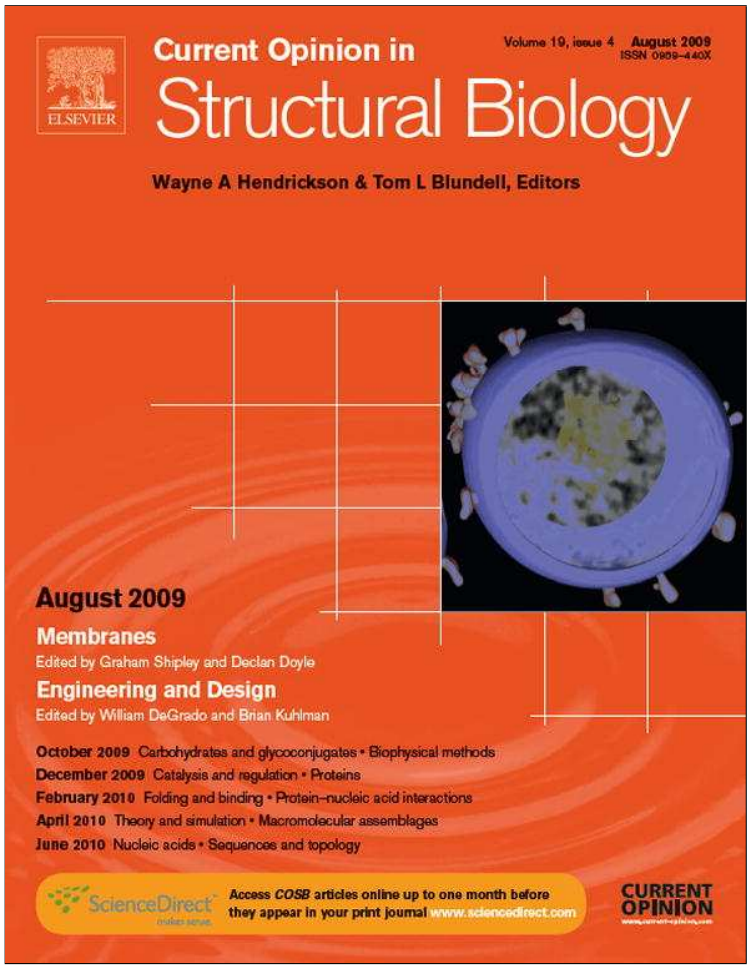

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Peptides as materials

Brian J Pepe-Mooney and Robert Fairman

This review focuses on the creation of electronically active peptide-based biomaterials and how such materials may be deposited onto surfaces to create integrated bionanocircuits. We describe recent efforts to add electronically active groups, such as metal complexes and various porphyrin derivatives, onto peptide-based materials. Having created such materials, the next challenge in creating a nanocircuit is to deposit these materials robustly and precisely onto appropriate surfaces. Methods for the deposition of peptides onto a variety of inorganic and organic surfaces are explored. Advances in patterning at the nanoscale are also described, focusing largely on softer methods appropriate for peptides. There are challenges yet to be overcome in realizing such peptide-based nanocircuits; these are discussed in our concluding remarks.

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Introduction

Peptides and proteins have been studied for their material properties for many decades, focusing first on the macroscopic properties of natural proteins such as collagen and spider silk. More recently, scientists have turned to creating materials with unprecedented properties through design efforts. These efforts take advantage of an increasing knowledge about the sequence/structure relationship in proteins and a deepening understanding of how proteins attain their functional three-dimensional structure. Within the last decade or so, scientists have been successful in creating short protein sequences, or peptides, that can attain macroscopic (and often emergent) properties, sometimes from design, sometimes from serendipity. Regardless, there is now an extensive body of work (including several excellent reviews) describing the use of peptides to create 1D, 2D, and 3D structures for a myriad potential applications. This distinct control over molecular structure has also allowed for the production of what have been called 'smart materials'. This 'smart' behavior derives from the ability of such engineered peptides to be reversible and responsive to their environmental conditions, which is a distinct advantage in the use of peptides as materials. Many of the structural designs, detailed in this review and several others, take advantage of the technique of self-assembly.

