

# Supramolecular biomaterials

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**Polymers, ceramics and metals have historically dominated the application of materials in medicine. Yet rationally designed materials that exploit specific, directional, tunable and reversible non-covalent interactions offer unprecedented advantages: they enable modular and generalizable platforms with tunable mechanical, chemical and biological properties. Indeed, the reversible nature of supramolecular interactions gives rise to biomaterials that can sense and respond to physiological cues, or that mimic the structural and functional aspects of biological signalling. In this Review, we discuss the properties of several supramolecular biomaterials, as well as their applications in drug delivery, tissue engineering, regenerative medicine and immunology. We envision that supramolecular biomaterials will contribute to the development of new therapies that combine highly functional materials with unmatched patient- and application-specific tailoring of both material and biological properties.**

Pressures arising from the increasing incidence of cancer and degenerative diseases, coupled with a focus to increase the quality of life for patients, have inspired the development of smarter and more creative healthcare solutions. The use of biomaterials — substances that are not foods or drugs and that are in contact with tissues or biological fluids<sup>1,2</sup> — has a long history dating back thousands of years to early wood prostheses<sup>3</sup>. In modern times, clinical uses of biomaterials have included the application of metals, ceramics and polymers to augment, repair or replace diseased, damaged or defective tissues. Over the past several decades, materials have been ubiquitous in the practice of medicine and have transformed orthopaedic joint replacement, vascular grafting, plastic surgery, dental augmentation and bone fusion and fixation, among many other clinical practices. Materials have also been used in the context of tissue engineering as scaffolds and delivery depots for cell-based regenerative medicine<sup>4,5</sup>. Traditional biomaterials offer the possibility of preparing structural analogues to native tissue, but their capacity to recapitulate native functional dynamics is limited.

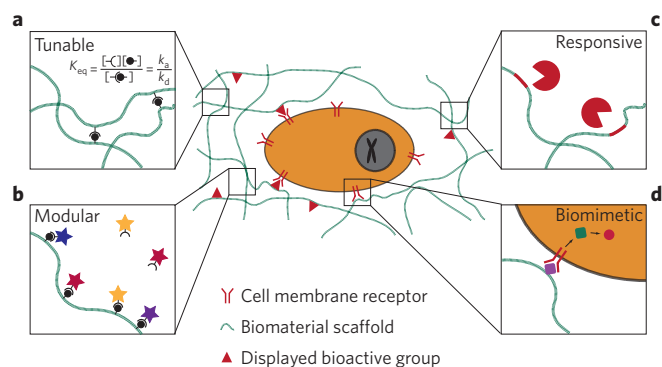
The development of next-generation biomaterials must include strategies to improve both structural and functional recapitulation of native tissue. In this regard, materials designed using supramolecular principles could have broad impact for use in the body. Supramolecular chemistry — defined as ‘chemistry beyond the molecule’<sup>6–9</sup> — is based on the rational design of specific, directional, tunable, reversible, non-covalent molecular recognition motifs that exploit hydrogen bonding, metal chelation, hydrophobic interactions,  $\pi$ – $\pi$  interactions, and/or van der Waals interactions. These motifs can be leveraged to create supramolecular materials whose properties follow from the dynamic nature of their constituents<sup>10,11</sup>, and thereby contribute distinct and useful properties that have thus far been unrealized in traditional biomaterials. Although individually the non-covalent interactions that give rise to supramolecular materials are weak, the summation and directionality of these interactions leads to materials with mechanical properties that were thought to be uniquely reserved for macromolecules and covalently

crosslinked materials<sup>12</sup>. Supramolecular biomaterials leverage motifs based on supramolecular chemistry in order to produce functional materials that have applications in therapy, diagnostics or devices to advance healthcare. The specific benefits of supramolecular biomaterials arise from the nature of these supramolecular interactions, which confer control over properties in a reversible, highly tunable, dynamic and modular fashion. Supramolecular biomaterials may also enable biomimetic recapitulation of native biological signalling or structural cues<sup>13</sup> (Fig. 1). As such, supramolecular biomaterials provide a diverse toolbox that could help to address important unmet medical needs.

## Synthesis of supramolecular biomaterials

Generally speaking, and on the basis of their supramolecular formation mechanisms, supramolecular biomaterials fall into two broad classes (Fig. 2): materials prepared from one-dimensional (that is, high-aspect-ratio) assemblies of molecular stacking motifs (Box 1), and materials prepared through chain extension of oligomers or through crosslinking of polymeric precursors by specific supramolecular recognition motifs (Box 2). The design of each of these classes of supramolecular biomaterials requires an understanding of the nature of the underlying non-covalent interactions that give rise to different supramolecular motifs. In preparing these materials, a number of specific, directional, non-covalent moieties have been developed. These are based on ordered non-covalent interactions such as hydrogen bonding, metal–ligand coordination, host–guest complexation and electrostatic interactions. Each interaction can be characterized by its equilibrium association constant ( $K_{\text{eq}}$ ) and binding kinetics, which is defined by the rate constants of association ( $k_{\text{a}}$ ) and dissociation ( $k_{\text{d}}$ ). Within every supramolecular material there exists a complex interplay between each of these parameters, as well as their binding mode, so that even when considering the same general non-covalent interaction (such as a hydrogen bond), each of these specific parameters must be considered in order to realize predictable material properties. The mechanisms of interaction that

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**Figure 1 | Supramolecular biomaterials are tunable, modular, responsive and biomimetic, as a result of the specific, dynamic, interchangeable and reversible motifs used in their design. a–d.** Examples of the ways in which the properties of supramolecular biomaterials could interface with a cell (such properties would also provide benefits across a number of other biomedical applications). Tunable nature of the affinity between molecular recognition motifs (a). Modularity enables the ‘mix-and-match’ creation of supramolecular biomaterials with, for example, conserved self-assembling motifs and variable bioactive groups (b). Supramolecular biomaterials can also respond to physiological cues if, for instance, they are engineered with enzyme-degradable segments (c). Supramolecular materials that exhibit structural and/or functional biomimicry can be made by, for example, displaying motifs that can activate receptors on cell surfaces and initiate intracellular signalling cascades (d).

underlie supramolecular motifs often cooperatively combine multiple such interactions, leading to additive implications for the kinetics and thermodynamics of the motifs.

