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More than just bare scaffolds: towards multi-component and decorated fibrous biomaterials†

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We are entering a new phase in biomaterials research in which rational design is being used to produce functionalised materials tailored to specific applications. As is evident from this Themed Issue, there are now a number of distinct types of designed, self-assembling, fibrous biomaterials. Many of these are ripe for development and application for example as scaffolds for 3D cell culture and tissue engineering, and in templating inorganic materials. Whilst a number of groups are making headway towards such applications, there is a general challenge to translate a wealth of excellent basic research into materials with a genuine future in real-life applications.

Amongst other contemporary aspects of this evolving research area, a key issue is that of decorating or functionalising what are mostly bare scaffolds. There are a number of hurdles to overcome to achieve effective and controlled labelling of the scaffolds, for instance: maintaining biocompatibility, i.e., by minimising covalent chemistry, or using milder bioconjugation methods; attaining specified levels of decoration, and, in particular, high and stoichiometric labelling; introducing orthogonality, such that two or more functions can be appended to the same scaffold; and, in relevant cases, maintaining the possibility for recombinant peptide/protein production. In this critical review, we present an overview of the different approaches to tackling these challenges largely for self-assembled, peptide-based fibrous systems. We review the field as it stands by placing work within general routes to fibre functionalisation; give worked examples on our own specific system, the SAFs; and explore the potential for future developments in the area.

Our feeling is that by tackling the challenges of designing multi-component and functional biomaterials, as a community we stand to learn a great deal about self-assembling biomolecular systems more broadly, as well as, hopefully, delivering new materials that will be truly useful in biotechnology and biomedical applications (107 references).

1. Introduction

1.1 Rationale for and objectives of this review

This review does not focus on any one class of biomaterial per se—there are excellent articles on these throughout this...

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1. Introduction

Derek N. Woolfson received his PhD from the University of Cambridge, UK, working with Prof Dudley Williams FRS and Dr Phil Evans on problems in peptide and protein folding. He did short post-doctoral stints with Prof Janet Thornton FRS (UC London), and Prof Tom Alber (UC Berkeley), where he honed skills in bioinformatics and biophysics to study sequence-to-structure relationships in proteins. For the past 15 years his independent group at the University of Sussex, and, since 2005, at the University of Bristol, has focussed on protein design and its application to bionanotechnology and synthetic biology. His group takes a multidisciplinary approach to its work.

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Themed Issue—though the ideas and concepts introduced will be illustrated by reference to specific examples. Rather, our aim is to layout, and to begin to address, what we see as a major challenge in biomaterials research today. Of course, there are multiple challenges in the general area: namely, the further introduction of truly rational approaches to the design of such materials; understanding, controlling and exploiting their dynamics; and the development of methods for producing biomaterials on large enough scales to be useful in real-life applications. However, the problem that we focus on here is the reliable addition of function to what are largely at present structural materials, or bare scaffolds.

This is challenging for a number of reasons: first, as supramolecular constructs, we have scant understanding of the atomic structures of many of these materials, which frustrates the processes of engineering and rational redesign; second, and what might seem contradictory to the first point, many of the scaffolds are so well folded, ordered and even partly crystalline that they effectively prohibit the inclusion of the altered or atypical components that might be used to carry function; thirdly, many of the materials are non-covalent assemblies and attempts to modify them post-assembly fail because of the underlying fragility of the systems, or the forcing conditions needed for some bioconjugations. Nonetheless, researchers are beginning to succeed in assembling multi-component systems, or decorating pre-assembled materials, giving considerable hope to addressing the challenge we have laid out.

1.2 Types of material and how these impinge on approaches to functionalisation

Mainly, we consider three types of peptide and protein fibrous materials here: (a) those based on amyloid-like structures, which can be formed from natural proteins, naturally derived peptides, or peptides of de novo design; (b) those based on α-helical assemblies, which usually employ peptides of de novo design; and (c) engineered peptide-synthetic hybrids, in particular peptide amphiphiles (PAs). This is primarily because these self-assembling systems have been developed sufficiently to allow their decoration and functionalisation. Other structures such as designed collagen-like assemblies and silks will be introduced as appropriate in later sections of this review.

(a) Amyloid-like structures. The term amyloid has a specific meaning: it refers to extracellular, insoluble, fibrous, protein aggregates associated with diseases such as Alzheimer’s. However, as many peptides and proteins—both natural and engineered, and implicated in disease or not—can be induced to form similar assemblies, including similar underlying secondary and quaternary structures,1 the more-general term “amyloid-like structure” is often preferred. In this generic structure, small model peptides, fragments of natural proteins, or even whole proteins, undergo conformational switches to largely β-structured states in which multiple β-strands hydrogen bond to form extended sheets, these pair to form the generic cross-β-structured fibrils. These may or may not bundle to form thicker fibres. In these structures, the β-strands run perpendicular to the long axis of the fibrils and fibres, Fig. 1a.1

Sequence-to-structure relationships in peptides that form amyloid-like structures are emerging, but the field is still in its infancy in this respect. This is somewhat different from the α-helical assemblies described next, where such relationships tend to be better defined partly because of the better understanding that we have for helical assemblies from work on free-standing and coiled-coil α-helical systems.4 Nonetheless, in terms of rational peptide design, rules of thumb for engineering peptides that form amyloid-like structures include: using a high proportion of β-structure-favouring residues; aromatic side chains; and employing sequence patterns in which hydrophobic (H) and polar (P) residues alternate, (HP)n.

(b) α-Helical assemblies. In amyloid-like structures, interchain backbone hydrogen bonding plays a significant role in the self-assembly process. For α-helical supramolecular assemblies this is not, indeed cannot, be the case. This is because the majority of the backbone hydrogen bonding of the α-helix is tied up locally within the helix. This has advantages and disadvantages for design: one advantage is that potentially promiscuous backbone-backbone interactions are avoided, or at least reduced, which increases the level of control possible compared with that for amyloid-like assemblies; a disadvantage might be stated in the question, then how are inter-chain interactions and higher-order assemblies achieved? The answer to this comes in part from clever protein designs, and partly through serendipity. The design aspect is that sequence-to-structure relationships for natural helix-helix assemblies are used, and slightly altered to drive new modes of helix-helix interaction that promote supramolecular assembly; essentially, α-helical peptides can be designed as building blocks, or tectons, for assemblies comprising many millions of organised peptides.5 The serendipity is that, when reduced to practice, rather than making long thin fibrils, most of these designs form long thick fibres. It turns out that this makes them more interesting, robust and potentially more useful than initially envisaged.