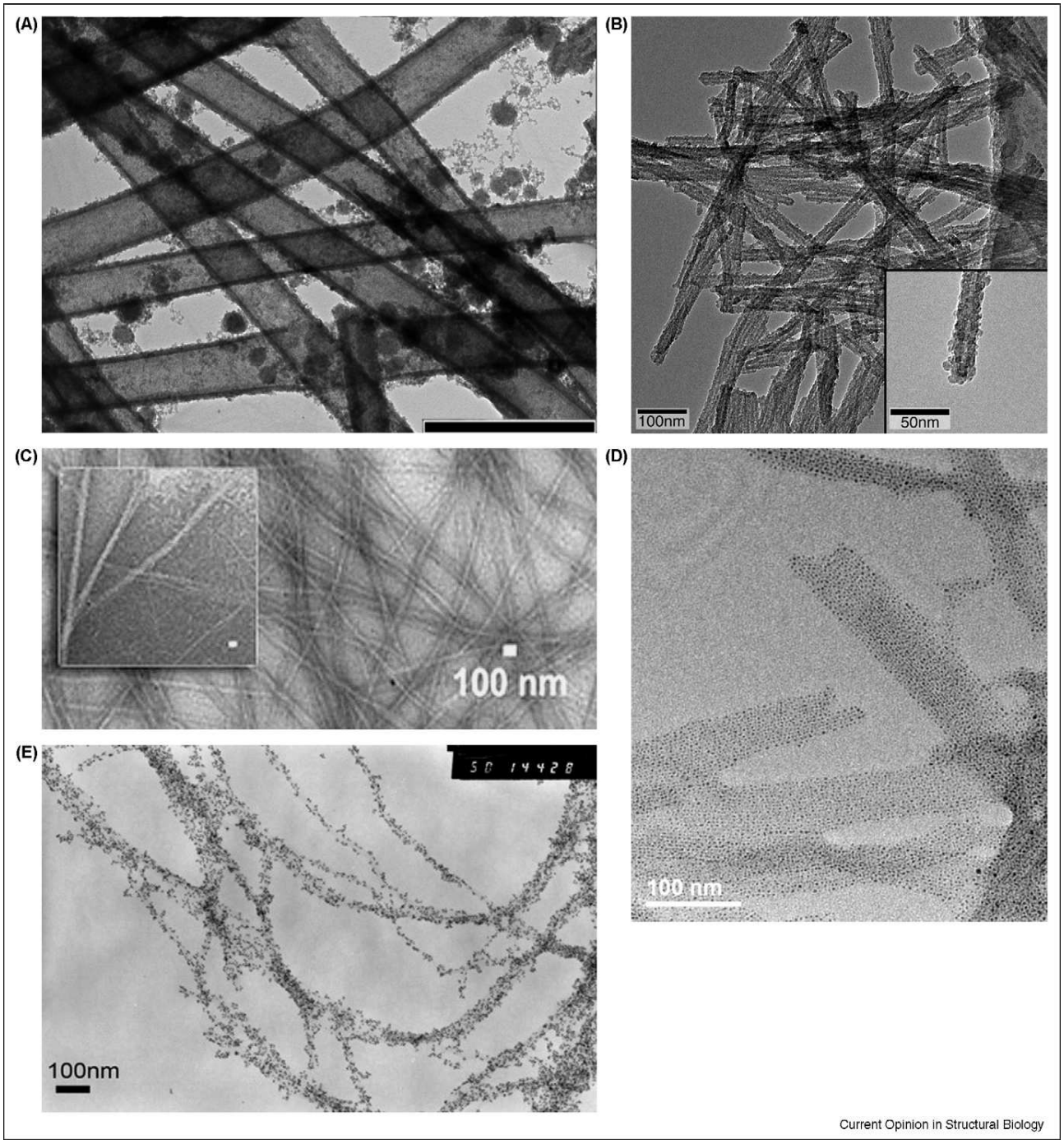
The focus of this review paper addresses the specific provocative question: How can peptides be used to design bionanoelectronic circuits? One quickly appreciates the interdisciplinary nature of the question in formulating a response: progress requires appropriate protein design efforts, the effective acquisition of photoelectronic functionality, preparation of surfaces that can bind peptides while retaining their function, and precise control of orientation and patterning. This outlines the analysis provided below of the state-of-the-art. Some aspects are still in their infancy (precise localized deposition of proteins on surfaces) while others are fairly well developed (creating 1D peptide materials in the form of filaments and fibrils).

Functionalization of supramolecular assemblies

There has been a long-standing interest in understanding the emergent properties of self-assembling peptides at the macroscale (10^{-6}–10^{-3} m). To address this interest, many scientists have taken a bottom-up approach to the design of structural and functional biomaterials, whose properties can be predicted with some fidelity based on atomic-level interactions. We refer to an excellent recent review by Ariga, who offers a thorough summary of the work in the field of material self-assembly [1]. We also refer readers to several other fine recent reviews on protein and peptide design of fibrillar assemblies [2–5].

Rather than offer a comprehensive review of work on peptide-based supramolecular assemblies, we focus specifically on interesting functionalization of assemblies relevant to the theme of creating bionanocircuits. Woolfson, one of the leaders in the field of biomaterials design, and his collaborators, have recently made advances in this area by using α-helical assemblies as templates for silica deposition (Figure 1A) [6]. They show how silica can be deposited on the exterior of a helical peptide assembly and how the removal of the peptidic component leads to forming silica nanotubes. Hartgerink and Yuwono also describe a method utilizing peptides as a scaffold for silica nanotube formation (Figure 1B) [7]. They synthesize hollow silica nanotubes by condensing tetraethoxysilane

Figure 1



Examples from the literature of functionalized peptide-based supramolecular assemblies. **(A)** TEM micrograph of silica nanotubes directed by α-helical peptide fibrils. Scale bar is 1 μm [6]. **(B)** TEM micrograph of silica-coated β-sheet peptide fibrils [7]. **(C)** Unstained TEM micrograph of α-helical fibers decorated with silver(I). Inset: high magnification of the small diameter fibrils (scale bar = 10 nm) [8•]. **(D)** Bright-field TEM micrograph of gold nanoparticles templated by β-sheet fibrils [9•]. **(E)** TEM micrograph of β-sheet peptide fibrils after incubating with a platinum solution [10].

on long β-sheet nanofibers formed by peptide amphiphiles. The ability to create nanoscale silica structures could allow for the creation of insulating materials that may be useful in the design of nanocircuits.

Another common functionalization that may have implications for the design of electronically active biomaterials is the decoration of fibril structures with metals. For example, Conticello and his colleagues describe the coating of peptide-based coiled-coil fibrils with silver ions (Figure 1C) [8•]. They tie the folding of the individual peptides to silver binding. In this way, silver ions are used to help drive self-assembly of these peptide-based fibrils, thus building responsiveness to metal environmental cues. Similar approaches have been taken by other laboratories to decorate β-sheet fibrils with silver, gold, and platinum (Figure 1D,E) [9•,10] There is a surprising paucity of examples of such work in the literature though, probably reflecting the difficulty of creating peptide-based fibrils that are sufficiently robust to such modification. We expect that many more examples will emerge in the next several years.