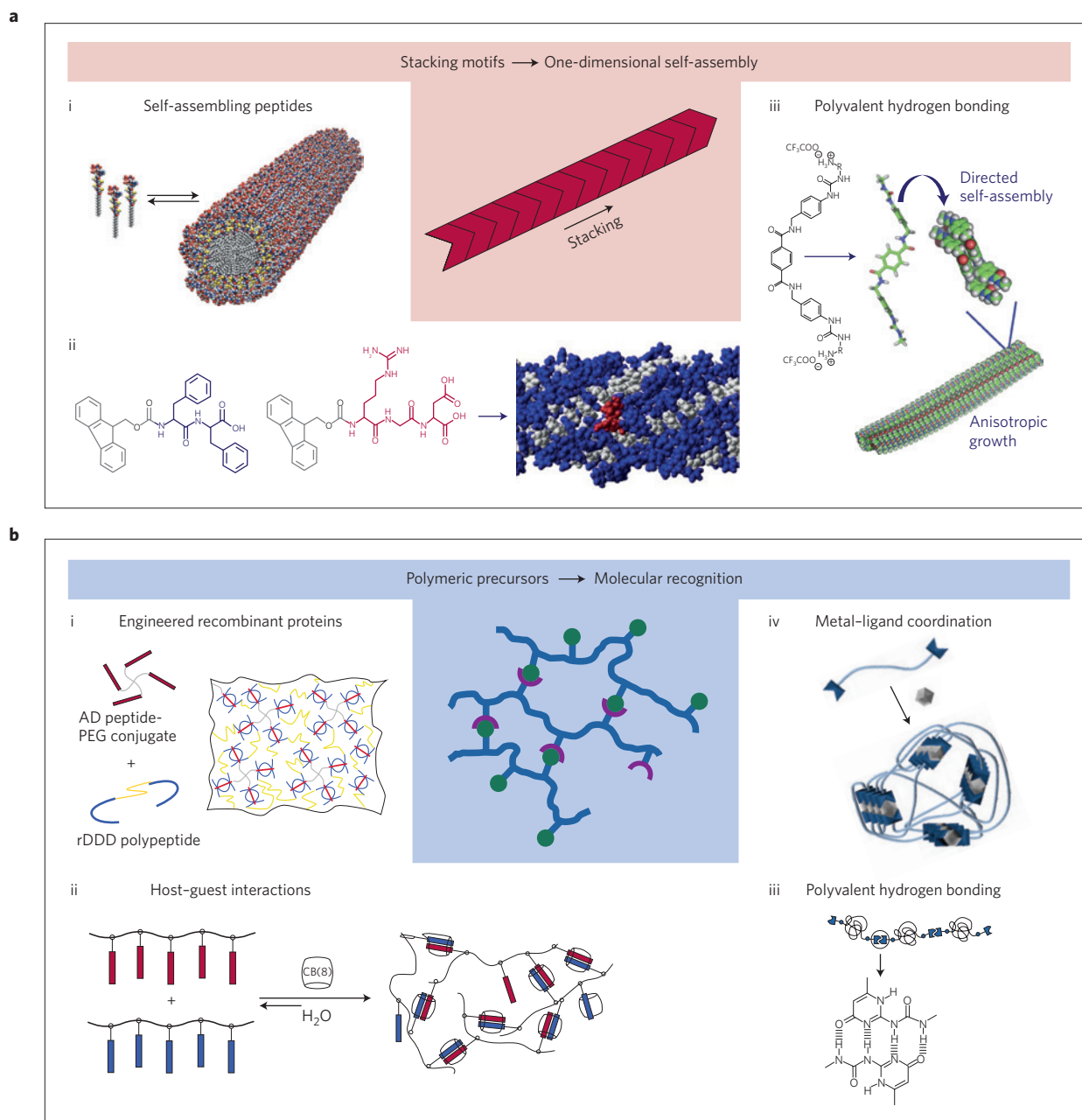
Seminal work in the field of supramolecular biomaterials has used an assortment of self-assembling peptide platforms to demonstrate the broad utility of these materials in biomedical applications<sup>14–16</sup>. Most commonly, self-assembling peptides leverage  $\beta$ -sheet-like hydrogen bonding to create peptidic molecular stacking motifs that form filamentous one-dimensional assemblies, with the axis of hydrogen bonding between molecules parallel to the long axis of these assemblies. At concentrations of the order of ~1% w/w in water, these high-aspect-ratio filaments can physically entangle to form hydrogels<sup>17–23</sup>. Peptide-based small-molecule gelators that leverage  $\beta$ -sheet motifs also include peptides hybridized with non-peptidic structural moieties, such as peptide amphiphiles bearing an alkyl domain<sup>16,24–26</sup>, and oligopeptides modified with small-molecule aromatic groups<sup>27–30</sup>. Hybridization of peptides with alkyl or aromatic groups gives rise to an additional directional driving force for self-assembly through hydrophobic interactions and/or  $\pi$ - $\pi$  conjugation. In particular, peptide amphiphiles have demonstrated particular utility as a modular platform of supramolecular biomaterials, and have enabled pioneering work across a number of different applications<sup>31</sup>. Self-assembling peptides prepared from  $\beta$ -sheet molecular stacking motifs can be further designed with triggers for hydrogelation, including pH, temperature, salt concentration, counterion addition, and the presence of enzymes or other biomolecules. Although molecular stacking via  $\beta$ -sheet formation is the most common way of preparing materials from self-assembling peptides, molecular recognition motifs that leverage other hydrogen-bonding interactions (for example, triple-helical collagen-mimetic interactions<sup>32–34</sup> and coiled-coil or leucine-zipper interactions<sup>35–39</sup>) have also been used to prepare biomaterials from oligopeptide building blocks. Peptide self-assembly provides a number of advantages when used to create biomaterials, including the ease with which discrete self-assembling small molecules can be synthesized, the broad structural diversity afforded by a range of natural and non-natural amino acid building blocks, routes for

natural biodegradation, and the possibility to mimic the structural and functional aspects of native matrix elements.

A different bioinspired strategy to create supramolecular biomaterials has been developed using *de novo* engineering of recombinant protein-derived molecular recognition motifs in order to crosslink polymers or recombinant polypeptides. Early work in this area demonstrated the feasibility of the strategy with designed synthetic proteins incorporating leucine-zipper domains along with non-structured flexible domains<sup>40,41</sup>. More specific protein-based interactions, such as molecular recognition through engineered receptor–ligand motifs, have also been leveraged as crosslinking domains within synthetic polymeric or recombinant protein backbones<sup>42,43</sup>. Although these types of materials have not commonly been discussed in the context of supramolecular materials, the specific, directional, tunable and strong nature of these non-covalent molecular recognition motifs resemble those of materials based on more typical supramolecular chemistry. Approaches based on recombinant engineered proteins benefit from the use of naturally derived building blocks, similar to small-molecule oligopeptide gelators, whereas recombinant production of polypeptides can produce high-molecular-weight building blocks with discrete mass exploiting the same established preparation and purification methods used in the bioengineering of protein therapeutics.

One of the most recognizable motifs in supramolecular chemistry is the macrocyclic host–guest interaction. One such macrocyclic host, cyclodextrin, has a long history as an excipient and solubilizing agent in small-molecule pharmaceutical practice<sup>44</sup>. The non-covalent interaction between host molecules like cyclodextrin or cucurbit[n]uril and small hydrophobic guest molecules has also been used as a recognition motif in order to crosslink polymeric precursors and prepare supramolecular biomaterials. Polymers routinely used as biomaterials, such as hyaluronic acid and polyacrylamide, can be grafted with either a host or its complementary guest molecule to prepare crosslinked hydrogel materials when host- and guest-bearing polymers are mixed, which endows these materials with self-healing and shear-thinning properties<sup>45–47</sup>. In addition to hydrogels, these interactions have been used to prepare nanoparticles<sup>48</sup> and can also be used to direct the assembly of three-dimensional macroscopic building blocks into controllable macro-scale ensembles<sup>49,50</sup>. Interactions between a polymer-grafted host with guest molecules can also enable the non-covalent incorporation of prosthetic groups into a material, which can be swapped with other such groups by selection of guests with stronger binding affinities<sup>51–54</sup>. Cucurbit[8]uril enables a unique strategy with which to prepare materials, as it can bind two separate guest molecules simultaneously and equivalently within its cavity, thus allowing physically crosslinked hydrogels to be prepared by mixing polymers containing pendant guest molecules with freely diffusible cucurbit[8]uril to form a ternary complex<sup>55–57</sup>. The benefits that arise from specificity of the host–guest interaction, and the ability to tune the strength and directionality of this interaction by selection of the guest, enable excellent control when designing supramolecular materials using host–guest binding to facilitate crosslinking.

Another supramolecular strategy for preparing biomaterials relies on specific polyvalent hydrogen-bonding moieties. The use of these moieties enables the preparation of materials that arise from the molecular stacking of small molecules containing these hydrogen-bonding moieties. Alternatively, complimentary or self-complimentary hydrogen-bonding moieties can be installed as crosslinking elements pendant to a polymeric backbone or can facilitate supramolecular polymerization through chain extension of end-functionalized oligomers. One of the first and most-used examples is the ureido-pyrimidinone (UPy) motif, a self-complimentary hydrogen-bonding group that can be synthetically appended to polymers to prepare modular, injectable thermoplastic elastomers for use as biomaterials<sup>58,59</sup> (Fig. 3). The addition of urea units — a



**Figure 2 | Supramolecular biomaterials created through the assembly of molecular stacking motifs and through engineered molecular recognition motifs for the crosslinking of polymeric precursors. a**, Examples of one-dimensional self-assembly enabled by stacking motifs: (i) one-dimensional supramolecular stacking of peptide amphiphiles; (ii) oligopeptides modified with aromatic groups; and (iii) synthetic small molecules with urea groups and aromatic groups that stack through a combination of hydrogen bonding and hydrophobic interactions. **b**, To prepare supramolecular biomaterials from polymeric precursors through molecular recognition, one can use: (i) engineered recombinant proteins leveraging receptor-ligand interactions for crosslinking, such as has been demonstrated with PEG modified with A-kinase anchoring protein (AD peptide-PEG) in combination with a recombinant protein containing a dimerizing and docking domain (rDDD); (ii) polymeric starting materials with pendant guests that facilitate crosslinking through host-guest affinity interactions and ternary-complex formation with cucurbit[8]uril (CB(8)) macrocyclic hosts; (iii) oligomeric precursors that elongate through the chain extension bestowed by terminal hydrogen-bonding moieties; or (iv) coordination of metals with end-terminated ligands to facilitate chain extension of oligomers. Figure reproduced with permission from: **a**(i), ref. 16, AAAS; **a**(ii), ref. 170, Elsevier; **a**(iii), ref. 61, NPG; **b**(i), ref. 42, Elsevier; **b**(ii), ref. 55, American Chemical Society; **b**(iii), ref. 58, NPG; **b**(iv), ref. 73, NPG.

common hydrogen bonding motif — to Uryl-containing supramolecular polymers facilitates orthogonal self-assembly via molecular stacking<sup>60</sup>. Bisurea moieties are also commonly incorporated into molecular-stacking motifs to promote orthogonal hydrogen bonding, leading to the creation of high-aspect-ratio nanostructures<sup>61–63</sup>. Other hydrogen-bonding motifs, including derivatives of benzenetricarboxamides<sup>64,65</sup> and guanines<sup>66</sup>, may be incorporated

into small molecules or oligomers to promote orthogonal hydrogen bonding and prepare supramolecular hydrogels. Leveraging hydrogen bonding afforded by rationally designed DNA base-pairing has received recent attention for its ability to prepare well-defined materials<sup>67</sup>, and this precision could give rise to unmatched spatial control over presented bioactive signals in supramolecular biomaterials<sup>68</sup>. Hydrogen-bonding motifs have demonstrated broad utility not only