Several groups have now succeeded in making α-helix-based fibrous systems,6–11 which have been reviewed by us elsewhere.12,13 However, the necessary details and differences between these systems are described in section 4 of this review. These designs rely on programming polypeptide sequences to form amphiphatic helices that associate—usually as dimers, trimers and pentamers. As such, the designs tend to be based on the well-understood α-helical coiled-coil motif,4 and have underlying heptad repeats, (HPPHPPP)n. However, with a couple of notable exceptions,10,11 unlike in natural coiled-coil assemblies, where the bundles are discrete or blunt ended, in the fibrous designs the helices are programmed to pack in slipped arrangements. This leaves overhangs, or sticky ends, which, in turn, assemble end-to-end to drive fibrillogenesis. This concept is illustrated in Fig. 1b.

(c) Peptide amphiphiles (PAs). Hartgerink and Stupp’s concept for the peptide amphiphiles (PAs),3 presents a very different approach to engineering soft materials: here self-assembling and functional units are combined in one molecule from the outset. In the original PA design,3 a
16-carbon alkyl chain is conjugated to the N-terminus of a polar ter-functional linear peptide with the sequence, Cys4-Gly3-SerP-Arg-Gly-Asp, Fig. 1c. The alkyl chain, and the overall amphipathicity of these molecules drives assembly into rod-like, fibrous micelles, in which the alkyl chains are buried and the C-termini of the peptides are exposed; these structures are stabilised by a corona of interchain disulfide bridges between the cysteine block; and the four C-terminal amino acids provide water solubility and function. Stupp’s team has confirmed these concepts and basic aspects of the design and assembly through a series of biophysics, microscopy and rheology studies over the past decade. 14,15

Each of the general classes of material a–e has advantages and drawbacks for decoration, which can limit or even dictate the optimal route(s) taken to functionalising the systems. For instance, the amyloid-like materials tend to have one or both of their N- or C-termini free. Thus, the functional moieties, be they small molecules, other peptides or whole peptides, can simply be appended to the amyloidogenic polypeptides prior to assembly. Similarly, the design principles for the PAs specifically considers the need to incorporate functional motifs with the molecular design. Clearly in both cases a and c, this carries many advantages, but the possible lack of control over assembly can be an issue; often these systems employ a single self-assembly unit, and are subject to spontaneous assembly, or thwarted by nucleation kinetics. As outlined in section 4, the decoration of at least some of the z-helical systems is less straightforward; as the termini are not necessarily free for conjugation for example. Nonetheless, the experience and knowledge that the community has in design and engineering z-helical systems when fully brought to bear in this area of materials could have a major impact.

2. Decoration of self-assembled, fibrous biomaterials

Broadly, there are two general approaches to making multi-component and decorated materials: top-down assembly, using a variety of patterning techniques; and bottom-up assembly, exploiting controlled self-assembly of monomeric units. Top-down processes are used extensively for the fabrication of phototactical devices, nanowires and so on. More recently, this approach has been used to pattern surfaces for biological applications.16 However, the approach presents limits for incorporating features at the nanoscale. This review focuses on routes in the bottom-up design of both straightforward and complex (multi)functional biomaterials via the self-assembly of peptide, and peptide-containing systems. Several routes have been taken here, which can be divided as follows. Firstly, there is the construction of monomeric units that contain both the instructions for self-assembly, and a functional moiety; we refer to these as Route 1, or co-assembly approaches. Secondly, in Route 2, or post-assembly approaches, bare scaffolds are assembled from more-basic building blocks, and then decorated either through covalent (Route 2a), or non-covalent modification (Route 2b). Thirdly, in Route 3, or templating approaches, pre-assembled fibrous materials are used as templates to draw down the assembly or condensation of inorganic or polymeric materials. These routes are illustrated in Fig. 2.
We find these classifications useful, but we recognise that they are broad and cover a wide range of different materials, chemistry and work from many groups. As a result, they can be split further. For instance, in the co-assembly approach for example, building blocks for assembly and function can be combined in the initial pre-assembled construct, as in Stupp’s peptide amphiphiles,15 or Barker’s amyloid-forming protein-cytochrome fusions.18 In the non-covalent approach, functionalised building blocks can be incorporated into the fabric of the assembling material if it is permissive, as exemplified by Yu’s work on collagen functionalisation,63 or through the design or selection of new binding motifs as we demonstrate for our own Self-Assembled peptide Fibres (SAFs).19 The final approach of templating is somewhat of a special case, which we split according to the types of materials that are deposited on the scaffold.

Finally, we give a short history cataloguing our attempts to decorate the SAFs, which have met with different levels of success; and following this we describe the work of others in this area who work on similar systems comprising z-helical building blocks.

2.1 Route 1: co-assembly of covalently linked structural and functional moieties

The first approach to generating peptide- or protein-based fibres that display a functional moiety is simply to construct a single molecule containing both the self-assembly and the functional components in question. There are three ways in which this might be envisaged: (1a) covalent attachment of the moiety to polypeptide during peptide synthesis; (1b) if the functional moiety is itself a peptide or a protein, gene fusions can be designed and expressed recombinantly; and (1c) hybrid peptide systems in which the peptide parts act as the self-assembling unit, the functional moiety, or both.

In all of these cases, functionalisation is covalent and performed prior to assembly. Of course, this mitigates some problems associated with incompatibility of bioconjugation chemistry and self-assembly. However, it places restrictions on the systems too: namely, and obviously, that the resulting constructs must remain competent for self-assembly; and, conversely, that the self-assembly process must not preclude or otherwise hamper the accessibility or activity of the attached functionality. For self-assembling peptides and proteins, this usually means having an available N- or C-terminus free for covalent chemistry; though, side-chain modifications are also possible. Indeed, the latter are becoming more-widely used with wider access to modern peptide chemistry, and the possibility of incorporating non-proteinogenic amino acids into proteins via new recombinant methods.