Design of photoelectronically active peptide materials

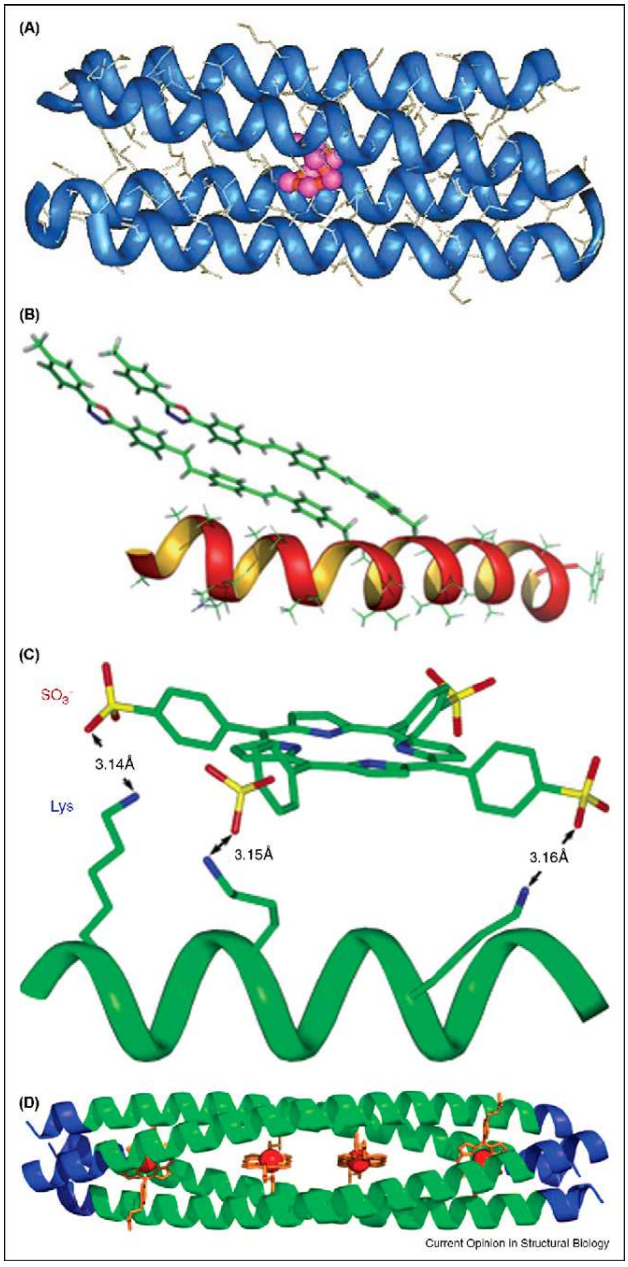
Peptides have long been used as model systems to study intrinsic chain electron transfer properties. The inspiration for these studies comes from a desire to understand the role that proteins play in capturing light energy, as exemplified in the photosystem protein complexes [11]. This early work has provided an important foundation for the more recent efforts in creating photoelectronically active biomaterials either in solution or on surfaces. We refer to an excellent recent review on the methods of electron transfer in peptides and proteins by Giese and Cordes [12]. One area of long-standing interest has been to understand how the intrinsic macrodipole of the α-helix directs potential electron transfer pathways and modulates the electronic activity of a variety of pendant groups. It is also now known that the rates of electron transfer depend critically on dynamics and orientation of the polypeptide chain relative to the applied electric field. Kolandaivel and colleagues have explored this recently on theoretical grounds [13–15]. They have applied density functional theory to explore electron and charge transfer in peptide model systems. Specifically, they detail the two important factors that are understood to contribute to the efficiency of electron transfer in a polypeptide: the hopping matrix element between amino acid subgroups and the site energy (the energy of a charge when it is localized on an amino acid subgroup) [14]. More recently, they have explored the way in which intramolecular charge transfer is dependent on the conformation of that peptide and conclude that electron charge transfer depends on the dihedral angles and conformations of neighboring amino acids [15]. The detailed mechanism of electron transfer for a given system, whether it involves tunneling or inelastic hopping, continues to be controversial and is discussed in the review by Giese and Cordes [12]. Electron transport has been explored experimentally in helical peptide self-assembling monolayers (or SAMs) and some of the work is described in the 'Peptide surface deposition strategies' section.

That peptides themselves (particularly in the helical conformation) have intrinsic electron transfer, or conductive, properties has led to the idea that peptide scaffolds might be used for inserting photoelectronic functions. Three strategies have been tested in the functionalization of peptides with electronically active capabilities: first, coordination of metals and metal complexes as electronically active species; second, covalent modification of sidechains to confer photoelectronic activity; and third, noncovalent association of chromophores with peptide scaffolds. One example of a designed metalloprotein is described in a recent review from Ogawa and his colleagues. They have shown photoinduced electron transfer in a coiled-coil model system in which cysteine residues act to coordinate a copper cluster (Figure 2A) [16•]. They observed novel photophysical behavior of their encapsulated multinuclear copper cluster, thus having obvious implications for the creation of a photoelectronically active biomaterial based on this structural motif. What is especially interesting in this work is that it did not require the chemical addition of complex redox factors to achieve their goals. However, there have been recent studies in the use of sidechain modifications to impart photoelectronic activity that are of great interest, and these are described below.

Kiick and her colleagues covalently modified a peptide using Oxa-PPV (oxadiazole-containing poly(phenylene-vinylene)) molecules to create an electronically active material (Figure 2B) [17••]. Similar to their earlier work with stilbene, they explored the relationship between the distance between the Oxa-PPV molecules and developed energy-minimized models, which give insight into the expected electronic properties of the material. These specific studies illustrate the possibility for a great deal of control over the photoluminescent or conductive properties of the hybrid peptide structure.