**Box 1 | Supramolecular stacking.**

The assembly of small molecules into one-dimensional supramolecular polymers typically relies on 'double-sided' motifs that are capable of facial association and stacking through a combination of various non-covalent interactions. The resulting filamentous assemblies, which can be thought of as polymers with a large persistence length, indeed capture many of the properties found in traditional polymers. In this mechanism of assembly, the non-covalent interactions from which molecular stacking arises can be controlled either kinetically (where assembly is highly pathway-dependent) or thermodynamically (where assembly is either driven by binding events that are isodesmic — that is, all bonds are of the same type — or cooperative). Often, the processing of these small-molecule moieties can lead to kinetically trapped aggregates — that is, aggregates that exist in a local free-energy minimum that is highly dependent on the aggregation pathway. In many cases, an external trigger can be exploited to nucleate the aggregation process in a controlled manner. For example, assembly can be triggered by changes in pH (where alteration of the solubility of the gelator is modulated around the  $pK_a$  of acidic or basic groups in the molecule), solvent polarity (where the solubility of the gelator is much higher in a water-miscible organic solvent than in water) or salt concentration (which can reduce the effects of electrostatic repulsion from charged groups on the gelator by reducing the Debye screening length). Moreover, enzymes capable of activating a stable pro-gelator species in order to trigger the formation of aggregates have been used as biocatalytic triggers for the fuelled assembly of small molecules. In contrast, some molecular-stacking motifs follow assembly pathways that are governed by thermodynamic equilibrium. These assemblies follow either an isodesmic polymerization mechanism marked by a linear increase in the molecular weight with increasing concentration, or a cooperative growth mechanism marked by drastic increases in apparent molecular weight of the aggregate after an initial nucleation step. In isodesmic polymerization, the degree of polymerization can be calculated directly from Carothers' equation for covalent step-growth polymerizations ( $DP = 1/(1 - \rho)$ ), where the conversion  $\rho$  is easily determined from the  $K_{eq}$  at a given concentration. It is important to consider pathway complexity in the cooperative nucleation–elongation mechanism. As almost all highly ordered filaments form through this mechanism, one must maintain an awareness of the large diversity in the structures obtained as a result of the complex energy landscape of the self-assembly process.

for facilitating the molecular stacking of discrete small molecules, but also for promoting chain extension or crosslinking of polymeric precursors through molecular recognition.

One final strategy that has been explored to prepare supramolecular materials is the incorporation of metal–ligand coordination bonds in order to crosslink polymeric precursors or create supramolecular polymers by chain extension of oligomers. Multiarm polymeric precursors terminated with metal-binding groups, such as histidine or catechol, can form hydrogels when mixed with metal ions, including  $Zn^{2+}$ ,  $Fe^{2+}$  or  $Fe^{3+}$ , among others<sup>69,70</sup>. Imidazole groups have also been grafted as side-chains of synthetic polymers for coordination with  $Zn^{2+}$ , leading to self-healing materials<sup>71</sup>. Ligands with enhanced specificity and tighter binding to metals have also been used to prepare self-healing, multistimuli-responsive supramolecular materials<sup>72,73</sup>. Although methods to prepare biomaterials based on metal–ligand coordination have been the least-explored of the material categories outlined here, they offer considerable potential for using predictable and strong interactions with biologically relevant ion species in order to prepare responsive and tunable supramolecular materials.

The assembly of small-molecule-stacking motifs is governed primarily by molecular structure and architecture. The resulting assembly can be described in part by using concepts from molecular packing theory. As an example, a peptide terminated with an alkyl or aromatic group has a molecular architecture that is similar to that of a surfactant. Like surfactants, those with a tapered shape are more likely to form structures with a high degree of interfacial curvature than those with a more cylindrical shape, which are more prone to form structures with a flat interface. Additionally, for these types of molecules the geometry of their aggregates is a result of the interplay of the various interactions incorporated in the molecular design. In the case of small-molecule gelators prepared from the fusion of hydrogen-bonding and hydrophobic domains (for example, a peptide amphiphile), the ratio of the relative interaction strengths in these two structural domains could be modified to alter the geometry of the resulting nanostructure. In the case where the hydrophobic interactions are dominant, spherical micelles of finite size are likely to result through a closed association pathway. However, in the case where hydrogen bonding dominates, the molecules would follow an open association pathway, leading to step growth of one-dimensional assemblies. In their typical manifestation, both forces contribute to the resulting geometry, and modulation of the interplay between these forces can direct the geometry of the assemblies.

One-dimensional nanostructures can further entangle to form hydrogels, yet the interactions that give rise to hydrogel formation are not usually supramolecular. Hydrogel formation requires a network of fibres, whereby interactions between fibres (for example, fibre bundling) arise either from their entanglement, branching or chemical crosslinking (for example, through addition of multivalent cations to form salt bridges). Furthermore, bundle properties (dimensions and stiffness) are critical parameters for the mechanical properties of the resulting hydrogels. These systems often mimic the chain bundling that is commonly observed for cytoskeletal filaments. In each of these cases, the crosslinks between fibres are also non-covalent, which highlights the reversibility of both polymerization along the fibres and crosslinking between them. The mechanical properties of these hydrogels thus depend on several factors, including the mechanical properties of the fibres themselves, the degree of fibre branching and the type and strength of the crosslinking between fibres.

**Benefits of supramolecular biomaterials**

Because of their specific, tunable and reversible character, supramolecular interactions confer a number of unique properties on materials across various length scales, from the nano- to the macroscopic. The properties outlined below (modularity, mechanical tunability, responsiveness and biomimicry) highlight some of the specific utility and benefits of supramolecular biomaterials over traditional biomaterials.

**Modularity.** Supramolecular interactions are highly specific, which allows systems to be created by conjugating a diverse array of functionalities without disrupting the self-assembly mechanism, kinetics or thermodynamics. This feature allows for the development of modular and generalizable material platforms, amenable to simple 'mix-and-match' assembly without significant alteration of the basic material properties. The modularity of supramolecular materials — a feature of materials based on both stacking motifs and polymeric crosslinking motifs — allows for precise control over material composition, functionality and bioactivity, while also enabling signals or targeting groups to be 'multiplexed'. To take a mix-and-match

**Box 2 | Supramolecular chain extension and/or crosslinking.**

Generally, polymeric materials derived from polymeric precursors take advantage of moieties appended to polymer chains that assemble via an isodesmic mechanism of molecular recognition. Within these materials, the thermodynamic equilibrium constant and concentration both directly affect the degree of association of a motif, and the binding kinetics governs the dynamic nature of these interactions. In these materials, supramolecular motifs generally assemble via either self-complementary (A:A) or complementary (A:B) interactions. When these motifs are conjugated to the ends of a polymer chain, their association leads to supramolecular chain extension and thus to large increases in apparent polymer molecular weight. Alternatively, when these motifs are pendant from a polymer chain, where there are many moieties per chain, their association generally leads to supramolecular crosslinking and network formation.

Consideration of the binding mode for a selected supramolecular motif is particularly important in the design and eventual properties of crosslinked supramolecular materials. A system that employs complementary binding motifs bound to separate polymer chains would generally promote interchain crosslinking, leading to stronger materials, whereas self-complementary binding motifs promote the formation of intrachain rings. The often-used quadruple hydrogen-bonding unit 2-ureido-4-[1H]pyrimidone (UPy) is notable because it is capable of self-complementary dimerization ( $K_{\text{dim}} = 6 \times 10^7 \text{ M}^{-1}$ ), yet can also undergo tautomerization (the migration of a hydrogen atom and the switch of a single bond with an adjacent double bond) to selectively form a complementary binding pair with 2,7-diamido-1,8-naphthyridine (NaPy;  $K_{\text{eq}} = 5 \times 10^6 \text{ M}^{-1}$ ). In the case of some metal–ligand binding pairs, such as metal–terpyridine interactions, whereby two moieties of the same type are complexed through a single metal ion (A:B:A), the supramolecular pair is complementary; however, the binding is effectively self-complementary with respect to the polymer backbone. The specific assembly mechanism of selected supramolecular crosslinking motifs therefore plays an important role in determining the behaviour and properties of the resulting supramolecular polymeric materials.

In these systems, whether utilizing supramolecular chain extension or crosslinking, the degree to which a supramolecular motif is associated (that is, the proportion of a species that is bound) is

strongly affected by both its concentration,  $c$ , and its equilibrium constant, and in general is proportional to  $\sqrt{(K_{\text{eq}} c)}$ . For example, hydrogel formation occurs at a specific ‘gelation transition’, whereby a contiguous network is formed from the evolution of a percolated structure at or above a critical concentration of associated moieties. This quality is particularly useful in the *a priori* tuning of crosslink densities in supramolecular polymeric hydrogels, which is achievable provided both the  $K_{\text{eq}}$  and the concentration of the associating moieties are known. In contrast, crosslinking in covalent systems typically can only be determined after the crosslinking reactions have taken place.

