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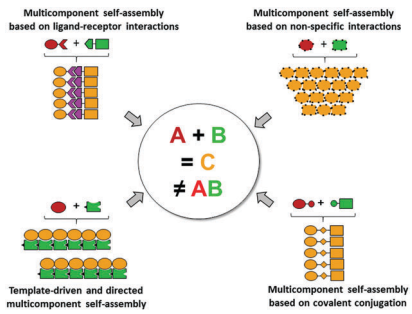

Multicomponent self-assembly as a tool to harness new properties from peptides and proteins in material design

Babatunde Okesola and Alvaro Mata

Nature is enriched with a wide variety of complex, synergistic and highly functional protein-based multicomponent assemblies.

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Multicomponent self-assembly as a tool to harness new properties from peptides and proteins in material design

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Nature is enriched with a wide variety of complex, synergistic and highly functional protein-based multicomponent assemblies. As such, nature has served as a source of inspiration for using multicomponent self-assembly as a platform to create highly ordered, complex and dynamic protein and peptide-based nanostructures. Such an assembly system relies on the initial interaction of distinct individual building blocks leading to the formation of a complex that subsequently assembles into supramolecular architectures. This approach not only serves as a powerful platform for gaining insight into how proteins co-assemble in nature but also offers huge opportunities to harness new properties not inherent in the individual building blocks. In the past decades, various multicomponent self-assembly strategies have been used to extract synergistic properties from proteins and peptides. This review highlights the updates in the field of multicomponent self-assembly of proteins and peptides and summarizes various strategies, including covalent conjugation, specific binding, templated/directed assembly and non-specific co-assembly, for driving the self-assembly of multiple proteins and peptide-based building blocks into functional materials. The use of multicomponent self-assembly as a platform to facilitate the emergence of new properties from the resulting nanostructures has also been discussed. The ultimate goal of this review is to highlight the importance of multicomponent self-assembly in protein and peptide engineering, and to advocate its growth in the fields of materials science and nanotechnology.

Introduction

Peptide and protein-based materials

The last couple of decades have witnessed increasing interest in supramolecular materials.[1] Self-assembling platforms based on non-covalent interactions have not only generated elegant nanostructures, but also advanced our understanding of biological systems.[2] Peptides[3] and proteins[4] have been fundamental to this progress both as a source of inspiration to new molecules and assembling mechanisms and as a rich resource of versatile building blocks. On one hand, proteins have inspired approaches that aim to dissect the information encoded in them and establish design rules for engineering intelligent materials.[5] Some examples include tough materials based on silk,[6] auxetic materials based on RhuA protein lattices,[7] flexible structures based on tropoelastin,[8] dynamic materials based on actin,[9] or bioactive materials based on collagen.[10] Peptides on the other hand provide a simpler structure allowing easier manipulation, a higher degree of control, and predictable assembly. Examples such as aromatic peptides,[11] peptide amphiphiles,[12] self-assembling peptides,[13] hair-pin peptides,[14] or multidomain peptides[15] have been used to generate nanostructures with the capacity to recreate elaborate quaternary structures,[3a,16] promote mineralization,[17] adhesiveness,[18] enzyme-mediated tunability,[19] or cell growth.[20]

Despite the increasing precision with which we are able to engineer these materials using self-assembly strategies, the level of complexity that has been achieved with molecular self-assembly is no way near the highly sophisticated and functional structures found in nature. This is so because in contrast to the generally practiced single component self-assembly, self-assembly found in nature involves the use of multiple distinct building blocks. For example, the proteasomes of yeast *Saccharomyces cerevisiae* result from the assembly of pairs of seven different proteins.[21] Consequently, there is an increasing need to move beyond the structure using single component self-assembly and instead develop systems with enhanced complexity and functionality.[22] The 2016 Nobel Prize in Chemistry awarded to Fraser Stoddart, Jean-Pierre Sauvage, and Ben Feringa for the design and synthesis of molecular machines is an exciting example of such aspiration. A number of groups are engineering increasingly complex molecular materials that enable new functionalities such as self-

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replication,[23] morphogenesis,[24] natural selection,[25] and tuneable mechanical properties.[26] However, the ability to transform molecules into functional macroscopic materials with practical applications remains a major challenge.[22b]

Multicomponent self-assembly in nature and synthetic systems

With this in mind, nature once again offers us guidance. Biological materials acquire most of their structural complexity and functionality as a result of their ability to assemble multiple types of building blocks into defined constructs. Proteins, arguably the most sophisticated building block of organisms,[27] rely on intermolecular interactions with other proteins or molecules to exhibit their remarkable functionality.[1d,28] Examples can be seen in many biological structures from the remarkable toughness of silk emerging from interactions between fibroin and sericin, to the strong and dynamic structures generated by actin and myosin; from the outstanding stiffness of enamel emerging from amelogenin and calcium phosphate interactions, to the precise motion arising from the interplay between kinesin and tubulin. These and many other biological structures attain their functionality from both the inherent properties of their components and their interactions and capacity to form highly ordered structures.[29]

Inspired by these examples, an increasing number of groups are engineering materials whereby peptides and proteins are used as multicomponent ensembles capable of generating new properties as a result of their interactions.[30] In this review, we focus on highlighting such systems, which through the self-assembly of multiple types of building blocks are able to create materials with emergent properties. We define these properties as those that are not present in the individual components but rather emerge from their interaction (Fig. 1). Furthermore, engineering materials in this manner can significantly enhance the diversity of the resulting structures, thus avoiding limitations on the emergence of complexity that homogeneity imposes.[31] In addition, multicomponent self-assembly can generate a wider range of more complex possible structures,[32] offers the possibility to enhance modularity[33] and enables the capacity for temporal control and a higher tuneability of properties.[34]