Finally, other chromophores have been incorporated noncovalently onto peptide scaffolds to impart photoelectronic behavior. In our own lab, we have developed a method of functionalizing a peptide en route toward creating a photoelectronically conductive biomaterial (Figure 2C) [18•]. We demonstrated the functionalization of a peptide by the noncovalent addition of an anionic porphyrin *meso*-tetrakis(4-sulfonatophenyl) porphine ($TPPS_4$). We took advantage of charged interactions between the sulfonato groups on the $TPPS_4$ with appropriately positioned lysine residues in the peptide to stabilize a helical conformation. As a final example,

Figure 2



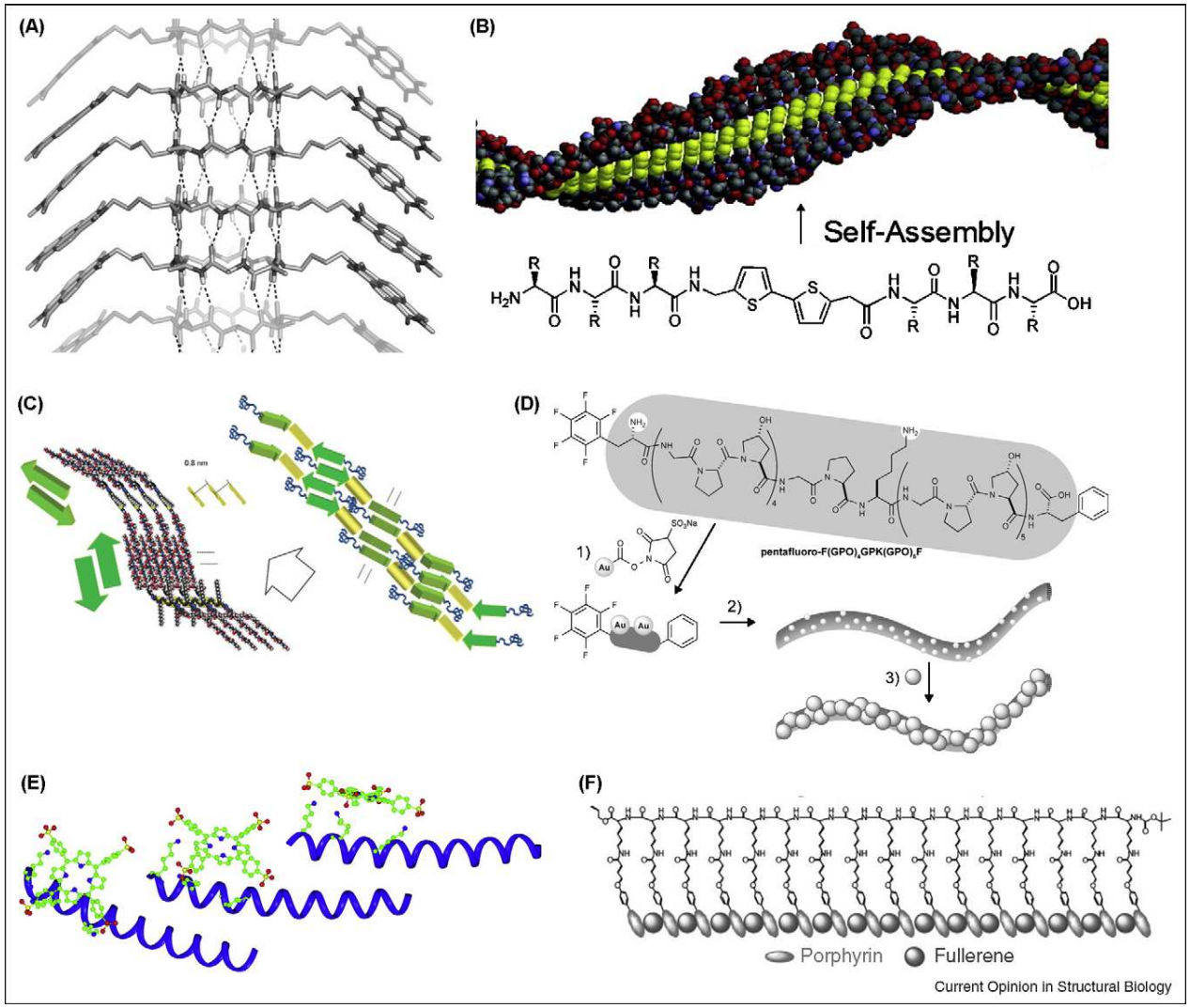
Recent examples of peptide designs that incorporate electronically active materials. **(A)** Computer model of the Cu(I) adduct of a four-helix bundle scaffold. The metalloprotein is shown encapsulating a tetranuclear Cu(I) thiolate cluster [16•]. **(B)** Energy-minimized structure of an α-helical peptide modified with two oxadiazole-containing poly(phenylenevinylene) oligomers [17••]. **(C)** Model of a 1:1 complex of *meso*-tetrakis(4-sulfonatophenyl) porphine/peptide. The porphyrin is shown bound to the α-helical peptide through charged interactions between anionic sulfonate groups and cationic lysine residues [18•]. **(D)** Model of a designed coiled-coil structure with four iron porphyrin cofactors bound [19•].

DeGrado and his colleagues describe the binding of a porphyrin to a peptide through coordination chemistry. They utilize a four-metalloporphyrin array which may be redesigned for the development of electronically conductive nanowires (Figure 2D) [19•]. These strategies offer great promise for the design of biomaterials with useful photoelectronic properties.