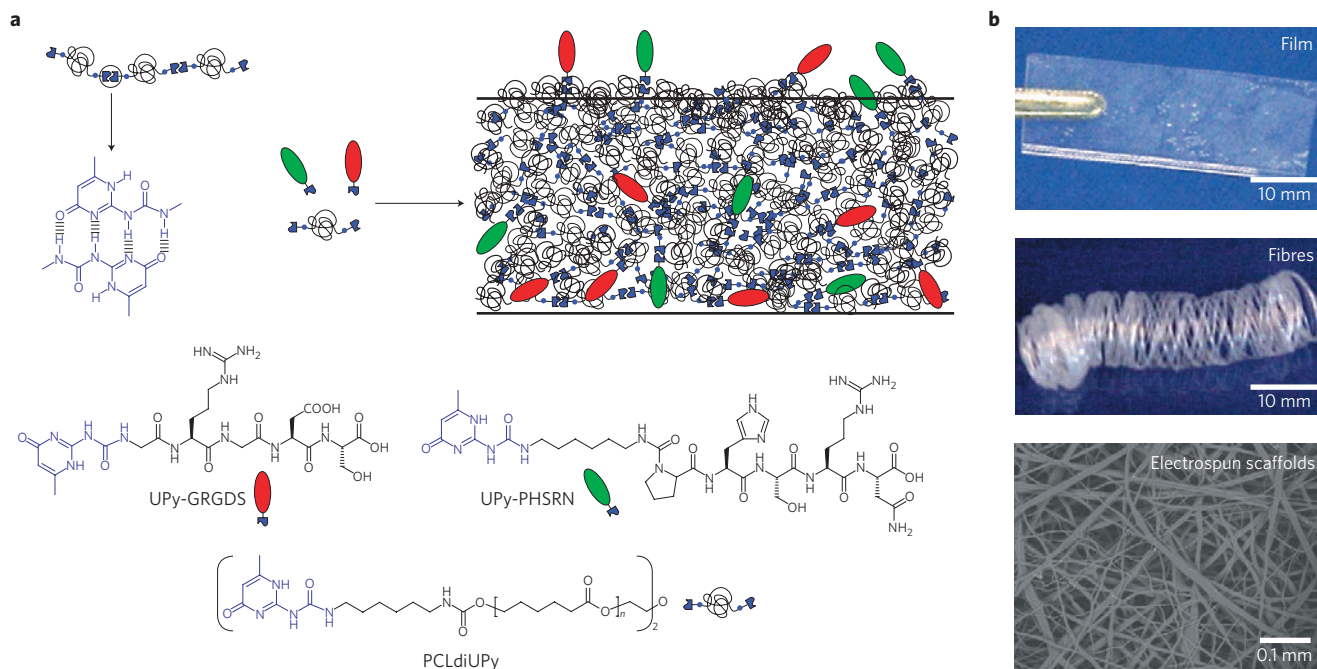
The timescale and dynamics of binding are another important characteristic of supramolecular materials. The interactions used to prepare these materials are inherently reversible — breaking and recombining on experimental timescales — and this interchange, as well as the lifetime of an associated complex, can have a large impact on the overall mechanical behaviour of the material. For example, some metal–ligand coordination bonds have extremely slow exchange kinetics (with lifetimes of the order of decades), and the corresponding materials exhibit macroscopic properties that are essentially equivalent to those of their covalent counterparts. Moreover, the activation energies for the associative and dissociative processes are important. Both Arrhenius theory and Eyring theory relate reaction rates of association and dissociation to temperature, thus providing important insight into processes such as the Gibbs free energy and enthalpy/entropy of activation. Building a comprehensive understanding of the role of binding dynamics and thermodynamics in the governing of the properties of the resulting material is currently underway, and should contribute to the development of new materials.

In addition to the multifaceted nature of supramolecular motifs, parameters that govern the properties of traditional polymeric materials — which typically include polymer molecular weight, polymer persistence length, extent of hydration and component concentration — also come into play in the design of supramolecular polymeric materials. An in-depth understanding of both supramolecular motifs and polymer physics is essential for the rational design and assembly, through supramolecular interactions, of polymeric systems with predictable macroscopic properties.

approach, one important consideration is to maintain the nature of the supramolecular motif (that is, all molecules should have the same equilibrium constant  $K_{\text{eq}}$ ), with structural modifications only implemented away from this motif. This method to multiplex activity in a biomaterial has been leveraged for targeted nanoparticles<sup>52,74,75</sup>, two-dimensional substrates<sup>51,76</sup> and hydrogels<sup>47</sup>. In each case, modularity from the supramolecular design brings about the opportunity of facile modification with diverse targeting ligands or bioactive epitopes by simply altering component formulation ratios. This approach has also been extended to the assembly of large proteins, directed by peptide-linked  $\beta$ -sheet motifs that stack into one-dimensional fibres<sup>77</sup> (Fig. 4). Similarly, the self-assembly of components at interfaces, including stacking systems based on peptide amphiphiles<sup>78</sup> and polymeric systems exploiting Upy<sup>60,79,80</sup> or cucurbit[8]uril-based<sup>81</sup> interactions, have been leveraged to build modular systems of hierarchically structured macroscopic materials and capsules for a broad range of applications (Fig. 5). Furthermore, stable radical species for electron paramagnetic resonance spectroscopy could be appended to a supramolecular building block; doping these groups into normal assemblies at various ratios allows for direct observation of the dynamics within a supramolecular

material without significant alteration of the self-assembly process<sup>25</sup>. The modular display of signals can also be modified as a function of time, as they can be installed through cleavable or degradable modules<sup>82</sup>, or swapped for different bioactive groups through the tuning of binding affinities<sup>51,60,79,80</sup>. It is important to note that the dynamic, non-covalent incorporation of bioactive components into materials, despite greatly benefiting from inherent modularity, is subject to chemical equilibrium and thus a small portion of the bioactive components remain inevitably unincorporated and free in solution. Because the presence of freely diffusible active entities may hinder applications in some cases, the supramolecular interactions used to design modular biomaterials must be carefully considered in the context of application-specific requirements.

**Mechanical tunability.** The classical network theory of polymeric gels provides strategies for tuning the mechanical properties of covalent polymeric materials in various ways, including modulation of the density or distance between crosslinks along a polymer chain<sup>83</sup>. These concepts can be translated to the design of supramolecular polymers, where the concentration of self-assembling units or the extent to which a polymeric backbone is functionalized with a



**Figure 3 | Modular bioactive supramolecular materials.** **a**, The self-complementary hydrogen-bonding UPy moiety can create supramolecular polymers capable of modular incorporation of UPy-functionalized bioactive signals (green and red moieties). For these materials, two UPy-functionalized peptides (UPy-GRGDS and UPy-PHSRN) were presented within a material based on UPy-functionalized polycaprolactone with UPy-units on either end of the oligomer, PCLdiUPy. **b**, Constructs prepared from these modular materials include films, fibres and electrospun scaffolds. Figure reproduced with permission from ref. 58, NPG.

molecular recognition motif can be easily tuned to modulate the properties of the resulting material. In addition, the rational design of specific molecular recognition motifs that leverage multifarious and synergistic supramolecular interactions in polymeric systems also enables control over the macroscopic properties of the resulting material<sup>11,56,84,85</sup>. Supramolecular interactions are inherently dynamic and are bound on specific timescales related to the rate of association/dissociation of the motifs. The interchange between active and inactive interactions, as well as the lifetime of a bound complex, can have a large impact on the overall behaviour of the resulting material, regardless of whether they arise from the stacking of supramolecular motifs or from interchain molecular recognition. Hence, the molecular weight of the polymer (or effective length of a one-dimensional assembly), valency and spacing of interacting units, binding mode for the interaction, and the thermodynamics and kinetics of binding interactions, all contribute to the mechanical properties of supramolecular materials (Boxes 1 and 2). Because these factors can be controlled in part through the rational design and selection of recognition motifs, supramolecular biomaterials have exceptional potential for tunability.

The properties that arise from the dynamic and reversible nature of supramolecular interactions could prove particularly useful for biomaterial applications, such as the generation of shear-thinning and self-healing<sup>22,42,46,70,71,86</sup> gels that enable minimally invasive implantation in the body via injection or catheter delivery. The self-healing character of supramolecular materials is the result of the controlled and tunable dynamics of the reversible bonding used to build these materials<sup>13</sup>. The molecular structure of the building blocks for supramolecular materials can also be altered, which can directly lead to changes in macroscopic properties. The rheological properties of hydrogels prepared from peptide-based supramolecular interactions can, for example, be tuned by altering the  $\beta$ -sheet propensity of the amino acid sequence<sup>87</sup>. Moreover, in polymeric systems, the component ratios of recombinant supramolecular protein assemblies<sup>88</sup>, the chemical structure of guest moieties in host-guest pairs<sup>56,89</sup>, or

either the ligand or metal in metal-ligand systems<sup>84,85</sup>, can be altered to tune macroscopic material properties. The tunability of mechanical properties has been exploited to modulate hydrogel erosion rate<sup>41</sup> (an important feature in a number of biological applications) as well as hydrogel strength<sup>90</sup>. Moreover, it is possible to build systems that exhibit material properties and behaviour comparable to a natural counterpart, where the modulation of cohesive forces can be leveraged to alter cytocompatibility<sup>91</sup>, or assemblies can be aligned to give rise to anisotropy for materials with long-range directionality<sup>92,93</sup> (Fig. 5). The specificity of supramolecular interactions also allows for facile incorporation of orthogonal chemistries leading to secondary crosslinking, which can impart new properties, including covalent crosslinking to stabilize polymeric materials that are templated by supramolecular interactions<sup>94,95</sup>.