In this context, it is exciting to think of the possibility to use multicomponent self-assembly as a tool to extract functionalities from peptides and proteins that would not transpire based solely on their individual structure. However, it is important to keep in mind that given the structural and functional differences between these molecules, the thermodynamic and kinetic factors that dominate their assembly are inherently different. Proteins are large chains of amino acids with complex 3D structures. Because of these characteristics, these molecules interact with others through multiple active sites that can be distributed over large areas. Together, these molecular interfaces act in a coordinated manner that orders the protein locally, increases its conformational rigidity, and consequently decreases the local entropy. However, the inherent structural heterogeneity of proteins[35] and difficulties associated with controlling their non-covalent interactions in a reproducible and hierarchical manner[27] have restricted their widespread applicability. On the other hand, peptides are shorter chains with up to around 50 amino acids and with a simpler and more predictable structure. In the context of multicomponent self-assembly, these properties enhance the capacity for programming, enable more precise interactions, and facilitate

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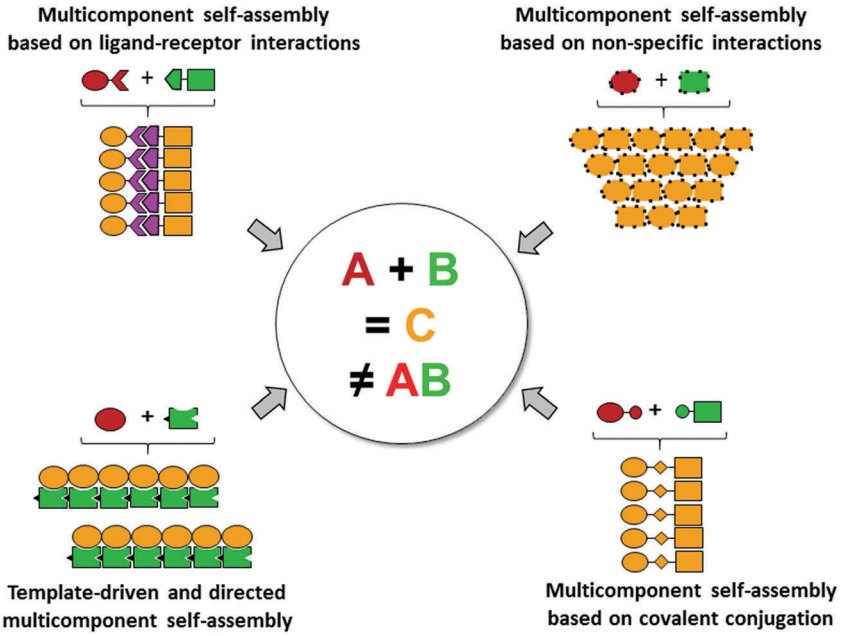

Fig. 1 Multicomponent self-assembly strategies for fabricating protein and peptide-based nanostructures.

entropically favourable processes. Nonetheless, the inherent simplicity of peptides has limited the capacity to control their assembly hierarchically[36] and form macroscopic structures that exhibit functions similar to the biological ones.

In this perspective, we argue that multicomponent self-assembly may offer a new way to build more complex and functional materials. In particular, we focus the discussion on peptide- or protein-containing multicomponent systems that, upon self-assembly, enable the emergence of new properties or phenomena (Table 1). This paper presents the body of work in four sections including (a) multicomponent self-assembly based on covalent conjugation, (b) multicomponent self-assembly based on ligand–receptor interactions, (c) template-driven and directed multicomponent self-assembly, and (d) multicomponent self-assembly based on non-specific supramolecular interactions (Fig. 1). We discuss how these approaches offer different advantages and exhibit distinct limitations, and demonstrate how new properties can emerge from synergistic interactions between different components (Table 2). Nature has evolved in a hierarchical manner through optimization of molecular interactions between multiple components.[5c] Similarly, we propose that developing materials through supramolecular engineering approaches that focus on intermolecular interactions, rather than solely on the individual building block structure, will lead to new discoveries on peptide and protein functions and opportunities for more functional and practical materials.

Multicomponent self-assembly based on covalent conjugation

Developing materials through molecular self-assembly offers outstanding opportunities such as molecular control, "error-editing", and constitutional reshuffling. However, covalent interactions could be used to further complement these properties. For many applications, molecularly defined multicomponent conjugates are particularly desirable because they confer improved stability, solubility, trafficking pathways and functionalities. As such, over the past decades, there has been increasing attention towards developing protein and peptide-based nanostructures with specific shape, size, and heterogeneity using multicomponent self-assembly driven by covalent conjugation. Covalent conjugation can range from irreversible bond formation to a transient and reversible interaction. This section presents how both of these strategies have been used to design multicomponent protein and peptide-based nanostructures with emergent properties such as increased protein activity, proteolytic resistance, and thermal and pH stability.

Irreversible covalent conjugation

While many approaches using irreversible covalent conjugation have focused on fixation[37] or modification[38] of pre-formed supramolecular architectures, numerous efforts have been devoted to "stitching" building blocks together in order to generate robust nanostructures with improved properties. Examples of such properties include enhanced proteolytic resistance as well as mechanical, thermal, and pH stability. In light of this, Luo and Kiick used copper-catalysed cycloaddition chemistry to conjugate an alkyne-functionalized elastin-like peptide (ELP) and an azide-functionalized collagen-like peptide (CLP) leading to self-assembly of the copolymer at different temperatures (Fig. 2b).[39] The synergistic benefits of the self-assembled copolymer include stability of the CLP triple helix at high temperatures (65 °C) due to the anchoring effects imposed by the ELP coacervation and the unexpected self-assembly of the ELP–CLP at very low temperatures as a result of the anchoring effects of the CLP triple helix on the ELP. Another example that demonstrates the capacity of

Table 1 Summary of key examples of multicomponent self-assembled architectures exhibiting new properties that solely rely on the interactions between various components that make up the systems