We offer a few examples of recent efforts to create peptide-based photoelectronically active biomaterials. Ghadiri and his colleagues described the design and utilization of a unique cyclic D,L-α-peptide containing 1,4,5,8-naphthalenetetracarboylic acid diimide (NDI) side chains, which can form supramolecular assemblies [20•]. They take advantage of hydrogen bond-directed assembly to stack the monomeric subunits into these assemblies to allow potential charge transfer between NDI side groups (Figure 3A). These assemblies were visualized using AFM. They describe the fabrication of nanotubes, which maintain charge-delocalized states. Furthermore, from their observations of the near-infrared absorption bands observed from this material, they conclude that the NDI radical anions are π-stacking and therefore electron transfer is highly probable. Another method used in the development of photoelectronically active biomaterials is described by both Tovar and his colleagues and Bäuerle and his colleagues. They incorporate the π-conjugated system of thiophenes into a polypeptide backbone that assembles to form a β-sheet scaffold. Specifically, Tovar and his colleagues illustrate the successful design and creation of a one-dimensional optoelectronic nanostructure using π-conjugated oligopeptides with bithiophenes (Figure 3B) [21••]. As a result of quenching seen in the emission spectra of the large nanostructure, they suggest that these oligopeptides, which maintain internal π-conjugated segments, have great potential to be used as conductive wires. Bäuerle and his colleagues use an oligothiophene segment that is substituted on either side by amino acid sequences commonly found in β-sheet structures. Using AFM and TEM, they show that these oligothiophene-peptide hybrid constructs form fibrillar structures. Bäuerle and his colleagues also suggest, based on quenching of the emission spectra, that these peptides have conductive properties because of the interactions of the π-systems incorporated into the peptide design (Figure 3C) [22•].

Progress has also been made on studying the electronic behavior of peptide-based biomaterials decorated with various metals. For example, Raines and his colleagues have decorated collagen-like peptide fibers with gold nanoparticles through binding to L-lysine residues within the peptide sequence (Figure 3D) [23•]. They then measured the conductivity of these 'nanoparticle-decorated' fibers at two terminal devices that were patterned after peptide deposition using electron-beam lithography.

Figure 3



Peptide-based supramolecular assemblies with electronically active properties. **(A)** Model of a cyclic D,L-α-peptide in a self-assembled configuration, highlighting the inter-β-sheet-like H-bonding, thus precisely positioning the stacking of aromatic 1,4,5,8-naphthalenetetracarboxylic diimide side chains [20•]. **(B)** Energy-minimized model showing helical twist of β-sheets and π-stacks as line drawings and space-filling models (thiophenes in yellow) [21••]. **(C)** Calculated model of an oligothiophene peptide conjugate as shown in a backbone representation (left) and as cartoon that emphasizes the antiparallel β-sheet conformation (right). The white arrows indicate the direction of fiber growth [22•]. **(D)** Scheme showing self-assembly of collagen-related peptides modified with colloidal gold nanoparticles, followed by self-assembly to collagen-like fibers, and electroless silver plating [23•]. **(E)** Model of coiled-coil assembly interacting with *meso*-tetrakis(4-sulfonatophenyl) porphine. The view emphasizes the overlapping nature of the helices with respect to one another [24•]. **(F)** Supramolecular organization between porphyrins and fullerenes in which the porphyrins are linked to a peptide backbone using an amide linkage [27•].

Our lab has also successfully developed a photoelectronically active fibrillar material [24•] which uses noncovalent association of $TPPS_4$, a sulfonated porphyrin derivative, with a peptide designed to form coiled-coil fibrils [25]. The peptides can associate to form a supramolecular assembly through offset coiled-coil interactions as illustrated in Figure 3E. Collaboration between our group and Åkerfeldt's group has resulted in the design of a similar hybrid biomaterial. We again used porphyrin as a conducting material, but in this case, the porphyrin was covalently attached to a short decapeptide complex. This biomaterial is able to form organized chromophore arrays and aggregates with exciton coupling between the porphyrins [26]. Another study that takes advantage of porphyrins is described by Fukuzumi and his colleagues (Figure 3F) [27•]. They constructed a series of porphyrin–peptide oligomers that are intercalated with fullerenes in the hopes that this would create a photovoltaic

device. They used TEM and SEM to reveal the three-dimensional nature of the biomaterial and carried out extensive characterization of its photoelectronic activity after deposition onto SnO_2 films.