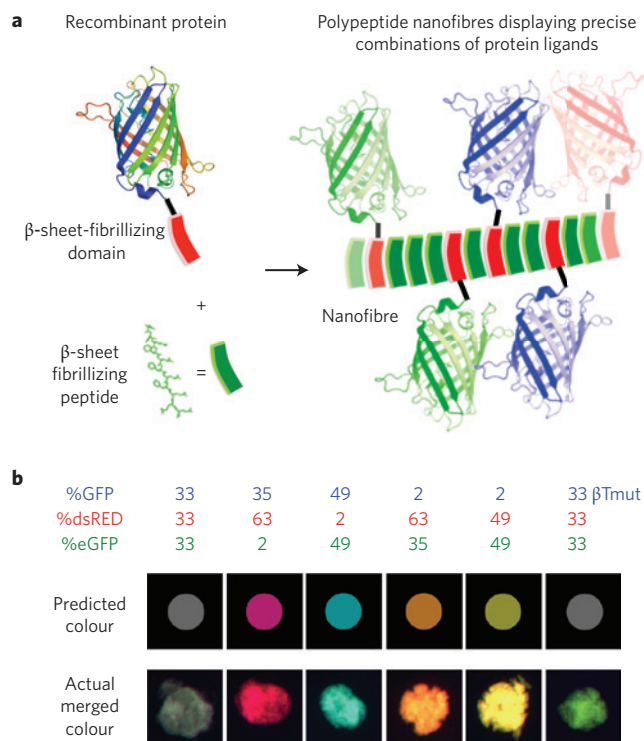
**Responsiveness and dynamic reciprocity.** Because of the dynamic nature of non-covalent interactions, supramolecular materials can rapidly respond to multifarious external stimuli, thereby recreating aspects of the dynamics present in living systems. Stimuli that have been used to control assembly include physical cues (such as temperature, light, voltage or magnetic field), chemical cues (such as pH, ionic strength, redox agent or competitive host-guest interactions) and biological cues (from enzymes or proteins, for example). Such responsiveness can be leveraged to engineer supramolecular biomaterials that allow for the modulation of properties in real time in response to externally applied stimuli, or that are capable of autonomously sensing and responding to environmental stimuli, thus ultimately yielding smarter therapeutics. Towards this goal, reversible enzymatic switches<sup>96-98</sup> and protease-responsive units<sup>99-103</sup> have been exploited to realize self-assembling systems that are under external biological control, whereby enzymes govern whether an oligopeptide is in a form amenable to one-dimensional molecular stacking. Many of the dynamic processes in living systems are regulated by complex and interdependent molecular-assembly and molecular-disassembly events, which are

often controlled by the manipulation of competing catalytic pathways under both thermodynamic and kinetic control. Recently described systems have used biocatalytic pathway selection between competing catalysis and hydrolysis reactions to drive assembly/disassembly in self-assembling peptide materials<sup>102,104</sup>. Similarly, the sequence-specific ‘self-sorting’ of peptide gelators in response to a slow decrease in pH enables the formation of higher-order, complex structures in a controlled manner<sup>105</sup>. Moreover, functional supramolecular components reversibly cluster in response to a multivalent binding partner (single-stranded DNA), thus enabling spatiotemporal control over the distribution of functional groups<sup>106</sup>. Responsive microcapsules and nanoparticles, prepared from supramolecular building blocks, have also demonstrated the triggered release of encapsulated payloads in response to an environmental stimulus<sup>79,81</sup>. The dynamic and responsive properties inherent to supramolecular systems could, therefore, greatly improve both the sensing and responsiveness of therapies.

**Biomimicry.** Biological systems are exceptionally complex, and there remains a great need to develop synthetic materials capable of recapitulating the structural and functional complexity of biological materials in order to create truly mimetic systems for enhanced therapeutic function. Supramolecular biomaterials can replicate aspects of structural and/or functional features of biological signal transduction. This is especially true for materials prepared from peptide or protein building blocks, whether assembled through molecular stacking or from polymeric precursors, as these systems are already prepared from the same amino acid coding elements used in native biological signalling. Biomaterials that can replace or recapitulate deficient native materials or signalling pathways could be especially useful for applications in regenerative medicine or tissue engineering. As synthetic scaffolds, supramolecular biomaterials can act as structural mimics of fibrous matrix components. For example, materials that can replicate the structure or function of native collagen (the most abundant component of native extracellular matrix) could be useful in the preparation of replacement cell scaffolds or in replicating the role of collagen in the nucleation of aligned biomineralization<sup>16,32,33</sup>. Functional biomimicry of protein signalling can be achieved through controlled display of bioactive epitopes — often protein-derived signalling sequences — on supramolecular biomaterials. This strategy promotes the selective differentiation of progenitor cells<sup>107</sup> and can be used to present specific cell-adhesion cues at defined densities<sup>108</sup>, or to mimic potent mitogenic proteins<sup>109</sup>. Some materials can simultaneously provide both structural and functional biomimicry, such as recombinant protein materials that combine elastin-like properties fused with functional cell-adhesive cues<sup>110–112</sup>.

### Applications of supramolecular biomaterials

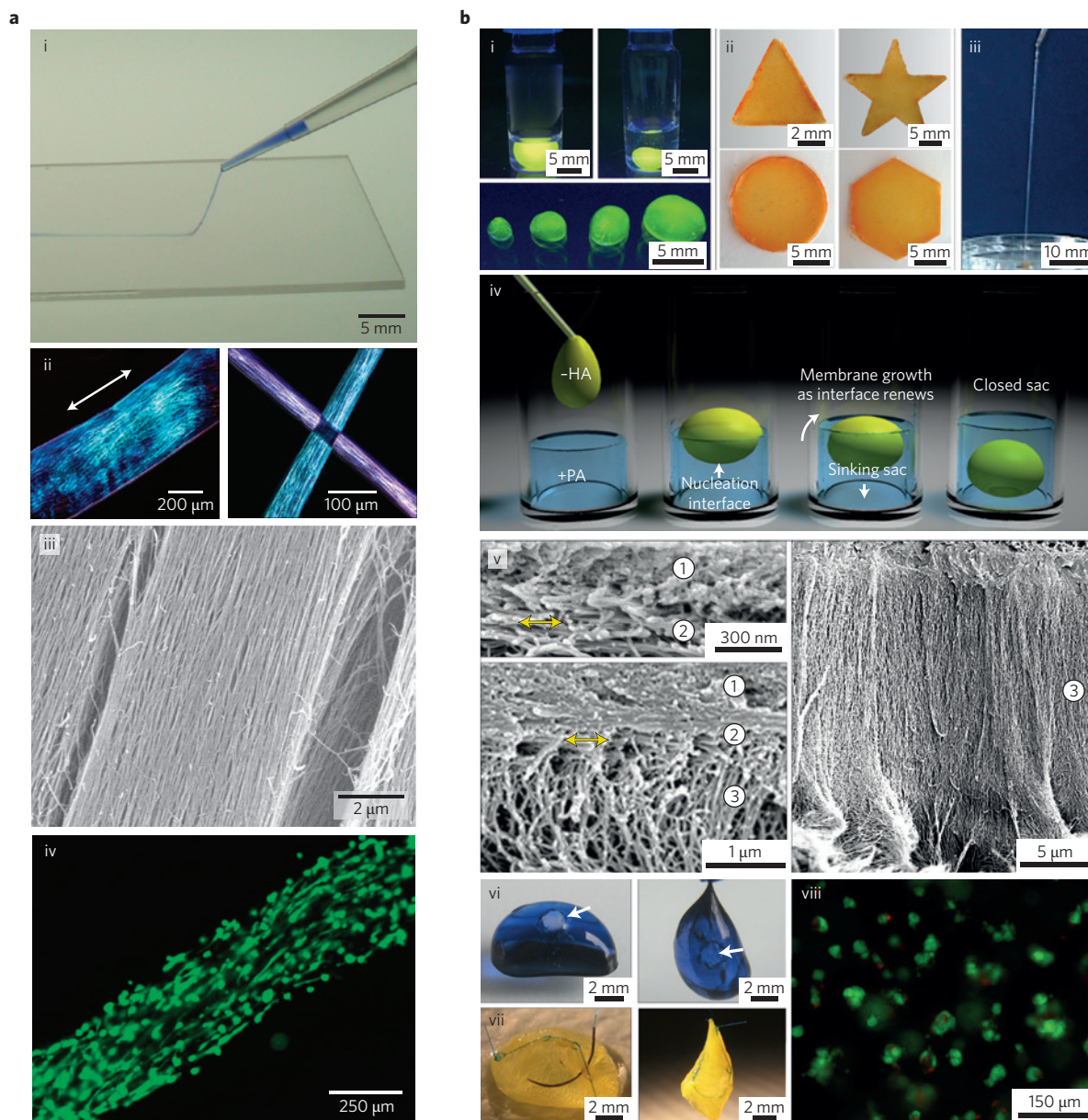
Examples of clinical implementation of supramolecular biomaterials are still limited. Yet many studies performed either *in vitro* or in small animal models have shown that these materials could function as possible therapeutics. The application of supramolecular biomaterials to date can be broadly categorized into four main areas: (1) drug delivery, where materials promote the solubilization, trafficking and/or controlled release of small-molecule pharmaceuticals, bioactive proteins or other therapeutically relevant payload, with specific applications in cancer therapy; (2) engineered cell micro-environments, where materials act as substrates or three-dimensional matrices to promote survival and function or to provide control of phenotype for therapeutic cell populations; (3) regenerative medicine, where materials provide structural or functional cues that promote repair, regeneration or healing of damaged, diseased or defective tissues or organs; and (4) immuno-engineering, where materials can be used to reprogramme the host immune system in order to induce immunity or promote tolerance. In each of these