Building blocks	Mechanism of self assembly	Emergent properties	Ref.
Peptide amphiphiles and elastin-like polypeptides	• Co-assembly based on hydrophobic and electrostatic interactions • Diffusion–reaction process • Compartmentalization	• Multilayer hierarchical structure of nanofibers • Formation of tubular membrane • Dynamic assembly–disassembly process • Self-healable	24
Antibodies-tagged peptide and spyligase	• SpyCatcher–SpyTag interaction	• Polymerizing capacity • High stability to boiling in sodium dodecyl sulfate • Enhanced magnetic cell capture	45
Fusion proteins CsgA-Mfp3 and CsgA-Mfp5	• Coiled coil interaction • Co-assembly of CsgA-Mfp3 and CsgA-Mfp5 • Self-polymerization of amyloidogenic fibril • Beta-strand lamination by lateral stacking	• Hierarchically ordered composite nanofibers and films • Strong wet bonding strength • Enhanced stability to auto-oxidation • Robust mechanical properties • Enhanced intrinsic fluorescence	125
Elastin-like peptide (ELP) and collagen-like peptide (CLP)	• "Click" chemistry • Temperature-switching	• Temperature-dependent dynamic structural formation • Stability of CLP to high temperature in the self-assembled structure	39
Biotinylated tumour-targeting and cell-penetrating peptides	• Avidin–biotin interactions	• Efficient DNA binding, high transfection efficiency, nuclear localization and tumour suppression	64
Enzyme–protein complexes (LDH–PDZ and FDH-PDZ) and their ligands (PDL)	• Receptor–ligand interaction	• Highly ordered 2D multilayer architecture • Multienzyme structures exhibit higher thermal stability, broader pH-tolerance, higher storage stability and efficient catalytic activities	68
Protein kinase A (PKA) and its anchoring protein (AKAP)	• Dock-and-lock interactions • Mixing-induced self-assembly	• Formation of self-assembled hydrogels • Tunable mechanical properties and erosion rate • Resistant to high yield strain • Self-recovery after deformation	69
Coiled coil peptide and double stranded DNA	• Templated self-assembly	• Formation of filamentous architecture	104
SP1 protein and quantum dots	• Electrostatic interactions	• Tunable nanostructures, shapes and sizes • Efficient energy transfer	121
Tyrosine-rich protein and buckminsterfullerene (C_m)	• π–π and hydrophobic interactions	• Formation of hybrid crystal suprastructures • High charge conductance	124

multicomponent self-assembly to generate new properties includes the conjugation of proteins with synthetic polymers.[40] In a seminal work by O'Reilly and co-workers, combination of "click" chemistry and protein engineering was used to synthesize a temperature-responsive bioconjugate system comprising a superfolder green fluorescent protein (sfGFP) and poly[(oligo ethylene glycol) methyl ether methacrylate] (PEGMA) in a site-selective manner.[41] It was observed that the polymer wrapped around the protein in a temperature-dependent manner, leading to highly ordered supramolecular aggregates. However, none of the individual components can access such a level of supramolecular aggregation in their own right. Another discovery of this study is that meticulous site-specific modification of the sfGFP using "click" chemistry enabled preservation of its inherent fluorescence property. For a proper understanding of how "click" chemistry has been used to conjugate peptides, we refer the readers to a review by Tang and Becker.[42]

SpyTag–SpyCatcher chemistry is another strategy that is currently emerging for conjugating proteins and peptides irreversibly.[43] SpyTag is a short polypeptide that spontaneously binds its protein partner (SpyCatcher), leading to the formation of irreversible isopeptide bonds under physiological conditions. SpyTag–SpyCatcher interactions are characterized by autocatalysis, rapid reaction rate, high yield, high thermal stability, mechanical stability, and protease resistance, making it an exciting strategy for designing multiplex protein assemblies and robust protein architectures.[44] Using this approach, Howarth and co-workers reported site-specific polymerization and covalent self-assembly of antibodies or antibodies functionalized with Tag peptides and a protein (spyligase).[45] Interestingly, while neither the SpyLigase nor the Tag peptide could polymerize, multiple binding of "spies" (tag and catcher) leads to assembly into polyantibodies. In addition to this emergent polymerizing capacity, the new product also exhibits high stability to boiling in sodium dodecyl sulfate (SDS) and enhanced magnetic cell capture. By fusing a blue fluorescent protein (mBFP) to the N-terminus of a split-Spy0128 and an enhanced green fluorescent protein (EGFP) to the N- or C-

Table 2 Strategies for designing multicomponent self-assembled nanostructures, their advantages and limitations

Self-assembly strategy	Advantages	Limitations
1a. Multicomponent self-assembly based on covalent conjugation: irreversible covalent conjugation	• Formation of mechanically and thermally robust nanostructures • Resistant to proteolytic degradation • Broad range of reaction toolbox (*e.g.* Michael-addition, click-chemistry, *etc.*) • Suitable for applications under a wide range of conditions	• Formation of thermodynamically unstable architectures • Lack of kinetic control • Irreversible bond formation, lack of error-correction • Requires multistep synthesis and purification • Impairment of functional units of proteins and peptides
1b. Multicomponent self-assembly based on covalent conjugation: reversible covalent conjugation	• Formation of responsible, adaptive and dynamic architecture • Chemically dynamic • Amenable to far-from equilibrium self-assembly process • Error correction possible • Kinetically controllable	• Labile interaction • Limited applications • Nanostructures highly susceptible to premature degradation
2. Multicomponent self-assembly based on ligand–receptor interactions	• Highly specific and selective • Driven my molecular recognition • Directional • Relatively straightforward for nanofabrication of protein/peptide-based nanostructures • Little or no modification required • Biomimetic • Formation of precisely well-defined architectures	• Limited usage • Limited sizes and shapes of nanostructures • Dependent on shapes and sizes of complementary partners • Affected by steric hindrance of receptor aggregates
3. Template-driven and directed multi-component self assembly	• Formation of well-defined structures by design • Possibility of controllable nanostructure geometry and spacing • Possibility for generation of hierarchical structures	• Dependence on properties of the template • The properties of the resulting structure may be affected by those of the template
4. Multicomponent self-assembly based on non-specific supra molecular interactions	• Complementary molecular interactions over large molecular surface • Formation of adaptive, responsive, and tunable structures • Capacity for controlled compartmentalization • Physically dynamic • Straightforward • Thermodynamically and kinetically controllable	Dependence on non-covalent interactions can limit mechanical properties Some of the interactions lack directionality Non-specific