Route 1a: peptide-small molecule constructs. Modern solid-phase peptide synthesis (SPPS) is opening a wide range of possibilities for bringing function to synthetic peptides and even small proteins.20 Peptides of up to ~50 amino acids can now be made routinely by SPPS. In these methods, the peptide chain is made by a series of catalysed condensation reactions between a resin-bound polypeptide with a free N-terminal amino group, and an amino acid in solution with a protected N-terminus and an activated C-terminal carboxylic acid. During these steps, the side chains of the growing polypeptide chain and the incoming amino acid are protected with chemistry that is orthogonal to that used to free the N-terminus and effect the condensation reactions. In this way, the peptide is synthesised from its C- to its N-terminus, and, at the end of the process, a complete, side-chain-protected peptide is attached to the solid support. At this stage, the N-terminus can be liberated separately—as can certain side-chain protecting groups—to allow a variety of functional groups to be added through straightforward acid-amine conjugation or alternative chemistries. Finally, the (remaining) side-chain protection can be removed, and the peptide cleaved from the resin simultaneously to produce the free peptide ready for self-assembly.

There are numerous examples of this chemistry being applied to add fluorophores, biotin (for later capture by streptavidins), small peptides and similar moieties to the N-termini of peptides, but here we focus on functionalising self-assembling peptides. This particular area has been reviewed by Channon and MacPhee recently,21 but we offer
up a few illustrative examples from the primary literature. Most of the examples centre on using amyloid-like assemblies.

For example, Channon et al. describe the assembly of modified peptides based on the 105–115 fragment from transthyretin (referred to as TTR), which forms amyloid-like assemblies. In this case, they add a fluorophore to the N-terminus of the peptide to give F-TTR. Assembly is driven solely by the peptide sequence, with the fluorophore still retaining its functionality, but not contributing to the assembly process; i.e., F-TTR assembles to 7 nm-wide fibrils as observed by transmission electron microscopy (TEM), which show meridional and equatorial reflections at 4.7 and 10 Å, respectively, in X-ray diffraction indicative of cross-β structure.22 The fluorophores in these assemblies exhibit new chiro-optical properties, which, the authors suggest, may find use or provide inspiration in the development of optoelectronic materials for photovoltaics or synthetic photosynthesis. Indeed, towards the second goal, the team has recently demonstrated the co-assembly of two independent luminescent moieties.23 In these cases the “conjugations” are straightforward, because the fluorophores are added to the N-terminus after peptide synthesis; indeed, in the first example, it is simply the Fmoc protecting group retained from peptide synthesis.

N.b. The development of shorter, Fmoc and similarly N-terminally conjugated peptides, is a rapidly growing sub-field being explored and reviewed well by Gazit, Xu, Uljijn, Adams, Zhang and others.24,25 Though we note in these cases the N-terminal aromatics and fluorophores usually do play a significant role in the assembly of these smaller peptides compared with the TTR-based systems.

Also somewhat related to the example from Channon et al., though strictly it comes under Route 2b in our categorisation, Welland and co-workers describe how concentrated amyloid-like fibres can be cast into ordered gels, that bind and orient fluorophores non-covalently.26 One advantage here is the use of relatively cheap, commercially available starting materials: the protein used is hen-egg lysozyme, and the fluorophore is Thioflavin T.

Extending this type of study in yet another direction, Gras et al. describe the construction of bio-active peptides based on TTR.27 In this case, the authors make C-terminal extensions with a Gly-Gly spacer followed by the integrin-binding peptide Arg-Gly-Asp (RGD). The resulting peptides still form amyloid-like assemblies that display the expected dye-binding tinctorial properties, fibre morphology by electron microscopy, and cross-β-structure in X-ray diffraction. Furthermore, dansyl fluorophore appendages, also at the C-termini, are used to demonstrate the exposure and availability of the C-terminus, and that blends of the parent TTR1 and TTR1-RGD can be made; both are confirmed by immunogold labelling using an anti-dansyl fluorophore antibody and visualisation by electron microscopy. The RGD-decorated fibres are used in cell studies with fibroblasts to demonstrate their use as substrates to support cell adhesion in tissue culture.

Gras’s work adds to a growing body of studies on designed and natural peptides that form amyloid-like assemblies, and particularly hydrogels, being applied in the burgeoning area of 3D cell culture and tissue engineering. On the design front for example, research groups like those of Zhang, Pochan & Schneider, and Aggeli are contributing here: Zhang’s group has added RGD-based peptides to his model amyloid-like gel-forming system, RADA16,28,29 though the MAX series of peptides from the Pochan and Schneider groups have not been engineered in this way, they do support 3D cell growth,30 and interestingly show antimicrobial activity;31 and Aggeli and co-workers have considered the requirements for expanding the field to real-life applications, including the need to move to recombinant production in place of peptide synthesis.32,33

Regarding other naturally derived peptides—in particular those not from proteins normally associated with amyloidogenic peptides or amyloidoses—Ohga et al. demonstrate that certain proteolytic fragments of laminin (a glycoprotein component of basal lamina) form amyloid-like fibres.34,35 These can be extended with N-terminal RGD-containing sequences, and retain the ability to form amyloid-like structures. The resulting functionalised materials support cell growth, and neurite-like outgrowth from a model rat phaeochromocytoma (PC12) cell line in culture. The second property is linked to the sequence motif Ile-Lys-Val-Ala-Val (IKAVV), which some of the peptides also contain making these assemblies multifunctional.

Route 1b: recombinant peptide-protein fusions. Recombinant DNA technology and, with it, the ability to clone, mutate and fuse genes for proteins, and then express these in tractable host cells is well established.33 Nonetheless, the increased efficiency and reduced cost of making synthetic DNA, together with the non-specialist kit or standardised approach to cloning and expressing such pieces, are influencing the development of the new field of synthetic biology. In essence, the aim here is to design de novo, or to engineer existing biological components, and to put these together in ways to make new and interesting systems and devices, which, hopefully, will have some new and useful properties.36 In these respects, the design and engineering of functional peptide- and protein-based materials, particularly when translated into recombinant production, can be considered a legitimate aspect of synthetic biology.5 However, it is one thing to sketch out ideas for basic biomolecular components, it is another to start fusing them together in predictable, and useful ways. Nonetheless, several studies offer hope and inspiration here.

For example, using the yeast protein Ure2p, Baxa et al. demonstrate that functional proteins can be piggybacked onto a self-assembly framework to give functional fibres.37 The N-terminal “prion” domain—either residues 1–65 or 1–80, depending on the construct used—of Ure2p protein is natively unfolded, but can assemble to form β-structured amyloid-like fibrils. Baxa and co-workers fused these regions to four proteins—three enzymes, barnase, carbonic anhydrase, glutathione S-transferase, and the green fluorescent protein. In all cases the soluble fusion proteins show unfolded Ure2p domains, but assemble to β-structured fibrils. Moreover, and though to different degrees, the appended natural proteins are all active. Similarly, Barker et al. have fused the gene for cytochrome b₅₆₂ to the amyloidogenic SH3 dimer, which is capable of independently forming fibrils.18 Addition of cytochrome b₅₆₂ does not interfere with fibril formation, and
cytochrome b$_{562}$ when displayed on the fibre retains functionality by binding metalloporphyrins, though only half of the potential binding sites are occupied.