Peptide surface deposition strategies

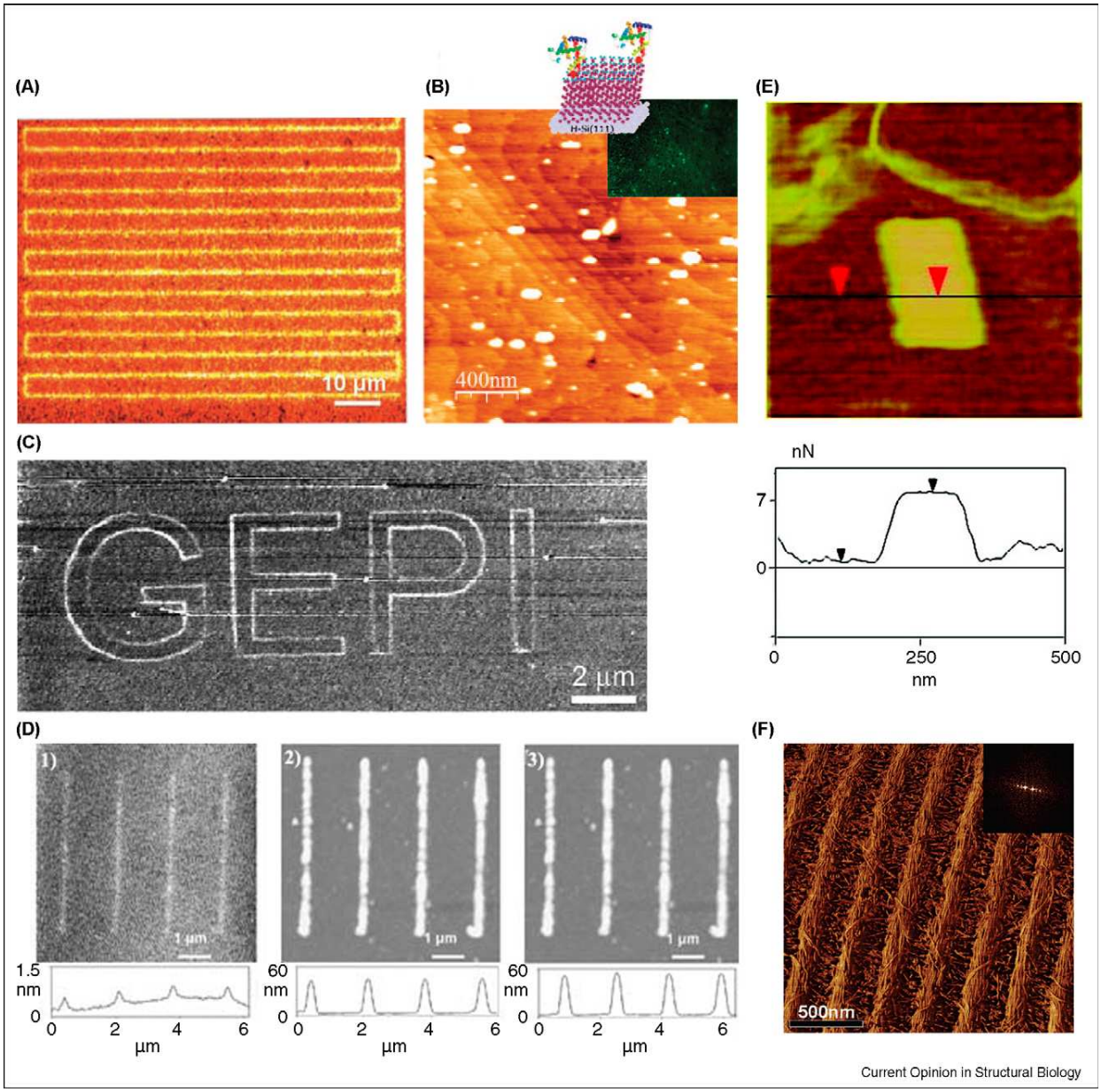
Having shown that photoelectronically active supramolecular assemblies can be designed using peptides as scaffolds, what techniques are available for depositing such assemblies onto surfaces, if our intent is to create bionanocircuits? Peptides have been deposited directly onto surfaces, such as mica and graphite, largely by taking advantage of noncovalent forces. However more robust, covalent (or coordination) methods have also been described. These methods take advantage of modified surfaces, containing metal (such as gold or platinum) or bifunctional molecules that can self-assemble to form monolayers (SAMs). Peptides themselves can form SAMs, and we discuss the study of the electronic properties of such peptide monolayers.

The need for precise positioning and patterning of peptides on a surface has required new approaches to covalent attachment and excellent progress has been made in this arena. Particularly, bioorthogonal reactions for covalent deposition have been reported that allow for deposition under near-native aqueous conditions and interfere minimally with biological function. Maynard and her colleagues have developed several bioorthogonal approaches to deposit proteins. These include the covalent attachment of proteins through oxime linkages created by the reaction of N-termini (modified with α-ketoamides) with surface aminooxy functionalities [28], and covalent attachment of antibodies crosslinked to electrochemically reduced aromatic NO_2 groups using appropriate homobifunctional linkers [29]. Both approaches have been used for patterning precisely defined areas on a surface. Waldmann and his colleagues report the use of a thiol-ene reaction for another type of bioorthogonal surface patterning [30•]. The authors first photochemically attach a biotin derivative to a thiol-functionalized surface (a procedure that has been exploited by others as well [31]). They then show that they can deposit streptavidin to the surface through its interaction with biotin and create precisely deposited assemblies (Figure 4A). Since streptavidin is often used as a fusion partner in genetic engineering, many proteins can be deposited in this fashion using this simple bioorthogonal approach. Other fusion approaches, designed to favor bioorthogonal reactions, have been studied as well. For example, Haruyama and his colleagues have used a His-Tag (which is often used as a fusion partner in recombinant proteins) in a bioorthogonal approach involving electrochemical deposition, resulting in reversible immobilization onto an electroconductive surface [32]. Electrochemical reduction of a His-Tag metal complex resulted in its deposition onto a redox-active platinum interface. Since the redox potential of their surface can be controlled, this deposition can be reversible, thus imparting 'smart' characteristics. They also comment generally on the value of bioorthogonal methods involving the fusion of tags as another valuable method to precisely control molecular orientation in order to optimize the accessibility of the protein functionality. Delle Site and his colleagues have taken an interesting computational approach to predict the energy associated with His-Tag and Phe-Tag adsorption to a Pt(111) surface [33]. Of particular note in their calculations is the differential influence of water adlayers on deposition energy of these two oligopeptides. Finally, a bioorthogonal method has been developed for protein deposition onto silicon surfaces, taking advantage of the strong Si–C bond. Silicon surfaces have been valuable because of their use in electronic detection methodologies so creating chemistries for the formation of SAMs on these surfaces is of great interest. Rogero and her colleagues have deposited alkyl organic monolayers terminated with an aldehyde group, which can then selectively react with peptide amino groups [34•]. The breakthrough that they have achieved is to deposit these aldehyde-modified SAMs without the aldehyde reacting with the silicon surface itself. Using AFM imaging, they show specific protein adsorption onto these organic SAMs in which the protein, TolIII-GFP, was immobilized (Figure 4B). This can easily by adapted for peptide attachment as well.