terminus of the isopeptag, Kamiya and co-workers were able to use Förster resonance energy transfer (FRET) to monitor the spatial orientation of the SpyTag–SpyCatcher fluorescent bioconjugate (Fig. 2a).[46] Arnold and co-workers also demonstrated how this modular strategy can be used to design protein topologies through *in situ* post-translational modification upon interaction between the two components.[47] This SpyX module has also been used to unlock properties inherent of spies-mediated protein bioconjugates. In a recent study, Zhang and co-workers used the assembly–reaction synergy to fabricate mechanically interlocked protein configurations using two recombinant proteins (ELP-SpyX and GB1 dimer).[48] Tunable topologies and enhanced stability to enzymatic digestion are some of the properties emerging from using this approach. Due to the inherent modular nature of SpyTag–SpyCatcher interactions, multicomponent and information-rich biomaterials with multiple functions such as cell adhesion and metalloprotease cleavability can also be envisaged.[49]

Genetic engineering represents another strategy for the irreversible covalent conjugation of protein/peptide building blocks to generate complex self-assembled nanostructures. Through this approach, van Hest and co-workers designed a block copolymer of a genetically fused protein comprising ELP and a viral capsid protein derived from the cowpea chlorotic mottle virus (CCMV).[50] Due to the pH responsiveness of the CCMV and thermal responsiveness of the ELP, the fusion protein exhibits two distinct self-assembling mechanisms leading to either large virus-like particles (when exposed to an acidic pH) or nanocapsules (when brought to high temperatures) (Fig. 2d). Again, neither the ELP nor the CCMV exhibits this polymorphic phenomenon but their co-assembly enables it. Similarly, Xia *et al.* demonstrated temperature-dependent self-assembly of a genetically fused resilin-like polypeptide (RLP) and a silk-like polypeptide (SLP) into nanoparticles. These nanostructures are able to show transition from a nanoparticle geometry to microscale fibers and finally into a self-supporting hydrogel in a time-dependent manner (Fig. 2c).[51] Interestingly, these transitions were temperature-independent, which suggests that self-assembly of the copolymer results from the interplay between both the RLP and SLP blocks. In this design, the SLP

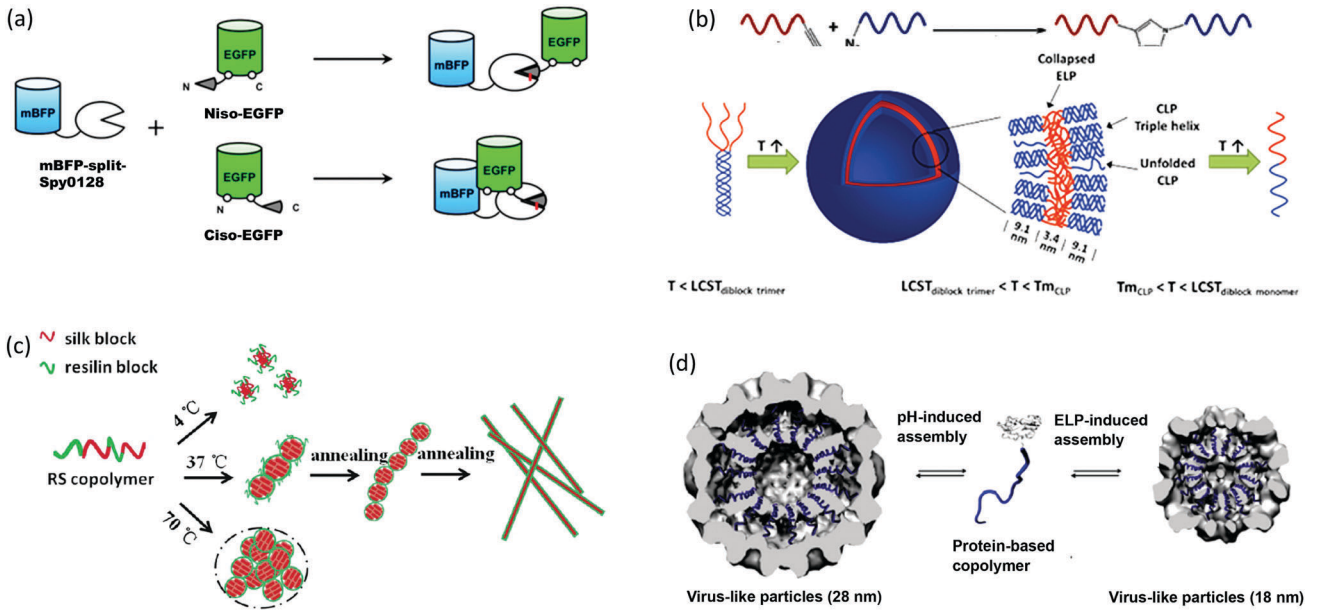

Fig. 2 (a) SpyTag–SpyCatcher interaction between mBFP and EGFP leading to enhanced energy transfer. (b) Click chemistry approach for preparing the ELP–CLP conjugate and subsequent self-assembly in a temperature-dependent manner. (c) Schematic representation of RLP–SLP conjugate self-assembly into nanoparticles and microscale fibres in a time-dependent manner. (d) Schematic representation of ELP–CCMV conjugate self-assembly into two different virus-like particles with different diameters in response to temperature and pH. Adapted with permission from ref. 46, 39, 51 and 50, respectively. Copyright 2013, 2015, 2017 and 2012 American Chemical Society, respectively.

block drives the assembly into nanofibres by β-sheet stacking at a physiological temperature while the RLP drives the assembly into nanoparticles at both low and high temperatures due to its temperature-dependent phase transition.