An area where recombinant production of materials has a distinct advantage is for large, repetitive and otherwise intractable fibrous proteins such as silks.$^{38,39}$ The rules relating to sequence-to-structure in these systems are only just being unravelled. Nonetheless, they have some superb materials properties ripe for exploitation as engineered biomaterials. The sequences are modular and this has been exploited, by the Kaplan and Scheibel groups for example, to produce and express synthetic genes. In terms of decoration, Kaplan’s group in particular has shown that these can then be used to introduce single and multiple cell-adhesion motifs near the termini of fibres to generate bioactive tissue-engineering scaffolds.$^{40,41}$ In this general area, others are turning their attention to other elastomers such as arthropod resilins,$^{42}$ though at this stage this work has focused on producing recombinant model proteins, and to our knowledge, these have yet to be decorated.

As noted by Kyle and co-authors,$^{33}$ the move towards recombinant production of self-assembled systems, either as independent moieties or functionalised as above, will become increasingly important if the field is to advance and fulfill expectations towards real applications. Though it is not without difficulties—amongst these is the overproduction of small, often ephemeral peptides; and the possible formation of cytotoxic oligomers or matured fibres—there is encouragement that the community is headed in this direction.$^{43}$

**Route 1c: hybrid peptide-containing systems.** Perhaps the classic examples of materials in which the components for self-assembly and function are directly and deliberately incorporated within one molecule are Stupp’s peptide amphiphiles (PAs), Fig. 3.$^3$ In the original PA, the polar part of the incorporated peptide sequence, -SerP-Arg-Gly-Asp, has a dual function: the phosphoserine is for hydroxyapatite templating; and, as mentioned above, the RGD sequence is for cell binding. Stupp’s team has increasingly demonstrated potential biomedical applications for the PAs.$^{15}$ For example, different PA fibres have been used as tunable scaffolds for tissue engineering,$^{44,45}$ including the capture and controlled release of growth factors,$^{46}$ and as bioactive surfaces for bone implants.$^{47}$

In a different approach to multi-domain amphiphiles Hartgerink and colleagues make ABA-type systems from purely peptidic blocks.$^{50}$ Here, the amphiphilic B block drives self-assembly into nanofibres around 6 nm in diameter, whereas the hydrophilic A block is composed of charged amino acids and determines the conditions under which assembly takes place. Moreover, functional motifs for cell-adhesion and enzyme cleavage can be placed in the central B block to create functional responsive bioactive gels.$^{51}$

### 2.2 Route 2: post-assembly decoration

Functionalisation performed after assembly of the scaffold poses different challenges to the assembly of combined structural and functional components. Moreover, the new functions can be added covalently (Route 2a) through bio-conjugation, or non-covalently (Route 2b) through binding. To make such post-assembly modifications requires accessible modifiable groups on the surface of the scaffold, which is pertinent to both Routes 2a and 2b; and the scaffold to be tolerant of the conditions used for functionalisation, which is usually more of an issue for covalent modification. Thus, there are potential restrictions and drawbacks of taking the post-assembly route. However, advantages of separating assembly and functionalisation in this way include the possibility of making general, and potentially more-versatile materials. These could then be applied in a variety of different applications without recasting the underlying self-assembled scaffold. i.e. For a given scaffold, and a robust, general way of appending cargo to it post-assembly, a wide variety of new functional biomaterials can be envisaged.

![Fig. 3 Functionalisation of peptide amphiphiles.](image-url)
Route 2a: post-assembly covalent modification. There are few examples in this subtopic because of the tension between the conditions needed to make covalent modifications, and those required to maintain the self-assembled materials. However, we suspect that click chemistry will transform this particular area, facilitating new routes to functionalised self-assembled peptide and proteins materials. Indeed, a search of Web of Science combining the terms “click chemistry” and “self-assembly” and protein/peptide returned less than 20 papers, most of which were from the last year (2009–2010).

Thus, it seems to be an emerging and promising area. However, most of the papers we found would be best placed in the previous section, as they involve the conjugation of peptides and, in one case proteins, to polymers to make diblock and triblock systems that subsequently self-assemble.\textsuperscript{53–55} Though a fascinating area for research, this field of peptide-polymer hybrids is broad with a large literature that is beyond the scope of this review. Therefore, we refer the reader to a number of excellent recent reviews, which expand this and the previous sections generally.\textsuperscript{56–58}

We examine the area of click chemistry as applied to purely peptide-based self-assembled systems with reference to our own work in the area in section 4. Click chemistry encompasses several types of reactions, of which the 1,3-dipolar Huisgen cycloaddition is the most popular because of ease of synthesis of the intermediates, relatively fast reactions kinetics and good yields. However, copper-based click chemistry has not been widely explored for functionalisation of biomaterials because of concerns over the toxicity of copper. However, we and others have shown that copper can be successfully removed from gels after functionalisation; additionally copper-free click is increasingly becoming accessible.\textsuperscript{59}

As an example for this section one recent paper is particularly noteworthy even if a little off the main topic of the review. DeForest \textit{et al.} describe an elegant modular hydrogel system that supports cell growth, Fig. 4.\textsuperscript{60} The components comprise: a “hub” with four poly(ethylene glycol) (PEG) arms each terminated by an azide functional group; a synthetic peptide “linker” incorporating multiple features including: cyclooctyne-containing terminal residues, a central alkene-containing side chain, and a protease cleavage site; and thiol-containing peptides that harbour fluorophores or other protease sites to provide “functional appendages”. Two orthogonal click reactions are employed in the assembly process: firstly, a copper-free variant of a 1,3-dipolar Huisgen cycloaddition between azides and alkynes is used to bring together hubs and linkers to produce a highly cross-linked material, which forms a physical hydrogel in aqueous buffer. Secondly, photocatalysed thiol-ene reactions are used to tag the alkene-containing hydrogel framework with the thiol-containing functionalised peptides. Impressively, this can be done in a top-down manner to allow cytocompatible photopatterning with tens of micron resolution.