We also focus on a recent method that allows peptide deposition onto monolayer surfaces to take place under relatively benign conditions. One technique, involving the deposition of peptides as hyperthermal ions onto neutral or reactive substrates (coined soft landing or reactive landing, respectively) has been recently described [35]. Laskin has done pioneering work in this area and most recently reported the reactive landing of a peptide resulting in covalent attachment to a COOH-SAM surface of a peptide containing reactive α-amino or ε-amino groups [36•]. The authors claim that, having established the feasibility of this new technique for depositing proteins both covalently and noncovalently, it will provide a valuable new approach for specific patterning of surfaces because of the ability to precisely control the positioning of the ion beam.

The regularity of peptide deposition on surfaces as SAMs and their detailed structural characteristics play a large role in electron transfer properties of such materials. A few novel approaches to the creation of peptide SAMs are highlighted first, before touching on their electronic properties. Gellman and Abbott explored the ability of helical β-peptides (peptides in which the amino group is bonded to the β carbon) to form well-ordered SAMs on gold surfaces [37]. Using such unnatural peptides for creating SAMs is helpful in preventing proteolytic (and other natural) degradation processes, an important

Figure 4



Images showing successful patterning of peptides and proteins at the nanoscale. **(A)** Scanning confocal fluorescence microscopy images of streptavidin binding to biotin, which has been photochemically patterned onto a thiol-modified surface [30•]. **(B)** AFM image of TolAIII proteins on a 1% aldehyde (1:99 C10CHO:C11) modified surface. A fluorescence image of this sample is shown in the inset [34•]. **(C)** Lateral force microscopy image of DPN-patterned silica-binding peptide on SiO_x [52•]. **(D)** AFM image and height analysis of G4-NH_2 dendrimer lines on Si/SiO_x. The reaction times for tryptophan addition are 0 hour, 4.5 hours, and 8 hours [53••]. **(E)** AFM lateral friction image of an area containing maltose binding protein in the presence of 1 mM maltose [56••]. **(F)** AFM image of aligned supramolecular nanofibers made up of a peptide amphiphile. The fibers were embossed from a 5-wt% solution into lines with widths of about 150 nm, and average heights of the lines of 33.3 nm. Note that the fibrils are largely aligned with the lines [57••].

criterion for creating robust materials. They show that amphiphilic structures are particularly useful for creating well-ordered arrays with many of the same properties as α-peptides. They suggest that β-peptides may provide more exacting geometric control that is needed for precise positioning of redox-active groups. Another interesting study is the work from Wöll and his colleagues [38]. They have been exploring the modification of surfaces to avoid the problem of nonspecific target protein adsorption by creating a proteophobic surface. A general strategy for

binding the peptide to a gold surface is described, taking advantage of a linker created by a copper-catalyzed cycloaddition reaction to incorporate a terminal thiol group (click chemistry). The peptide sequences were designed to be hydrophilic but not charged. Resistivity to protein adsorption was compared to other adlayer materials such as octane thiol.

Several laboratories have probed the conductive properties of peptide SAMS, principally to gain insight into polypeptide chain electron transfer processes, but with clear implications for biomaterials design. Most of the work has focused on using regular arrays of α-helices. Recently, Nichols and his colleagues published a study looking at the pH dependence of the electrochemical properties of a peptide monolayer, in which protonation states of the glutamates in the sequence dictated the conformation of the peptide (helical at low pH and extended at neutral pH) [39•]. This pH triggering ability allowed them to study the effects of kinetics and dynamics on electron transfer upon helix acquisition in the monolayer. Kraatz and his colleagues have studied the influence of the helix macrodipole on the responsiveness of helical SAMs and collagen-based SAMs upon application of a redox potential [40]. They also note the role of H-bonding in enhancing electron transfer [41]. Sidechains also play a role in potentiating electron transfer in helical peptides as SAMs, as shown by Venanzi and his colleagues. They performed cyclic voltammetry and photoelectronic experiments to show that aromatic residues have peculiar effects on electron transfer that are not fully understood [42]. We would be remiss if we did not recognize the important contributions from Kimura and his colleagues in the study of the electron transfer properties of helical SAMs and we cite some of his more recent works [43–45,46•,47]. One set of interesting studies involves understanding the effects of precise geometric deposition on helical peptide SAM electronic activity [44,46•]. Their goal was to test the ability of an α-helical structure to transfer energy from a redox-active ferrocene attached at one end of the peptide down to the underlying gold surface through a sulfur-linkage. They examine both the molecular properties of various peptide SAM derivatives and their associated conductivity. A careful analysis of their various peptide constructs, using FTIR and voltammetry methods, reveals evidence of intermolecular electron transfer as a preferred mode because of geometric and dynamic influences. This study is valuable in that it provides important insights into the relationship between secondary structure, monolayer geometry, and conductivity.

Patterning methods for directing peptide deposition

The development of peptide-based nanocircuits will require the controlled deposition of peptides into well-ordered arrays, thus creating a precisely patterned surface. This is an area that has not been explored deeply and represents an important future challenge for this field. The goal is to position relevant biomaterials in precise locations with exact geometries while avoiding nonspecific binding (by creating high resistivity regions). SAMs have been particularly helpful in creating surfaces with binding sites and high resistivity nonbinding sites, as described earlier. Several methods have been developed for patterning surfaces that are amenable to precise biomaterial deposition. Work in this area has been extensively reviewed. Such methods include dip-pen nanolithography (DPN) involving both direct and indirect deposition (with indirect methods involving deposition of surfaces that can bind protein) [48], nanoshaving and nanografting [49], and nanocontact printing (a stamping method) [50,51].