Enzyme-mediated irreversible covalent conjugation is another widely practised approach for stitching proteins and peptides together. Enzymatically driven self-assembly of peptides and proteins is particularly fascinating under the mild and physiological conditions involved. Enzymatic methods involving *thermolysin*,[52] *transglutaminase*,[53] and *sortase*[54] have been used to conjugate proteins and peptides into hybrid nanostructures that exhibit emergent properties as a result of co-assembly.

It is noteworthy that despite the account of favourable self-assembly driven by irreversible covalent conjugation,[55] formation or cleavage of covalent bonds always attract enormous amount of energy and the associated lack of bond flexibility could limit dynamic properties desirable in specific applications.[56] More so, the products of a self-assembled system should represent a thermodynamic minimum of the system, enabling the possibility for self-correction of errors. This selective reproducibility is the hallmark of self-assembled systems. However, formation of irreversible covalent bonds is thermodynamically unfavourable under normal conditions. In this case, using transient or reversible conjugation could be a better approach towards the self-assembly of proteins/peptides into thermodynamically stable nanostructures void of defects.

Reversible covalent conjugation

Ideally, chemical conjugation has to fulfil certain requirements for the engineering of protein and peptide nanostructures. For example, the newly formed bond should be (i) stable across a broad range of biological environments, (ii) highly selective and specific, *i.e.* no random reaction between two reacting moieties, (iii) small enough to minimize steric hindrance, (iv) bioorthogonal, and (v) reversible on-demand. Reversible covalent chemistry such as hydrazone and imine bond formation, disulfide bridge formation, thioether exchange, and enzyme-mediated transamidation meets some of these parameters. Therefore, these chemistries are the hallmark of constitutional dynamic chemistry (CDC) and directed molecular assembly of a variety of molecules such as polymers, oligomers, peptides, and proteins.[57] Multicomponent systems would particularly benefit from these transient chemical interactions to facilitate the connection and disconnection of the different building blocks in response to specific stimuli.[58] In this way, this approach would enable the creation of nanoarchitectures with dynamic properties. As such, reversible covalent interactions hold great promise for nanofabrication of protein and peptide-based structures with wide chemical diversity and innovative properties.

Hydrazone chemistry has particularly been useful for conjugating biomolecules due to the small size of the bond and thus elicits no perturbation to the native state of the proteins or peptides. Due to the speed of the reaction, hydrazone chemistry can also facilitate rapid conjugation under physiological conditions.[59] In a recent study, Anslyn and co-workers used hydrazone-based dynamic covalent chemistry between aryl aldehyde and acyl hydrazide functionalities to fabricate peptide-based complex quaternary structures by simple mixing of the two components.[60] These complex architectures also exhibit amplified antimicrobial activity.

Unlike hydrazone chemistry, disulfide bridge formation is a naturally occurring approach which can be used to direct the self-assembly of peptides and proteins into nanostructures with defined quaternary structures, sizes, and functions. Due to sulfur redox chemistry, when cysteine-containing proteins or peptides are subjected to oxidation at high pH, the thiol groups form a disulfide bridge, which is reversible upon reduction.[47] Taking advantage of this, a multi-thiol system with multiple disulfide exchanges is able to generate dynamic nanostructures with the capacity to change morphology and self-replicate while exhibiting high stability.[61] Furthermore, through a similar approach, Otto and co-workers reported two macrocycles created through oxidative disulfide formation from a dynamic combinatorial library of peptides with pendant thiol groups.[23b] As expected, these nanostructures exhibit self-replication only when multiple thiols were used. Another reversible covalent conjugation strategy that has attracted a lot of attention is the transamidation reaction.

Given the antithetical functions of enzymes, some proteases have the capacity to reversibly catalyse the formation of the amide bond as well as catalyse the hydrolysis of peptides in aqueous systems. As such, in the past decades, enzyme-mediated transamidation has remained an attractive platform for designing a dynamic combinatorial system capable of generating multicomponent self-assembled structures. In this case, exchange of amide bonds between the constituting building blocks results in an *in situ* generation of new molecules, complex systems, and thermodynamically stable nanostructures.[19,62] For example, Ulijn and co-workers demonstrated that the use of reversible enzyme-catalysed reactions to induce self-assembly can facilitate (i) self-correction-fully reversible self-assembly under thermodynamic control, (ii) component-selection ability to amplify the most stable molecular self-assembly structures in dynamic combinatorial libraries and (iii) spatiotemporal confinement of nucleation and structure growth.[21] In this way, enzyme-mediated self-assembly can provide control in the bottom-up nanofabrication of nanomaterials with a high level of complexities and fewer defects.

The transient nature of reversible covalent conjugation makes it a supramolecular strategy with great potential to engineer robust, yet dynamic, protein and peptide nanostructures. However, despite these advantages, it also suffers from inherent problems associated with covalent conjugation such as the lack of specificity, poor control of molecular modifications, need for multiple synthetic steps and purification of the building blocks, and limited compatibility with living systems due to the use of nasty chemicals.

Multicomponent self-assembly based on ligand–receptor interactions

While covalent conjugation has been elegantly used to fabricate protein and peptide assemblies, doing it in a way that does not affect the intrinsic properties of these molecules is a challenge given their structure–function relationship. To overcome this challenge, a number of groups have taken advantage of the ligand–receptor that peptides and proteins provide in order to design hierarchical architectures that can be assembled with a high degree of molecular precision.[63] Such ligand–receptor interactions including avidin–biotin binding, protein pairing, dock- and lock, carbohydrate–protein, Watson–Crick nucleobase pairing and antibody–antigen are widespread in nature. The macrocyclic host–guest interaction has also been widely exploited as a synthetic alternative to the natural specific binding interactions for driving protein/peptide-based multicomponent self-assembly.