In this way, the authors prepare a number of hydrogels that can be visualised, patterned, used to support cell growth and enzymatically degraded.

Route 2b: post-assembly non-covalent binding. This route is attractive for the same reasons that biology employs weak non-covalent interactions—usually combinations of hydrogen bonds, van der Waals’ forces, and salt bridges—to assemble functional complexes: essentially, such forces allow for control, specificity and reversibility; and they present possibilities for building up complex (multi-component) systems. In biomaterials design, they offer another potential advantage of mild, biocompatible conditions for decoration. Balanced against this, it is often difficult to rationally design specific binders for a substrate (even if its surface structure is known); and any binding will be reversible to some extent, and the dynamics of cargo binding, release and, therefore, longevity on the substrate may present problems.

Non-covalent decoration via hydrophobic interactions. Peptides that form well-ordered supramolecular structures are good candidates for non-covalent decoration, and make even the notion of rationally designing binding peptides

Fig. 4 Functionalisation of polymer networks using copper-free click chemistry. A polyethylene glycol hub with terminal azides (a) is cross-linked to cyclooctyne-bearing functional groups (b) to produce a physical hydrogel (c) with regularly arranged functional groups ($R_2$; green). Reproduced in part from ref. 60, with permission of Macmillan Publishers Limited, copyright © 2009.
possible. For example, Mihara’s group have reported a peptide design, F1, that self-assembles to form straight, highly ordered, tubular amyloid-like structures.61,62 Interestingly, F1 first assembles into protofibrils 5–17 nm in diameter, these align side-by-side to form ribbons, which twist to form straight fibres 80–130 nm in width and ~10 µm in length. This packing gives striations in negative-stain TEM—indicative of a high level of order—and it is proposed that it exposes some of the hydrophobic residues within the fibre. On this basis, the group has designed anchor molecules to interact with these hydrophobic regions non-covalently. 17 These consist of biotin linked to a hydrophobic dodecyl group with or without a spacer unit. Assembly of F1 is not perturbed by either anchor, and subsequent incubation with gold-conjugated antibody shows that the anchors were successfully recruited to the surface of the fibres. To expand this style of non-covalent anchoring, the dodecyl component can be replaced with Ile-Ile or Phe-Phe dipeptide units to similar effect. Moreover, these dipeptide binders can be extended via β-alanine linkers with imidoacetate moieties for Ni-chelation and subsequent capture of hexahistidine-tagged proteins. Using these constructs, the group present some striking images of nanoparticles arranged with regular periodicity along the edges of fibres, Fig. 5.17

Non-covalent decoration using structural mimics. Yu and co-workers describe how peptides that mimic collagen sequence and secondary structure, so-called collagen-mimetic peptides (CMPs), can be incorporated into the triple-helical structure of collagen during the assembly process and so be used to bring function to this natural scaffold.63 Naturally occurring fibrous collagens form right-handed triple helices due to underlying Pro-Hyp-Gly sequence repeats (where Hyp is hydroxyproline), Fig. 1d. The CMPs are short, synthetic peptides made up of such repeats. Yu and colleagues reason that CMPs should associate with partly denatured collagen, and demonstrate this as a series of fluorophore-labelled CMPs with varying melting temperatures ($T_M$): long, for instance (ProHypGly)$_{10}$-based CMPs with high $T_M$s interact with collagen films equilibrated at 80 °C, but not at 25 °C; shortening the core sequence, to (ProHypGly)$_6$, lowers the $T_M$ to 25 °C, and this modified CMP interacts with collagen films at 30 °C. Importantly in the second case, the temperature is lower than the $T_M$ of collagen itself (37 °C), indicating that denaturation of CMP is more critical for the interaction than denaturation of collagen. To study the binding site of CMPs, (ProHypGly)$_7$ has been conjugated to gold nanoparticles via a cysteine residue.64 This shows preferential decoration of type I collagen fibres in the gap regions, which arise from staggered packing of the triple-helices along the long fibre axis, and appear as dark bands every 67 nm in negative-stain TEM. The group suggest that the gaps correspond to regions low in hydroxyproline, and that the CMPs supplement this acting as triple-helix stabilisers and, hence, have increased affinities for these regions. CMPs may be exploited in tissue engineering. For example, it is possible to make hybrid PEG-CMP hydrogels, which retain cell-secreted collagen, thus allowing the cells to grow in an environment more-closely mimicking the natural extracellular matrix.65 Further modifications to these gels using CMPs have been tested, including the addition of multiple anionic charges to the N-terminus to recruit VEGF.66 The VEGF-CMP charged complex is released from the gel at a sustained rate, allowing the development of Human Umbilical Vein Endothelial Cells (HUVEC) with a tubular morphology indicative of three-dimensional growth.

Non-covalent decoration using complementary pairing. Related to Stupp’s earlier PA work, Li and Stupp have engineered new PAs after Yamada and co-workers.67 In these
constructs, fatty acid-like dialkyl-chains and monoalkyl chains are linked through a tripeptide unit. These wedge-shaped molecules assemble to nanofibres in organic solvents burying the monoalkyl moieties and leaving the dialkyl-chains exposed. In this case, Li and Stupp append thymine to the terminus of one of the latter chains. This can then be non-covalently decorated with gold nanoparticles functionalised with diaminopyridine (DAP), which forms complementary hydrogen bonds with the base, to render long, linear gold nanowires.

Returning to water-soluble PAs, Guler et al. and Stupp, have decorated yet another variant of the PA nanofibres post-assembly using a biotin-avidin label. These designs differ in a number of respects from the original PAs: first, the alkyl chain, which drives assembly of the rod-like micelles, is appended to a C-terminal side-chain of a central polypeptide; second, at the N-terminus of the polypeptide two branches are spurred off from lysine side chains. These branches are RGDS peptides with N-terminal biotins. The resulting PAs are again wedge-shaped and form nanofibres that gel. The surface biotin moieties can then be decorated with BODIPY-NeutrAvidin.

2.3 Route 3: templating hard inorganic materials on soft biomolecular assemblies

The final approach to decorating self-assembled peptide- and protein-based biomaterials that we discuss is templating. This bridges the gap between soft, self-assembled scaffolds, which afford control over assembly and nanostructure at the 2-D and 3-D levels, and systems that cannot, or do not readily self-assemble, but bring new functions to the resulting composite materials. In general terms, this is another broad field, which we cannot do justice to here, so we present a small number of illustrative examples.