Scanning probe microscope (and atomic force microscope) techniques have been developed as important tools in nanolithography and are particularly well suited for soft nanolithographic methods (those involving primarily biomaterials). DPN, one type well adapted to soft materials, involves inking an AFM tip with the material of interest and then depositing it on a surface either in scanning mode or in tapping mode (the advantages of tapping mode for soft materials are discussed below). This has proven to be well suited for precise deposition of peptides and has been successful in cases where spatial confinement strategies may fail (see below). The degree of precision that is possible with DPN techniques is beautifully illustrated by work from Ginger and his colleagues, in which they inked both gold and silica surfaces with peptides designed to bind with high selectivity to these types of inorganic surfaces (Figure 4C) [52•]. They show that these peptides, when derivatized with biotin, can be exploited for binding fluorescently labeled streptavidin, thus acting as biomolecular anchors. A second paper, by Zhang and his colleagues, addresses the problem of precise deposition of preformed supramolecular assemblies by describing a method in which the assemblies instead can be grown on a surface [53••]. They first ink a Si/SO_x surface using a tip coated with amine-derivatized dendrimers. The amino groups on the dendrimers can then be used to react with tryptophan-*N*-carboxyanhydride to generate oligopeptides *in situ* and they show tight regulation of the oligopeptide lengths through control of the reaction times (Figure 4D). The use of tryptophans suggests the possibility of photoelectronic behavior. However, adapting this method to incorporate other types of amino acids remains a challenge.

More recently, nanoshaving and nanografting have been used to deposit proteins and peptides either directly onto solid supports or indirectly onto specifically functionalized SAMs [49,51]. Nanoshaving is an AFM technique that is used to remove a resist layer (usually a SAM) in

order to create nanometer scale regions that peptides can bind to, typically through noncovalent bonds or through metal coordination. Nanografting is an extension of nanoshaving, in which the surface where the layer is locally removed is immediately replaced with a different, but related, material. The newly deposited material will now contain a molecule that can still be involved in forming a precisely oriented surface but has been functionalized to bind the peptide of interest. Alternatively, the peptide itself can be grafted directly to the surface. Several labs have recently reported success using these techniques to create peptide-patterned surfaces in which their function has been verified. One example is from Scoles and his colleagues, where they have created dithiol monolayers on gold surfaces using nanografting [54]. Later, they show that nanografting can be used to replace one alkanethiol with a shorter alkanethiol [55]. An important technical contribution made in this work is to replace contact mode methods with tapping mode methods for nanografting, since this newer approach is less damaging to proteins. They follow up this work by demonstrating a nanografting procedure in which the maltose binding protein, engineered with cysteines near its amino terminus, is deposited directly onto a gold surface, resulting in patterned and properly oriented deposition (Figure 4E) [56••]. They verify the proper orientation using a functional assay for the maltose binding protein.

Nanocontact printing is another method used for the patterning of surfaces designed for peptide deposition. Hung and Stupp have published work recently describing the patterning of peptide-amphiphile nanofibers using this spatial confinement method (Figure 4F) [57••]. This work is important because it allows for self-assembly from solution to occur simultaneously with patterning via spatial confinement; in the past, this has been a severe limitation of the technique. The authors demonstrate that they can pattern these nanofibers over large areas using a technique called sonication-assisted solution embossing, and that, upon solvent evaporation, specific alignment of the biomaterials can be achieved.

We have chosen to highlight recent papers that focus on specific deposition and patterning advances particularly suitable for peptide materials. While there is a growing literature of applications of these advances to specific peptide designs, there is as yet little work on deposition and patterning of preassembled biomaterials onto surfaces. Nevertheless, this is clearly an emerging area and we expect that, within the next three to five years, many papers will demonstrate the ability to pattern surfaces with peptide-based biomaterials that contain novel electronic functionalities.

Conclusion and outlook

The ability to functionalize peptide biomaterials has come to the fore, particularly since there are now a great variety of approaches available in creating well-defined supramolecular assemblies. Of particular interest in this review has been those assemblies that form fibrillar type structures, as they offer the most obvious form for creating components of integrated nanocircuit designs. We have described several recent efforts in creating electronically active assemblies [20•,21••,23•,24•,26,27•] but there is room yet for more research in this area. There are certainly other chromophores that could act as useful photoelectronically active additions. This would increase the diversity of such biomaterials and we suspect that in the next three years, there will be significant advances in this arena. Another critical technology that has been extremely well developed is ways in which peptides can be deposited onto a wide variety of surfaces, whether inorganic or organic (e.g. organic SAMs). In conjunction with the appropriate deposition methods, bioorthogonal chemistry methods have been developed to provide covalent attachment under aqueous, or otherwise gentle, methods that allow the peptides to retain their structure and function [28,29,30•,33,36•]. The ability to create organic-based and peptide-based SAMs has been largely mastered and has already provided many opportunities to study interesting peptide functions on appropriate surfaces. Many groups have used such strategies to greatly increase our understanding of intrinsic electron transfer properties [39•,40,42,44]. The major challenge that will need to be addressed, however, is how to precisely pattern such supramolecular assemblies. While recent success in patterning peptides as individual blocks has been achieved, most notably using patterning methods such as DPN, nanocontact stamping, and other AFM methods such as nanoshaving, nanografting, and nanografting [52•,53••,56••], these have not been applied yet in any significant way to preformed supramolecular structures. The one exception to this is the nanocontact stamping work of Hung and Stupp [57••]. It will be exciting to see other labs use techniques such as this to deposit peptide-based electronically active materials. We are certainly working toward this goal in our own research, using our recently developed biomaterials. The key to success has not been clearly defined, since alignment of the fibrillar assemblies is still a major challenge. Deposition technologies will need significant refinement to achieve such a lofty goal. Another challenge that has not yet been solved is the ability to address these patterned materials to test their photoelectronic characteristics. Such work has been done for other organic-based biomaterials but, to the best of our knowledge, has not been adapted for peptide-based supramolecular assemblies.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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