Avidin–biotin mediated self-assembly

Selective interaction between the protein avidin or its homologues and biotin and some of its homologues is one of the most widely used strategies for creating high affinity peptide/protein pairs. Zhang and co-workers designed tumour targeting gene delivery constructs by interacting a pair of biotinylated peptides (CREKA and R8) with avidin (Fig. 3d).[64] In addition to the intrinsic tumour-targeting (CREKA) and cell-penetrating (R8) properties of the individual components, the hybrid nanostructures exhibited efficient DNA binding, high transfection efficiency, nuclear localization, and suppression of tumour growth in both *in vitro* and *in vivo* models. This approach has also been used to engineer supramolecular protein complexes.[65] For example, Kamiya and co-workers demonstrated the use of avidin–biotin interactions to fabricate one-dimensional assemblies of functional proteins.[66] In this case, tetrabiotinylated *endoglucanase* and cellulose-binding module units were self-assembled into cellulosome-like architectures by mixing them with streptavidin. Interestingly, the co-assembly of these components enabled tunable breakdown of cellulose (saccharification) by varying the ratio of the protein units. Despite the versatility of the avidin–biotin complex, avidin is a tetrameric receptor and the majority of its applications are limited to a maximum valency of four, restricting the size and complexity of the resulting molecular assemblies. To overcome this, orthogonal self-assembling approaches involving avidin–biotin and SpyTag–SpyCatcher interactions (SpyAvidin hub) can offer a promising alternative to the use of avidin–biotin interactions alone.[67]

Protein or peptide pairing interactions

High affinity interactions between proteins and other biomolecules (peptides, saccharides, and nucleotides) can be used to design functional multicomponent systems. Using this ligand–receptor approach, Wei and co-workers developed multicomponent enzyme–protein complexes with redox properties able to catalyse the synthesis of *tert*-leucine.[68] In this case, a protein (PDZ) derived from the metazoan cells and its complementary ligand (PDL) were genetically fused with *leucine dehydrogenase* (LDH) and *formate dehydrogenase* (FDH). LDH is an enzyme that catalyses oxidation of NADH to NAD^+ while FDH is another enzyme that catalyses regeneration of NADH. Due to the interaction between PDZ and PDL, the protein–enzyme complexes (LDH–PDZ and FDH–PDL) were assembled

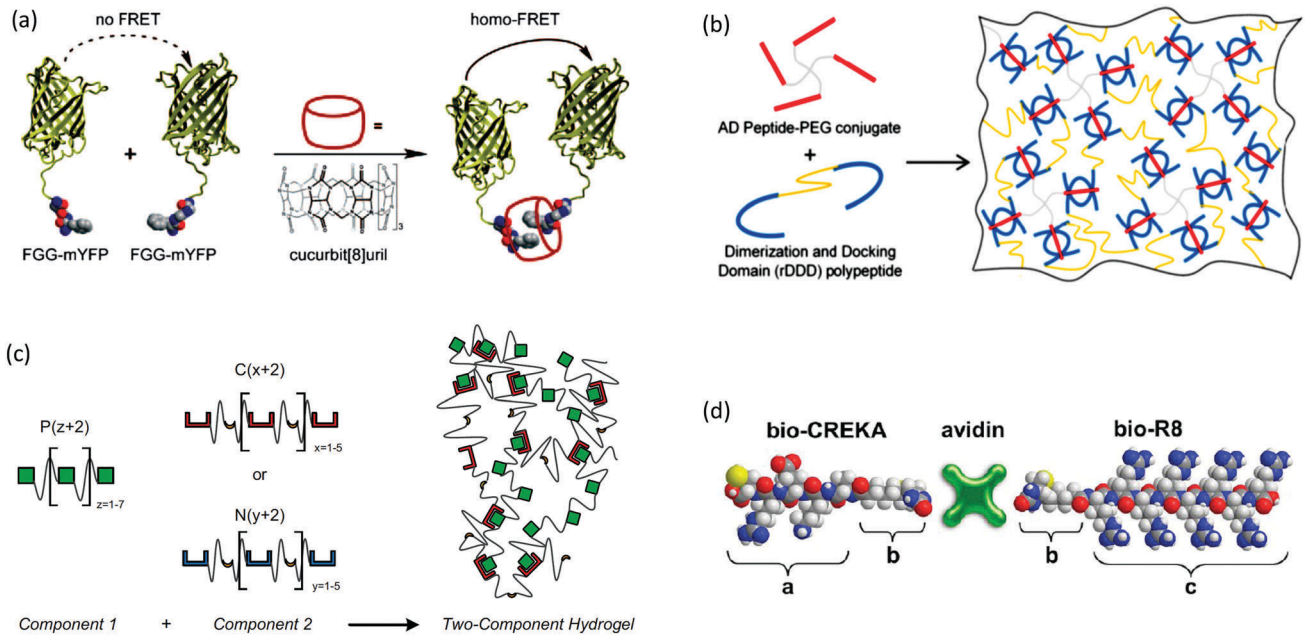

Fig. 3 (a) CB[8]–FGG interactions induced protein heterodimerization with enhanced FRET. Adapted with permission from ref. 81. Copyright 2009 Wiley-VCH Verlag GmbH & Co. KGaA. (b) Co-assembly of docked protein kinase A (PKA) and PEGylated A-kinase protein in hydrogel fibres. Adapted with permission from ref. 70. Copyright 2012 Elsevier. (c) Mixing-induced two-component co-assembly based on the interaction between the WW domain and the proline-rich peptide. Adapted with permission from ref. 75. Copyright 2009 National Academy of Sciences. (d) Self-assembly of tumour-targeting (CREKA) and cell-penetrating (R8) peptides facilitated by the avidin–biotin interaction. Adapted with permission from ref. 64. Copyright 2013 American Chemical Society.

into highly ordered 2D structures with enhanced properties compared to its component enzymes. These emergent properties include enhanced thermal stability, broader pH-tolerance, higher storage stability, and higher efficient catalytic recycling of NADH.