In templating, usually inorganic materials—though there are examples for organic and polymeric materials—are deposited on the surfaces of the scaffolding materials. This is either done passively, which usually relies on recruitment of charges akin to layer-by-layer deposition, or more-actively, which often involves the inclusion of thiols (usually cysteine residues) into the peptide and protein scaffolds. Materials that have been combined with biomolecular assemblies in this way include, gold and silver nanoparticles and metals, clays and silica, and polyelectrolytes. The challenge is to template these in a controlled fashion, to form functional conducting wires or biomimetic substitutes for example.

Route 3a: towards metallic nanowires and related bionanomaterials

Building on amyloid-like assemblies, Reches and Gazit have exploited the self-assembling properties of aromatic diteptides to create silver nanowires with uniform dimensions, Fig. 6. At high concentrations, diphenylalanine precipitates out of hexafluoro-2-propanol as self-assembled hollow nanotubes with outer diameters of ~100 nm. The hollow of these tubes can be filled with silver ions, which are subsequently reduced to silver metal using citric acid as a mild reducing agent. Moreover, the outer peptidic shell can be removed with proteinase K to leave free-standing silver nanowires of ~20 nm diameter and almost microns in length. Thus, the authors have succeeded in passively casting metal wires using a peptide scaffold.

Similarly, Scheibel et al. use a variant of the N-terminal-to-middle, “NM”, region of yeast prion protein Sup35p, which assembles into fibres ~10 nm wide and up to microns in length, to template gold nanoparticles. In this case, the initial recruitment is actively encouraged by using a cysteine-modified variant of NM. Again, metal wires are produced by reduction of either silver or gold ions from solution. In this case, the resulting silver and gold nanowires coat the surface of the fibres, and are ~100 nm across. With both metals, the wires show low-resistance and ohmic metallic properties.

Further on this theme, other early pioneering studies in this area come from Belcher and co-workers. Along with others, such as Naik, this group show how metal recruitment can be specified and controlled more tightly using peptide aptamers selected using phage display against the target metal or inorganic material. In these studies, the bacteriophage M13 is used both as a vehicle for peptide selection, and as a scaffold for display and decoration, Fig. 7. M13 is a single-stranded DNA virus widely used in molecular biology. The virus particles are filaments with dimensions ~7 nm in diameter and ~1 μm in length, which display a number of different proteins on their surfaces in copy numbers ranging from ~3–3000. The genes for these proteins can be modified to allow peptides and proteins to be displayed on the viral surfaces; hence the term phage display. Belcher, Naik and others have used this system to display libraries of peptides and to select from these those that specifically and tightly bind inorganic surfaces. The cunning tricks that allow this are: (1) that binding phage can be enriched and amplified through rounds of selection and infection into Escherichia coli; and (2) that the sequences of the selected peptides can be determined via DNA sequencing as they are barcoded in the viral DNA. In this way, Belcher’s team, for example, has developed phage-templated silver, and gold and cadmium nanowires. Recently, the group has focussed on developing more-ambitious phage-templated systems as a basis for highly conductive lithium-ion batteries, and porphyrin assemblies for light-driven water oxidation.

Other recent examples of note in this general area include: the production of metal-insulator-metal, trilayered, coaxial nanocables from Gazit’s group. In this case, the aforementioned diphenylalanine tubes that sequester silver are supplemented by cysteine-containing peptides that bind the surface of the structures to provide a second, outer template for metalation. Recently, Ostrov and Gazit have turned to the prokaryotic tubulin homologue, and filamentous protein Z (FtsZ), which, in the presence of GTP or calcium, assembles into nanometre-scale fibres. Through genetic engineering, various metal-binding peptides with different metal-binding affinities have been fused to the N-terminus of the protein, allowing a series of nanowires to be generated. Mitraki et al. used the self-assembling octapeptide peptide Asn-Ser-Gly-Ala-Ile-Thr-Ile-Gly (NSGAITIG) from the fibre protein as a template. This forms amyloid-like structures, and N-terminal cysteine variants can be used to sequester gold, silver and platinum nanoparticles to impressively high coverage. Yet another structure has been explored as a template by Raines
and co-workers. They use collagen-like self-assembling peptides and electroless silver plating (silver enhancement) to generate nanowires. The basic collagen repeat of Pro-Hyp-Gly is modified by replacing one of the hydroxyprolines by lysine, which, in turn, is used to recruit amine-reactive gold nanoparticles, followed by silver enhancement.87

**Route 3b: templating minerals on soft materials.** As already covered, the original PAs from Hartgerink and Stupp template the mineralisation of hydroxyapatite via the phosphoserine incorporated in the peptide component.3,88 PAs also assemble into gels when incubated with calcium, which decorates the outside of the fibres to form nucleation points for hydroxyapatite mineralization. This system has been expanded further to template hydroxyapatite crystals in 3-D using the enzyme alkaline phosphatase.89 Additional rigidity and stability can be added to the system by filling these amphiphile gels into titanium foam scaffolds.47

Similarly, Hartgerink et al. have used PAs bearing an RGD motif and a enzyme-cleavable sequence to encapsulate two types of dental stem cells.90 When grown in the presence of osteogenic supplements, these PAs support growth of both soft cells and osteoblastic cells, which mineralize the matrix, making this a useful scaffold for dental tissue regeneration. On a related theme, Aggeli and co-workers have used their amyloid-like β-tapes as a scaffold to promote hydroxyapatite crystallization and tooth mineralization.91

**4a. Anecdotes from studies towards multi-component and decorated SAFs**

Over the last decade, we have explored the design, assembly and application of a Self-Assembling peptide-based Fibre (SAF) system.7,92-94 Unlike the majority of the systems described above, which tend to employ a single self-assembling molecule, the basic SAFs comprise two peptides that only assemble when mixed. The peptides are of completely de novo design, based on well-established sequence-to-structure relationships for α-helical coiled coils.7 A key difference between the SAFs and natural, or other designed coiled coils is that they are designed to form offset, or staggered heterodimers.7 These so-called sticky ended dimers are the building blocks for fibrillogenesis. We have performed a detailed analysis of the folding and assembly of the SAFs (at 100 μM in each peptide, 20 °C, pH 7).95 essentially, the two peptides combine instantly to form the sticky ended dimer; this is followed by a lag phase on the order of tens of minutes during