**Figure 4 | Recombinant-protein supramolecular nanostructures and hydrogels.** **a**, Recombinant proteins (in this case fluorescent proteins) fused with a  $\beta$ -sheet-fibrillizing domain, when combined with short  $\beta$ -sheet-fibrillizing oligopeptides, enable the creation of self-assembling peptide nanostructures with the graded and ratiometric incorporation of multiple proteins. **b**, Ratiometric mixing of different fluorescent proteins (green fluorescent protein, GFP; red fluorescent protein, dsRed; enhanced GFP, eGFP) results in graded colouring of the final hydrogel. In the case where the  $\beta$ -sheet-fibrillizing fusion domain is mutated ( $\beta$ Tmut), the observed hydrogel colour does not match the predicted colour, which indicates that the  $\beta$ -sheet-fibrillizing domain is essential for protein incorporation. Figure reproduced with permission from ref. 77, NPG.

areas, specific features or properties of supramolecular biomaterials can be leveraged to bring about opportunities for new therapies that otherwise would not be possible through traditional approaches for the preparation of biomaterials.

**Drug delivery.** For the controlled delivery of drugs, proteins and other biological payloads, often the structural components that give rise to supramolecular biomaterials can be leveraged to control the release of a drug. For example, drugs can be incorporated as prosthetic groups onto self-assembled peptides through hydrolytic linkages, which facilitate sustained and localized drug release upon hydrogelation<sup>113</sup>. Peptides hybridized with aliphatic chains can also encapsulate poorly soluble drugs within their hydrophobic core, thus serving as efficient drug carriers<sup>114</sup>. A number of strategies have also tuned the strength of supramolecular interactions to control release kinetics directly, in particular by modulating the strength or dynamics of the specific supramolecular interactions that give rise to the material itself. For example, hydrogels prepared through macrocyclic host-guest complexation, when used to encapsulate and control the release of model drugs or proteins, exhibit release rates that can be easily tuned by altering the dynamics of the host-guest interaction through selection of the guest<sup>89,115</sup>; alternatively, the release of biomolecules from materials prepared by molecular recognition of engineered recombinant proteins can be tuned by modifying the erosion rate of the hydrogel<sup>42,94</sup>. Other approaches involve the



**Figure 5 | Supramolecular interactions can be harnessed to make supramolecular biomaterials with hierarchically organized structures. a**, Thermal annealing of a  $\beta$ -sheet-forming peptide amphiphile induces anisotropy in solutions of peptide gelator molecules. When these solutions are subjected to modest shear while simultaneously exposed to counterions that facilitate gelation, as can be achieved by extrusion from a pipette (i), massively aligned bundles of nanofibres can be created as observed through polarized light microscopy (ii; arrow indicates axis of alignment) and scanning electron microscopy (iii), thus enabling the preparation of aligned hydrogels that can encapsulate cells and direct their orientation (iv). **b**, Interfacial complexation of a self-assembling peptide with a high-molecular-weight biopolymer can produce a variety of morphologies, such as liquid-containing sacs of various sizes (i), structured membranes with controlled shape (ii) and strings (iii). To achieve this, a solution of the biopolymer (such as hyaluronic acid, HA) is dropped into a viscous solution of a peptide amphiphile (PA; iv) so that a structured membrane results at the interface of complexation. Scanning electron microscopy (v) of this interface shows an amorphous layer on the side of the biopolymer (1), a region of parallel alignment at the biopolymer-peptide-amphiphile complexation (2) and a region of perpendicular fibres that grow from this interface over time (3). This assembly mechanism enables the formation of enclosed sacs that are self-healing (vi; arrow indicates the site of a defect), can be sutured (vii) and can encapsulate cells (viii). Figure reproduced with permission from: **a**, ref. 92, NPG; **b**, ref. 78, AAAS.

use of properties of the specific drug as a driving force for supramolecular assembly; this was done, for example, in the preparation of a self-assembling amphiphile through the covalent attachment, by means of a degradable linker, of a hydrophobic drug to a hydrophilic  $\beta$ -sheet fibrillizing domain<sup>116</sup>. Also, the drug or bioactive payload could itself be modified to contain a molecular recognition motif; this was done, for instance, in the creation of recombinant therapeutic proteins fused with engineered protein recognition domains<sup>117</sup>.

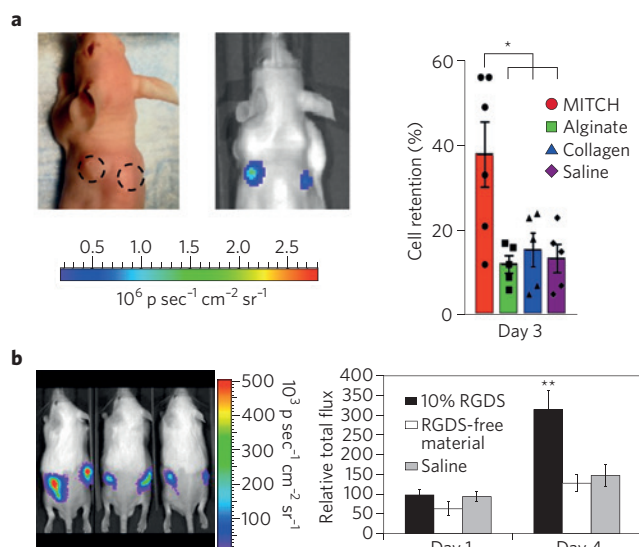
Another strategy for delivering protein drugs involves the presentation of a specific binding site at high densities on the supramolecular material, as exemplified by the incorporation and presentation of heparin to facilitate specific binding to growth factors<sup>118</sup>, and the presentation of phage-display-derived binding sequences<sup>119</sup>.

Cancer is one area where supramolecular biomaterials have been applied both in the context of canonical drug-delivery principles and more recently in creative therapeutic approaches. Supramolecular



materials based on host–guest interactions could provide many opportunities for the delivery of chemotherapeutics or other poorly soluble drugs carried within excess macrocyclic portals<sup>120</sup>. Host–guest interactions can also be used to install modular prosthetic groups for targeting, as demonstrated in human clinical trials for siRNA delivery to solid tumours using cyclodextrin-containing polymeric nanoparticles endowed with adamantyl-linked targeting groups<sup>75</sup>. Supramolecular materials with hydrophobic domains can act as carriers to solubilize chemotherapeutics<sup>121</sup>, and the encapsulation of camptothecin within peptide nanofibres has demonstrated significant inhibition of tumour growth in a mouse model<sup>122</sup>. Supramolecular building blocks could also double as anticancer signals, thus enabling a new therapeutic axis for supramolecular materials. For example, the high-density display of a cytotoxic peptide sequence on a supramolecular peptide nanofibre promoted significantly increased cancer-cell death compared with the free cytotoxic peptide alone<sup>123</sup>. This same sequence, when combined with a PEG-bearing module to facilitate improved circulation time, significantly inhibited tumour growth in a mouse xenograft model without the addition of drugs<sup>124</sup>. Enzymes produced by cancer cells can be used as a trigger to control supramolecular assembly<sup>125</sup>, for example by inducing a conformational change in nanostructure that causes the release of an encapsulated chemotherapeutic in response to a cancer enzyme<sup>96,126</sup>, or through the catalytic induction of assembly from small-molecule precursors in order to inhibit cell growth by promoting gelation of the extracellular environment<sup>103,127</sup>. Supramolecular peptide nanofibres may also contribute to cancer-cell death by acting intracellularly in a prion-like role and impeding cytoskeletal dynamics<sup>128,129</sup>; this phenomenon was demonstrated to inhibit tumour growth in a concentration-dependent manner when self-assembling peptide precursors were injected into the peritumoral space<sup>130</sup>.