Dimerization and docking of protein kinase A (PKA) and the anchoring A-kinase protein (AKAP) have served as inspiration for designing self-assembled multivalent nanostructures.[69] Burdick and colleagues harnessed the natural dock-and-lock mechanism of PKA and AKAP to prepare hydrogels by mixing the two components at physiological pH and temperature (Fig. 3b).[70] Some of the emergent properties of this two-component hydrogel include tunable mechanical properties and erosion rate, resistance to high yield strain, and self-recovery after deformation. Parameters such as the AKAP peptide sequence, the concentration and ratio of each component, and the number of peptides on the cross-linking polymer were used to facilitate this tunability.

Assembly between proteins/peptides and polysaccharides represents another class of ligand–receptor interaction. This approach offers a unique opportunity for designing extracellular matrix (ECM)-mimetic scaffolds without impairing bioactivities of the components, which makes it suitable for developing self-assembled matrices for growth factor delivery[71] and for tuning the viscoelastic properties of hydrogels.[72] Protein pairing interactions also enable the incorporation of inorganic components to create directed complex colloidal assemblies.[73] Complementary interactions between a WW domain and proline-rich peptide is another biorecognition strategy with high binding affinity and potential exploitation in multicomponent self-assembly. The WW domain is a naturally occurring anti-parallel β-sheet forming peptide with two highly conserved tryptophan residues.[74] Consequently, Heilshorn and co-workers employed this specific interaction to develop a mixing-induced two-component hydrogel (MITCH) suitable for cell encapsulation and delivery of growth factors (Fig. 3c).[75] Like any other non-covalent interaction, this protein–peptide binding interaction is transient and, as such, results in a hydrogel with shear-thinning, self-healing and injectable properties. Interestingly, because the association is normally found intracellularly, the interactions are highly strong and are not disrupted by the presence of additional molecules in the environment.

Macrocyclic host–guest interactions

Host–guest interactions represent a synthetic alternative to natural specific recognitions owing to their selectivity, high binding affinity, reversibility, and responsiveness. Synthetic macrocyclic molecules such as cyclodextrin (CD) and cucurbit[n]uril (CB[*n*]) has been widely exploited over the last decades as host molecules for non-natural supramolecular complexation. Integrating synthetic host–guest molecules with proteins and peptides can enhance the control of co-assembly and functionality of the resulting materials such as facilitating protein recognition,[76] signaling regulation,[77] amyloid inhibition,[78] phase transfer[79] and protein assembly.[80] In a pioneering study by Brunsveld and co-workers, the strong host–guest interaction between β-cyclodextrin (β-CD) and

lithocholic acid (LA) was used to induce dimerization of two sets of cyan (CFP) and yellow (YFP) fluorescent proteins into heterodimers with strong binding affinity, an enhanced FRET effect, and the possibility to be reversed in a stepwise fashion (Fig. 3a).[81] The ''pumpkin-shaped'' CB[8], which is capable of forming ternary complexes with its complementary ligands such as the tripeptide phenylalanine–glycine–glycine (FGG) motif, is another strategy that has been used to trigger the formation of self-assembled protein–protein complexes[82] and nanomaterials[80b,83] with new properties. In general, host–guest interactions can serve as an additional link between the co-assembled components, aiding the native weak protein–protein interactions and generating stronger assemblies.

Other approaches

As demonstrated in these examples, the use of peptides and proteins within self-assembling systems allows the generation of diverse nanostructures with flexible molecular composition, architectural versatility, and structural robustness and complexity. The possibility to use tools based on specific recognition and directional interactions facilitates programmable self-assembly and control over the material properties. Considering the specificity and directionality of Watson–Crick nucleobase pairing, hybridizing the intrinsic properties of peptides/proteins and nucleobases could be a useful strategy for designing novel multicomponent nanostructures.[84] Furthermore, antibody–antigen interactions hold great promise as a platform for directing supramolecular protein/peptides assemblies.[85]

Template-driven and directed multicomponent self-assembly

While specific interactions can be used to aid the design of self-assembled structures, this approach provides limited capacity to guide assembly beyond the molecular scale. The use of predetermined ordered structures that serve as templates can be an efficient and controllable strategy for assembling protein/peptide building blocks into complex hierarchical architectures. Using a defined framework for templating can equally enable the growth of protein and peptide nanomaterials at predefined positions on the templates. This approach can eliminate the need for post-assembly manipulation and enhance connectivity or precise spacing within the nanostructure, which are essential features of opto/electronic devices[86] and biological processes.[87] Templated self-assembly is usually driven by initial specific recognition (as discussed in the previous section) between protein/peptide building blocks and well-defined templates followed by nucleation and statistical growth.[88] This process is a form of directed self-assembly and is reminiscent of natural biomineralization[89] and tunnelling nanotube-templated nucleation and propagation of prions.[90] In the past decades, nanoparticles, polymers, supramolecular structures, and DNA have been used as templates to guide self-assembly of proteins and peptides.