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**Fig. 6 Self-assembly and functionalisation of peptide nanotubes.** (a) Diphenylalanine assembles into peptide nanotubes. (b) Schematic of metal nanowires and coaxial cables templated from these nanotubes. (c) SEM image of vertically aligned peptide nanotubes useful for photoptic applications. Reproduced in part from ref. 73, copyright © the American Chemical Society. (d) SEM image platinum-coated NSGAITIG nanotubes (adapted from ref. 74). (e) Expression and self-assembly of filamentous protein Z (FtsZ) into nanofibres. (f) TEM image of FtsZ filaments coated with silver particles. Reproduced in part from ref. 75 with permission of Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, copyright © 2010.
which aggregates comprising \( \sim 8 \) sticky ended dimers nucleate; over the next few hours fibrillogenesis occurs both longitudinally and laterally from these nuclei; subsequent fibrillogenesis is essentially one-dimensional leading to matured fibres \( \sim 50 \text{ nm} \) thick and tens of microns in length. In addition, we have a good working model for the molecular structure and organisation in the equilibrium, matured fibres.\(^{93}\) This design concept, together with our understanding of the mechanism of assembly and the equilibrium structures of fibres, place us in a strong position to build on and exploit the SAF systems; specifically, they allow considerable control over assembly, and present possibilities for introducing additional components either at the start of folding and assembly, or during fibrillogenesis, Fig. 8.

Rather than seeking decoration of the SAFs \( \text{per se} \), we began by investigating the addition of non-standard, or special peptides, to the standard SAF mixtures in order to alter the morphology of the fibres. This included introducing T-shaped peptides and half-peptides connected through flexible linkers to bring branches and kinks, respectively, to otherwise straight and stiff fibres.\(^{96-98}\) One surprise from this early research was that the special peptides incorporated into the fibres only

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**Fig. 7 Using M13 bacteriophage virus to create ordered nanostructures.** (a) Noncrystalline nickel, rhodium and cerium templated on M13 for production of hydrogen (adapted from ref. 82). (b) Schematic and TEM images of M13-templated gold and silver nanowires. Reproduced in part from ref. 83 with permission of the American Chemical Society, copyright \( \odot \) 2010. (c) SEM image of Kevlar fibres coated with M13 phage bearing a peptide that interacts with gold nanoparticles (reproduced in part from ref. 84 with permission of WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, copyright \( \odot \) 2007.)
sparingly; in some cases although stoichiometric mixtures of the two parent peptides and the specials used to initiate fibrillogenesis (that is, 1 : 1 : 1 mixtures), the resulting fibres incorporate special peptides to massively sub-stoichiometric levels. We understand this now from our structural studies: the individual SAFs are crystalline, and as such are not permissive of incorporating specials that are significantly different from the parent peptides; in other words, the introduction of special peptides is like introducing defects into a crystal lattice. Nevertheless, these new peptides do insert into, and do cause the desired, albeit limited, morphological changes.

Furthermore, using this strategy, we were also able to incorporate peptides harbouring biotin and FLAG-peptide tags into the fabric of the SAFs. These allowed the subsequent recruitment of streptavidin and anti-FLAG antibodies, respectively, which could be visualised using nanogold probes and TEM. Finally in this approach, we have demonstrated that fluorophore-labelled peptides assembled into the fibres. This facilitates direct visualisation of the fibres by light microscopy (LM), which, in turn, has led us to explore the epitaxial (polar) growth of the fibres, and the late stages of the kinetics of assembly. In these cases, fluorophores can be introduced after peptide synthesis via bioconjugation, or during peptide synthesis using modified amino acids. One note of caution, however, because the SAFs carry an overall positive charge they recruit negatively charged fluorescein non-specifically; therefore, exclusively we use positively charged rhodamine to label peptides.

As with the aforementioned systems, the SAFs can be used as templates for the deposition of inorganic materials.

Fig. 8 Functionalisation of Self-Assembling peptide Fibres (SAFs). (a) SAF-p1 and SAF-p2a, which when mixed together in equimolar concentrations at pH 7.4 assemble to form long straight fibres tens of microns long and tens of nanometres thick. (b) Non-covalent decoration using short 11-residue peptides (SAF-tags), which bind via electrostatic interactions. Here decoration is visualised using rhodamine attached to the N-terminus of the tag. (c) A click-chemistry approach for post-assembly decoration of fibres incorporating azide side chains. Here decoration is followed by reacting the surface azide moieties with biotin-alkyne, followed by visualisation with 5 nm streptavidin nanogold.
We have demonstrated this by depositing silica on both standard linear SAFs and some of the morphological variants.\textsuperscript{101} Here, and in contrast to amyloid-like and PA-based systems in particular—where the templating material has to be removed by calcination\textsuperscript{102,103}—the SAF templates can be removed under mild, ambient conditions by proteolysis to leave silica replicas.

Returning to decoration with specific chemical and biological moieties: most recently we have explored two other routes to decorating the SAFs (ref. 19 and Mahmoud et al. & Woolfson, unpublished work). This has been done for several reason: (1) to tackle the aforementioned problem of low levels of inclusion of special peptides into SAFs, in particular, to increase these to stoichiometric incorporation of labels and the subsequent high degrees of decoration; (2) to improve the biocompatibility of the labelling; and (3) to begin addressing issues in temporal and spatial control of functionalisation. Both methods are post-assembly routes (Route 2 in the above rationale), and therefore demand mild conditions.

The first approach is entirely non-covalent (Route 2b), with the aim of engineering small peptides that bind the outer surface of the matured SAFs.\textsuperscript{19} The rationale is that, as the fibres exhibit a high degree of structural order and carry an overall positive charge, one might be able to select peptides that bind the surfaces. Our initial attempts used peptide libraries displayed on M13 phage. This failed for a variety of reasons related to the generally acknowledged stickiness of M13 phage, which led to high background binding. However, this, along with the aforementioned observations with fluorophores, suggested an approach employing short, negatively charged, synthetic peptides. Briefly, we find that peptides of the type DEDEDE bind strongly to the fibres; neutral peptides, AQAQAQ, bind less weakly; and positively charged peptides, KRKRKR, do not bind at all. Moreover, the DE- and AQ-based peptides can be tagged, and used to recruit cargo to the SAFs, as demonstrated with nanogold particles visualised by TEM, and with fluorophores visualised directly in solution by LM, Fig. 8b.\textsuperscript{19} The advantage of these so-called SAF-tags is that they could be readily added to target proteins for recruitment to the SAFs. A disadvantage is the potential for highly dynamic and non-specific binding with such simple sequences; though in the studies we have conducted we see long-lived binding and very little evidence for non-specific interactions.