**Engineered cell microenvironments.** Using supramolecular principles to design scaffolds for the support and delivery of cells enables the creation of modular artificial matrices with tunable bioactivity, mechanics and material form. The reversibility of supramolecular interactions could be of particular use in creating cell scaffolds that can be administered through minimally invasive routes and then establish a preferential microenvironmental niche for therapeutic stem or progenitor cells. Technologies that have demonstrated utility in this regard are typically three-dimensional matrices prepared from peptides or engineered proteins, as the supramolecular building blocks for these materials can also act as bioactive elements<sup>43,108,131</sup>. The presentation of epitopes for integrin adhesion is a strategy that is often used to provide cell-adhesive cues to supramolecular biomaterials<sup>38</sup>. The fibronectin-derived RGDS adhesion epitope, for example, has been incorporated into recombinant engineered proteins to facilitate enhanced support of induced pluripotent stem cells or adipose-derived stem cells (Fig. 6)<sup>132,133</sup>. Modular supramolecular biomaterials prepared through molecular stacking facilitate tunable and high-density presentation of RGDS, along with cell-specific optimization of RGDS density, leading to injectable materials that can retain and support therapeutic cells within a target tissue<sup>134</sup>. In addition to adhesion cues, modular supramolecular materials can present a variety of other static or soluble signals that direct cell differentiation and cell phenotype. For example, high-density presentation of a laminin-derived epitope on the surface of a supramolecular peptide nanofibre promotes selective differentiation of neural progenitor cells into neurons<sup>107</sup>, and dual-stage availability of growth factors from a recombinant protein hydrogel promotes adipogenesis of encapsulated progenitor cells<sup>135</sup>. Another approach used supramolecular materials produced from assembled peptides presenting saccharides on their surface in order to prepare a polyglycan-mimetic artificial matrix that facilitated the growth, expansion and zygote formation of embryonic stem



**Figure 6 | Supramolecular materials can be used as injectable scaffolds that support the survival of therapeutic cell populations.** **a**, Recombinant-protein materials (labelled MITCH) significantly improve the support and survival of transplanted cells *in vivo*, as evidenced by a significant increase in the signal (when compared with alginate, collagen or saline) arising from transplanted luminescent cells when implanted within these materials *in vivo*. Data presented as mean  $\pm$  s.e.m., normalized to day 1;  $n = 5$  or 6;  $*p < 0.0001$ . **b**, Self-assembling peptide materials presenting the fibronectin-derived RGDS sequence support the survival and proliferation of bone-marrow-derived progenitor cells in comparison to an RGDS-free control and saline, as shown by the signal arising from the transplantation of luminescent cells within a wild-type mouse. Data presented as mean  $\pm$  s.e.m.;  $n = 11$ –15;  $**p < 0.01$ . Left, 10% RGDS; middle, RGDS-free material; right, saline. The colour scales in **a** and **b** show the radiance. Figure reproduced with permission from: **a**, ref. 133, Wiley; **b**, ref. 134, Elsevier.

cells<sup>136</sup>. Microscale features of supramolecular biomaterials, which can often be imparted through printing or fabrication techniques, could also be leveraged to direct the fate or phenotype of encapsulated cells. For example, massively aligned self-assembling peptide bundles facilitate directional cell migration and aligned outgrowth of neurites when neural progenitor cells are encapsulated<sup>137</sup>.

**Regenerative medicine.** Studies primarily performed in preclinical animal models have shown that supramolecular biomaterials can regenerate a variety of tissues and organs. A number of different therapeutic modalities have been explored by using materials as scaffolds to direct endogenous tissue repair<sup>138–140</sup>, deliver soluble growth factors or drugs<sup>141–147</sup>, mimic potent signalling proteins<sup>109</sup>, deliver therapeutic cell populations<sup>148,149</sup> or reconstitute functional tissue<sup>150</sup>. Regeneration of the central nervous system represents a particular challenge because this tissue type is known to have limited endogenous regenerative capacity. Strategies for neural regeneration have included the use of injectable supramolecular peptides to promote neural reconnection following injury. Promising results have been demonstrated in mouse models, with vision restored through promotion of axon elongation after severing of the optic nerve<sup>138</sup>. High-density presentation of a pro-neurogenic bioactive sequence was also successful at restoring hind-limb motor function in a mouse with spinal cord injury by facilitating axon elongation and reducing glial scar formation<sup>139</sup>. The use of aligned peptide scaffolds in combination with encapsulated mitogenic proteins has led to the regeneration of peripheral nervous tissue, restoring erectile function in a rat model of a crush injury to the cavernous nerve<sup>151</sup>. The treatment of diseases

such as Alzheimer's, Parkinson's and multiple sclerosis are pressing areas where supramolecular biomaterials could have broad impact within the nervous system<sup>15</sup>.

Cardiovascular diseases, including ischaemic heart disease, peripheral arterial disease, stroke and non-healing wounds, represent some of the most burdensome health challenges in the world today. The hallmarks of many emerging therapeutic strategies for the treatment of cardiovascular diseases comprise the promotion of the growth of new blood vessels (angiogenesis) and the regeneration of damaged or necrotic tissue. Supramolecular peptide nanofibres that present a high density of heparin on their surface have been used as injectable hydrogels that could bind and deliver angiogenic growth factors. This strategy led to improvements in cardiac function following induction of an acute infarct in a mouse model, and to the growth of new blood vessels in a rat model of peripheral ischaemia<sup>143</sup>. In addition, the controlled delivery of angiogenic growth factors from UPy-based materials promoted cardiac regeneration in a chronic infarct pig model<sup>142</sup>. Inspired by the use of angiogenic growth factors, a minimalist approach achieved functional biomimicry of an angiogenic growth factor through the display of mimetic sequences on the surface of a peptide nanofibre<sup>109</sup>. This approach restored function and reduced necrosis in a model of peripheral ischaemia, a result that was only achieved through high-density display of the mimetic sequence on supramolecular nanofibres. In the context of autologous cell therapy, supramolecular peptides have been used as scaffolds to deliver therapeutic cell populations to ischaemic tissue, resulting in enhanced blood perfusion and limb function in a mouse ischaemic hind-limb model, as well as in enhanced cardiac regeneration in a myocardial infarct model<sup>148,149</sup>. In each case, the injectable supramolecular peptide facilitated improved functional performance of the therapeutic cells and contributed to stabilization in the harsh ischaemic environment. Another promising approach involves the use of supramolecular biomaterials to prepare engineered heart valves or vascular grafts (their clinical evaluation through surgical implantation into human paediatric patients is presently underway<sup>152</sup>). Supramolecular biomaterials could therefore be used to augment and potentiate growth factor or cell-based strategies for cardiovascular regenerative medicine, and could even contribute to the reconstitution of functional cardiovascular tissue.

Supramolecular strategies have also been used to promote the regeneration of hard tissues, such as bones and teeth. The use of primarily soft hydrogel-based materials in these tissue sites represents a departure from traditional strategies, which have historically used rigid metal or ceramic constructs. Supramolecular biomaterials have instead been used as inductive materials for pro-healing functions or the delivery of growth factors that stimulate endogenous tissue regeneration. For example, the delivery of bone morphogenetic protein-2 (BMP-2) from peptide-nanofibre gels displaying a BMP-2 binding sequence was used as a low-dose strategy for spinal fusion in a rat model<sup>119</sup>. Heparin-presenting peptide nanofibres as part of a composite material with a collagen scaffold for enhanced mechanical integrity have also been used to deliver BMP-2, and were found to bridge critically sized femoral defects in rats<sup>144</sup>. Bioactive peptide scaffolds have also induced stem-cell-based regeneration of dental pulp<sup>141</sup>. A different bioactive peptide scaffold promoted *de novo* production of enamel — the hardest tissue in the body — in ectopic tissue sites when used within the transplant bed for a mouse incisor<sup>140</sup>. Osteoarthritis, another orthopaedic target that has become increasingly important, was reversed in a rabbit cartilage defect model through the use of a supramolecular peptide hydrogel that presented binding sequences for a potent growth factor<sup>145</sup>. The self-healing properties of supramolecular materials have also been used to fuse a pro-osteogenic supramolecular hydrogel with a pro-chondrogenic supramolecular hydrogel, for application in regeneration of the osteochondral junction<sup>153</sup>. The use of soft, injectable

materials that are pro-healing represents a change of paradigm for orthopaedic and dental biomaterials. Moreover, leveraging the bioactivity brought about by rationally designed supramolecular materials could contribute to significantly improved regeneration in hard tissues.

**Immuno-engineering.** There has been recent interest in developing therapies to modulate the immune system, for example in prophylactic vaccination or in the reduction of inflammation and autoimmunity. Supramolecular systems could contribute in this regard, through both soluble drug delivery and controlled presentation and delivery of immunomodulating signals. For example, the controlled delivery of cytokine IL-10 from engineered recombinant protein hydrogels led to significant anti-inflammatory protective effects in a model of chronic obstructive nephropathy<sup>146</sup>. Supramolecular peptide assemblies, when used for the presentation of T- and B-cell epitopes, can have potent adjuvant properties, leading to both cellular and humoral immune responses to the presented epitopes<sup>154–156</sup>. Such a potent immune response arises specifically from high-density epitope display on otherwise non-immunogenic supramolecular peptide fibrils, and obviates the need for a traditional adjuvant in order to promote a robust immune response. This concept can be extended to the display of natively folded whole-protein antigens by using a supramolecular fibrillizing domain as a template, leading to a robust immune response to the displayed antigen, strictly by virtue of its supramolecular presentation<sup>77,157</sup>.

