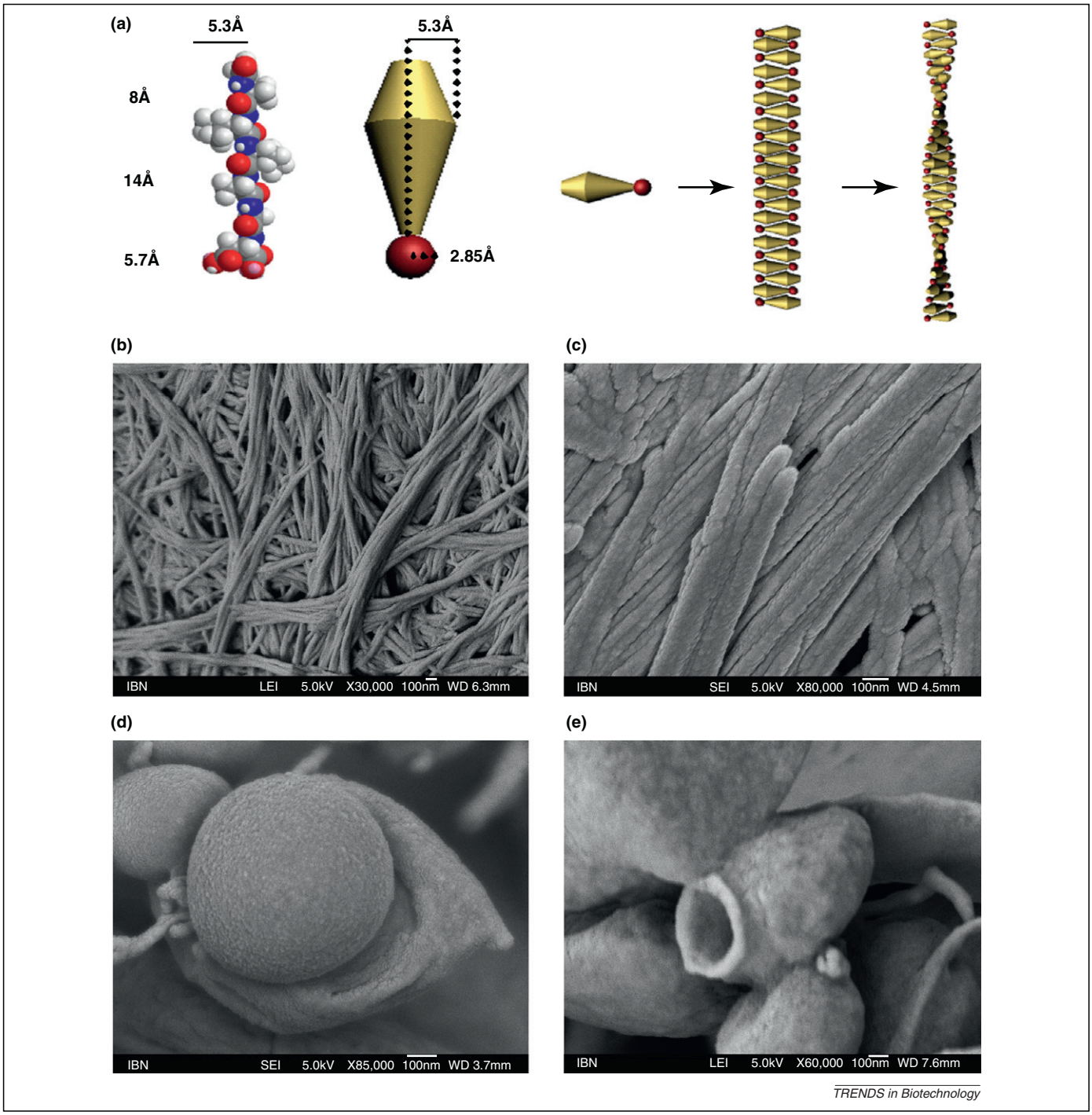

Figure 5. Mechanism of self-assembly and supramolecular structures formed by rationally designed ultrasmall peptides **(a)**. Schematic representation of the formation of single fibers by stacking of peptide monomers using Ac-AIVAGD ($Ac\text{-}AD_6$) as a model system. **(b)** Morphological characterization of the self-assembled peptide nanostructures by FESEM. Condensed helical fiber networks of $Ac\text{-}ID_3$ (L) at a concentration of 15 mg/ml. **(c)** Aligned fibers of $Ac\text{-}ID_3$ (L) at a concentration of 20 mg/ml. **(d)** Spherical structures of $Ac\text{-}AD_6$ (L) at a concentration of 5 mg/ml. **(e)** Visible hollow nanospheres formed by $Ac\text{-}LD_6$ (L) at a concentration of 0.1 mg/ml. Adapted with permission from [77], Copyright Elsevier.

stabilization of membrane proteins not only for structural and functional evaluation, but also for the development of GPCR-based nanodevices [75].

Rationally designed ultrasmall peptides

Recently, a diverse range of nanostructures were formed in aqueous solution via self-assembly of a unique class of trihexapeptides (Figure 5) [76]. Despite their small size, these peptides show a secondary conformational transition from structurally unorganized monomers into metastable α-helical intermediates that terminate in cross-β structures. The peptides have a characteristic sequence motif that consists of an aliphatic amino acid tail of decreasing hydrophobicity capped by a polar head, which makes them amphiphilic (Figure 5a). Molecular recognition, probably via parallel–antiparallel pairing, results in dimers that stack on top of each other to form fibers (Figure 5a) that ultimately condense into hydrogels [76,77]. These hydrogels have high, tunable mechanical stiffness (10^3–10^5 Pa) and are temperature resistant up to 90 °C [77]. The self-assembled nanostructures formed by this peptide class include long helical as well as straight fibers (Figure 5b,c) and hollow nanospheres (Figure 5d,e) [77,78], which could be used as templates to make conductive wires, nanoparticle arrays, hybrid spheres and superstructures for nanodevices. Modifying the peptide by introduction of functional groups could allow binding to specific elements that can be exploited in making biosensors and conductive elements at the nanoscale. The robust peptide hydrogels could serve as an attractive platform for making biosensors by physical entrapment of enzymes and inorganic elements such as quantum dots within the self-assembled matrix. More crucially, ultrasmall amphiphilic peptides could also serve as surfactants for stabilization and production of GPCRs and other enzymes for the construction of biosensors and nanodevices.

Conclusions and outlook

There is a broad range of literature available on the different applications of self-assembling peptides as scaffolds for tissue engineering, nanocarriers for drug delivery, models for studying amyloidosis, and even drugs to cure amyloid-associated disorders [7,79–84]. In this review, we have focused on the emerging role of self-assembling peptides in making organic templates and nanoscale components for the next generation of biosensors, as well as functional electrochemical and optoelectronic devices. The formation of diverse nanostructures by short, linear, self-assembling peptides paves the way for large-scale bionanotechnology based on simple building blocks that have a diverse chemical profile and can be synthesized in large quantities. With the design of platforms such as microfluidic chips for the controlled synthesis of biological self-assembled peptide nanotubes and nanoparticles [85], as well as techniques such as SASE [64], the process of directly integrating self-assembled structures into functional devices is fast becoming a reality. However, it is important to keep in mind that many of the current studies are either proof of concept or small-scale production of such self-assembled devices in the laboratory. Translation to an industrial scale will eventually require cheap and large-scale production of self-assembling peptides that can be met by biotechnological methods such as recombinant production [79].

Conflict of interest

The authors declare no conflict of interest.

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