Our second approach involves small chemically reactive groups suitable for biocompatible “click” reactions being directly introduced into the SAF peptides during synthesis (Route 2a). The modified peptides are then co-assembled with standard SAF peptides to produce matured fibres. These can then be decorated with a variety of organic and peptide reagents harbouring a complementary click group. To demonstrate this, we have synthesised SAF peptides incorporating azide and alkyne side chains. Importantly, and unlike our earlier approaches,\textsuperscript{99} the modified peptides incorporate stoichiometrically with the standard SAF peptides. The resulting modified SAFs can be functionalised through conjugation with copper-catalysed azide-alkyne and thiol-ene click reactions, respectively. This allows high levels of incorporation of the modified peptides (up to 100%), subsequent high coverage of the SAFs with cargo to be achieved, and the proportion of the label to be varied. Moreover, as the aforementioned click reactions are orthogonal and both modified peptides can be co-incorporated into a single fibre preparation, dual functionalisation of the SAFs is possible. We have demonstrated all these possibilities by incorporating either or both nanogold and fluorophore labels, Fig. 8c. Of course, this approach requires the synthesis of non-proteinogenic peptides, which are not amenable to standard recombinant DNA expression. However, as the incorporation rates are very high, relatively small amounts of the modified peptides are required to get respectable levels of decoration, and, so, these could be used sub-stoichiometrically with standard peptides; also, side chains that incorporate groups for the click reactions, and diazo transfer reagents that work in water are becoming more available.\textsuperscript{104} Therefore, in our view, this is the most promising approach to making functionalised SAFs that we have investigated so far.

4b. Decoration of other coiled-coil-based fibrous systems

A number of other groups have presented designs for fibrous materials based on $\alpha$-helical, and in particular coiled-coil building blocks,\textsuperscript{6,8-10} and some of these have decorated or functionalised their systems. Notably, Kajava and colleagues offer a system similar in some respects to the SAFs; however, it is based on pentameric coiled coils, and the individual helices “slip” to give sticky ended assemblies, which then associate end-to-end and side-by-side to form thickened fibres depending on the conditions. In a modified design they incorporate the integrin-binding RGD sequence, to render fibres that support cell growth in culture.\textsuperscript{106} In another SAF-related design approach, Dublin and Conticello describe a sticky ended trimeric coiled coil with buried histidine residues. In this case, these imidazole side chains coordinate, and facilitate the preparation of silver nanowires shrudded in peptide fibre.\textsuperscript{107}

Conclusion

Through this review, we hope to have outlined the current activities and trends in decorating, or functionalising, soft biomaterials, in particular fibrous assemblies. We confess that, whilst we appreciated that there was a strong and growing base of literature in the area, we hadn’t expected to find the depth and breadth of new, exciting and varied research that we did. Indeed, this review, and the literature cited in it, could have been expanded considerably had we had the time and space to do so. As a result of the growth of this field and its associated literature, rather than simply list and review the papers that we came across—say, chronologically, by materials type, or research group—we have tried as far as possible to see past individual papers and onto generalisations that are emerging. Specifically, we have categorised the work in terms of routes to functionalising fibrous biomaterials. Clearly there are limits to this: some examples are not so easily pigeon-holed, others span more than one route, and, of course, there will be future ground-breaking pieces of work that challenge dogma, and force us to reconsider the categorisation.
Nonetheless, we find this analysis useful, and we hope that others will find it helpful too.

During the course of our reading and writing, it became clear that common themes, approaches and methods that represent best practice are emerging in the field. For example: (1) the exploitation of natural self-assembly systems, or peptides derived from these often present a good starting point. However, this can present restrictions in terms of control over self-assembly, and also asks the question, *just how does one functionalise materials pre- or post-assembly without interfering with the self-assembly process itself?* This raises the next two key points from our perspective. (2) That the design or engineering of the self-assembling and functional components of the system should be orthogonal. And, related to this, (3) that this orthogonality should be designed or engineered into the system, or at least considered, from the outset of materials development. Perhaps the best examples that illustrate these two points to date are the peptide amphiphiles from Stupp and colleagues. (4) Rational peptide design also represents a good approach in these respects, but it does rely on having good rules that relate sequence, structure and assembly. Our understanding for certain protein-folding motifs, such as the coiled coil, is headed in the right direction, but this is by no means the case universally for protein folding.

Finally (5) there are many examples of designed and engineered self-assembled systems, and it is likely that some will be better suited to appending certain functions and in specific applications than others. It would seem prudent, to choose the right tool for the job in this respect, and not to be wedded to any one material type. Ideally, as the field develops we would have an open-source or synthetic-biology ethos, and a toolkit of basic materials with which to build will emerge.

Somewhat related to this, it is quite understandable that different groups have adopted similar strategies to demonstrate that they have achieved decoration. The common methods are “functionalisation” with a fluorophore of some description followed by visualisation using light microscopy; or the addition of gold nanoparticles (GNPs) followed by visualisation using electron microscopy. Clearly, these are the best options for demonstrating that decoration has been achieved, but usually they do not represent functionalisations in themselves; though examples such as the formation of metallic nanowires following the recruitment of gold nanoparticles are cases where functional materials are being developed in this way. Nonetheless, true functionalisation would be to impart some biological activity such as cell-binding properties onto the scaffolds. Of course, there is strong research in this endeavour. However, this is and must remain one of the tenets of both materials science and synthetic biology: that is, whilst as a community we must develop the very best understanding of natural and design self-assembling systems, as materials scientist and/or synthetic biologists, we must consider applications that put our materials to use.

In the case of soft fibrous biomaterials, the key applications areas that are being explored here are scaffolds for 3D cell culture and tissue engineering, and as sacrificial templates for the organisation of functional, often hard, materials that are less readily self-assembled. In many respects, the studies that we have highlighted have laid the groundwork for this development, and it is now time to translate further this basic research and deliver functionalised materials for specific real-life applications. There are many challenges ahead, including issues of large-scale materials production, biocompatibility of methods used in functionalisation, and, for tissue engineering, immunogenicity of the final materials. However, we feel that these and other issues are being tackled and will be surmounted.

In summary, the current state of basic research in functional fibrous biomaterials is buoyant, and provides a strong basis for translation into real-life applications.

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