### The future of supramolecular biomaterials

In coming years, the advancement of supramolecular biomaterials will probably leverage new methods in supramolecular chemistry to improve the precision of supramolecular structures, leading to more defined or highly controlled biomaterials. One vision for the future of supramolecular biomaterials would seek to more closely mimic both the structural and functional aspects of native extracellular matrix. Evidence from studies in mechanobiology point to an expanded understanding of the role of matrix mechanics in governing the function and phenotype of cells<sup>158</sup>. In addition, the native matrix provides spatiotemporal control of bioactivity, chemical composition and mechanical properties as tissues develop or cells mature. The exposure of cryptic sites in native collagen through specific enzymatic cues<sup>159</sup>, for example, inspires efforts toward matrices that exhibit dynamic reciprocity — that is, able not only to sense biologic cues but also to respond by modulating both its mechanical and biological properties. Truly mimicking this complexity will entail materials that are 'smarter' than those available to date, and will require advances that go beyond the simple presentation of cell-adhesive epitopes or the modulation of mechanical properties. Achieving truly dynamic reciprocity may only be possible by relying on the same dynamic non-covalent interactions that are present in natural matrix materials — that is, by using supramolecular principles. Success in the creation of bioactive materials would enable the preparation of more highly functional surrogate matrices, which, when coupled with advancements in stem cell biology, could lead to great improvements in stem cell therapy. It is noteworthy that the majority of supramolecular biomaterials evaluated so far have been soft materials with relatively fast erosion rates, and are furthermore often degraded or cleared after a few weeks *in vivo*<sup>160</sup>. It may be desirable for biomaterials used as surrogate cell matrices, and for other applications in regenerative medicine, to achieve longer-lasting function. Efforts for improving the stability of supramolecular materials, perhaps through secondary-covalent-crosslinking methods, could help in this regard.

The use of supramolecular biomaterials in drug delivery and cancer therapy could also benefit from improved nanostructural precision. Nanostructure shape, size and surface chemistry are well-known to govern both circulation behaviour and biodistribution. So

far, many supramolecular materials have used molecular stacking to prepare high-aspect-ratio drug carriers, and although such geometry may benefit from enhanced circulation time<sup>161</sup>, the dramatic length polydispersity inherent to such assemblies could complicate efforts to achieve predictable circulation and biodistribution. It is postulated that the controlled growth or templated assembly of such carriers should lead to more consistent performance *in vivo*<sup>162,163</sup>. Another important area is that of improved strategies for spatial control over therapeutic deployment. Efforts to prepare stimuli-responsive systems, as well as those leveraging biocatalytic control of assembly, could contribute to improved therapeutic efficacy in the delivery of drugs or biologics. Also, supramolecular biomaterials that can locally sense disease state and respond to disease-biomarker triggers could be used to precisely control dosing and limit off-target effects, thus leading to broad-ranging applications such as the detection and treatment of metastatic cancer, inflammatory diseases, atherosclerosis, and a number of other diseases characterized by local changes in the extracellular microenvironment.

As outlined in this Review, the preparation of biomaterials through supramolecular design principles offers many advantages over traditional approaches for material design and synthesis. The supramolecular design of materials could also translate to benefits in their clinical use. Indeed, in combination with developments in genomics, proteomics and related data-driven diagnostic areas, modular supramolecular therapies could contribute to the vision for the future of personalized medicine<sup>164</sup>. Facile modular platforms of discrete building blocks could make possible the engineering of customized therapies with patient- and/or disease-specific tailoring of chemical, biological and mechanical properties. Another benefit would leverage developments in the field of high-throughput screening of materials, as modular supramolecular biomaterials could be mixed ratiometrically to prepare libraries that could be screened for the desired therapeutic effect on a patient- or disease-specific basis<sup>165</sup>. The preparation of biomaterials from rationally designed building blocks also improves predictive efforts for biodegradation products, thus limiting unintended side effects that can arise during material processing in the body and streamlining the toxicology screening of the materials and their degradation by-products. Many supramolecular biomaterials are shear-thinning and self-healing, which enables minimally invasive administration routes, and could thus be translated to work with routine patient-administered methods of delivery. For clinical utility, supramolecular biomaterials must be designed so as to ensure consistent application, predictable properties on deployment in the body and reliable performance with tolerance for the myriad variables possibly encountered when used as therapeutics. Because most of the formation mechanisms for supramolecular materials are kinetically driven, these materials are sensitive to environmental or procedural variables; ensuring consistency within a reasonably expected range of circumstances is critical.

There are a variety of drawbacks and hurdles that must be overcome prior to the broad translation of many supramolecular biomaterials. Strategies to prepare materials from small-molecule precursors often entail rigorous and/or low-yielding synthetic procedures, and improving both the scale and efficiency of these syntheses are important considerations. Supramolecular materials based on peptides must also address issues of cost and scale, although precedents exist for the industrial-scale and economically viable production of peptide drugs<sup>166</sup> (for example, Enfuvirtide — a 36 amino acid anti-HIV drug). The industrial-scale production of polymers synthesized by a variety of methods is effectively routine at this point; however, the attachment of molecular recognition motifs and the subsequent purification step needed to prepare supramolecular building blocks remain to be optimized. Efforts to leverage non-covalent interactions in the preparation of materials have demonstrated the use of low-cost components available

in bulk<sup>167</sup>, and such simplification of material building blocks is another strategy for reducing cost and increasing scale. Besides up-scaling synthesis and manufacturing, and the many material-specific considerations, the future development of supramolecular biomaterials will require methods for non-damaging sterilization and packaging that provides reasonable shelf-life.

As supramolecular biomaterials advance toward clinical use, there are regulatory hurdles that must be overcome. In this regard, the clinical successes of traditional biomaterials offer a possible roadmap for navigating the regulatory process *en route* to clinical use<sup>168,169</sup>. In the context of the US regulatory system at the Food and Drug Administration, many of the supramolecular biomaterials discussed in this Review would have characteristics from many different classes of therapeutics, and thus may have to traverse multiple regulatory pathways simultaneously. The material itself may constitute a medical device, but it might also contain biologic entities, small-molecule drug entities and/or tissue (that is, encapsulated cells). This complicates the regulatory framework and, although precedents exist for tissue-engineering constructs and other combination products, it is likely that many strategies discussed here necessitate case-specific tailoring of the regulatory pathway<sup>4</sup>. Ultimately, ensuring complete safety as well as robust efficacy must be the focus of any such efforts to translate biomaterial-based therapeutics, supramolecular or otherwise. Although we do not expect that the supramolecular materials discussed in this Review will introduce safety considerations that are unique and different from those of other biomaterials, there remains a possibility that, due to material composition or form, complications could arise. Immunogenicity is a potential concern, especially for peptide- or protein-based materials. In light of the dramatic effect seen for the high-density display of immunogenic signals on supramolecular peptides used for vaccination<sup>154–156</sup>, care must be taken to ensure that materials for other applications avoid the selection of predicted B- or T-cell epitopes.

In spite of promising proof-of-concept demonstrations for supramolecular biomaterials, so far there has been limited demonstration of their clinical use. Towards clinical translation, early stage efforts are presently being taken to evaluate a number of strategies, including supramolecular materials for cardiovascular tissue engineering, cyclodextrin-containing nanoparticles for cancer therapy, and self-assembling peptides for regenerative medicine. We hope that these early efforts chart the course for future supramolecular biomaterials to enter clinical practice. However, the path to market for new therapies is sometimes tortuous, and many of these early technologies could fail along the way. Lessons learned from the development of traditional biomaterial platforms suggest that, from concept to realization, this process can take upwards of decades. In the context of this timeframe, the development of supramolecular biomaterials is a relatively nascent endeavour. Technologies that are under development now and in the coming years will therefore be crucial in determining whether the benefits of supramolecular biomaterials can be brought to bear in the clinical setting.

Progress in the development of supramolecular biomaterials over the past decade has established, in principle, their broad utility for the design of highly controllable and highly functional materials for biomedical applications. In the coming years, we expect that an increasing number of strategies based on supramolecular materials will advance into clinical practice, and that these efforts will further establish the utility of the supramolecular toolbox for the treatment of disease. Continued fundamental advancements in the molecular engineering of new supramolecular motifs, as well as functional studies evaluating materials prepared from these motifs, will broaden and deepen the technology available in the supramolecular arsenal. Coupled with expanding efforts in cell biology, high-throughput screening of materials, and data-driven ‘-omics’ fields, we anticipate that the rational design of supramolecular biomaterials will lead to thus-far unrealized therapeutic impact.

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### Competing financial interests

The authors declare no competing financial interests.