Metal nanoparticle templates

Metal nanoparticles are attractive templates for guiding the self-assembly of proteins and peptides because of their inherent electronic properties, large surface areas, and high aspect ratio. These properties make them amenable to different chemical functionalization with for example ligands having different surface charges, which can promote multiple binding and consequently guide subsequent assembling steps.[91] Among numerous possible metal nanoparticles, gold nanoparticles (AuNPs) have attracted a lot of attention as a template for protein and peptide self-assembly. Hayashi and co-workers pre-functionalized gold nanoparticles with ligands which were further covalently conjugated with heme moieties.[92] By harnessing the heme–heme pocket specific interactions, dendrimer-like supramolecular architectures with clusters of AuNPs were fabricated. In contrast, rather than using this protein specific interaction to guide assembly, Dragnea and co-workers synthesized highly homogenous, symmetric, and ordered virus-like particles (VLPs) using nanoparticle templates. Here, an initial electrostatic interaction is used to coat PEG-functionalized AuNPs with brome mosaic virus (BMV) proteins, which together serve as templates capable of guiding subsequent crystallization through protein–protein interactions.[93] The VLPs exhibit icosahedral packing and pH-induced swelling transition, and emergent properties that resemble those of the native viruses and which are not observed in the individual components. In a later study, the authors demonstrated how the diameter of these nanoparticle–protein templates can be used to control the structure of the capsid crystals.[94] Wang and co-workers also employed AuNPs functionalized with metal-chelating structures bearing Ni–NTA chelates to hierarchically self-assemble protein nanocages into a discrete nanostructure.[95] In general, nanoparticles can assume different shapes and sizes by design and, as such, they remain amazing templates for engineering protein and peptide nanostructures in a scalable manner.

Polymer-based templates

Polymer-based nanostructures represent another class of templates for guiding the self-assembly of proteins. Polymers remain a universal structure-building platform because they can be designed with different molecular sizes, diverse topologies, and with a variety of pendant chemical functionalities. As such, the use of polymer-based templates can facilitate control over the structure and function in hierarchical assemblies. This is a particularly attractive approach given the challenge to control the complex structure of proteins. In light of this, Wooley and co-workers synthesized nanoparticulate copolymers based on biotinylated poly(acrylic acid)-*b*-poly(methylacrylate) (PAA-*b*-PMA) and non-biotinylated PAA-*b*-PMA nanoparticles through co-micellization.[96] The nanoparticles were used to facilitate a dense assembly of avidin-based nanostructures in a controlled manner. A further step towards actualizing the emergence of new properties with the use of a polymer-based template is the use of multivalent precursors. Multivalency is a powerful tool nature uses to facilitate high-affinity molecular recognition, particularly in the biological system.[97] Therefore, complex and

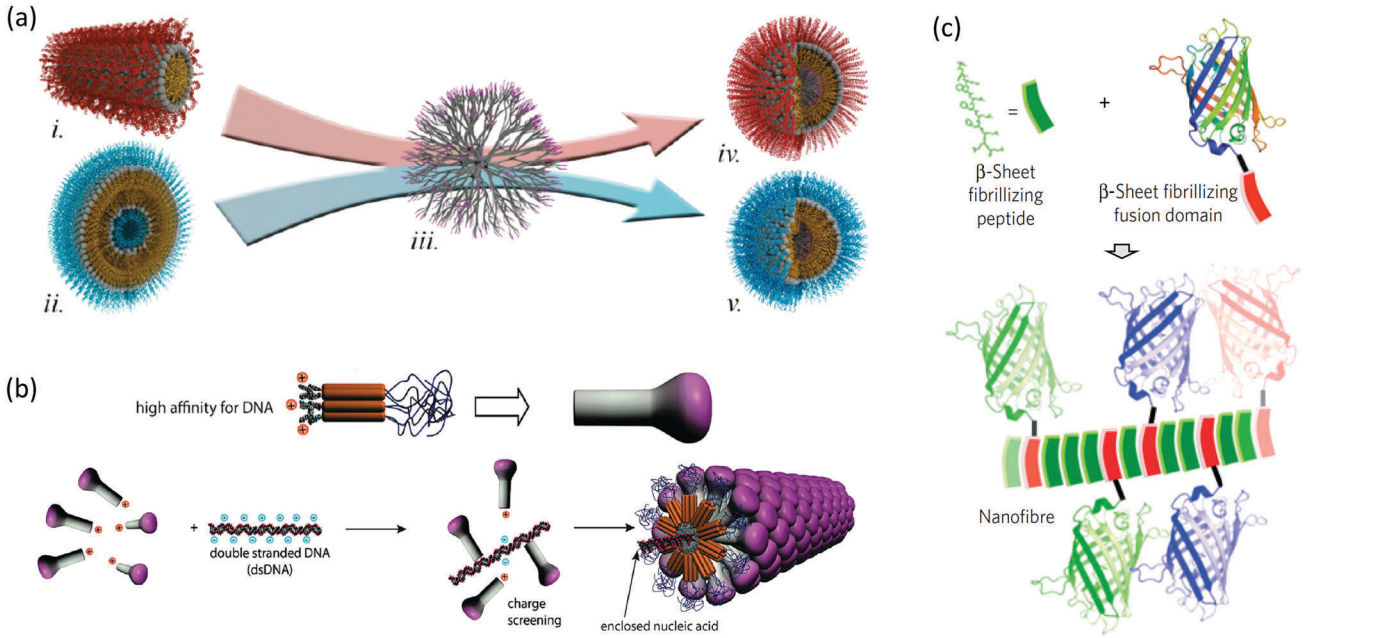

Fig. 4 (a) Schematic of dendrimer-templated co-assembly of peptide amphiphiles into spherical architectures. Adapted with permission from ref. 99. Copyright 2011 American Chemical Society. (b) Schematic representation of a strategy to prepare self-assembled mushroom-shaped nanostructures of PEGylated coiled coil peptides and subsequent assembly into a filamentous virus-like architecture using a DNA template. Adapted with permission from ref. 104. Copyright 2013 American Chemical Society. (c) Schematic representation of self-assembly of multiple β-sheet nanofibers and subsequent templating of fluorescent proteins. Adapted with permission from ref. 105. Copyright 2014 Springer Nature.

highly branched polymer structures such as phthalocyanine-based dendrimers with multiple ligands could also permit enhanced templating control of protein assembly compared to linear polymers.[98] Based on this concept, Tirrell and co-workers reported a modular fifth generation dendrimer that can serve as a platform for templating the self-assembly of cylindrical and bifunctional peptide amphiphiles (PAs) into stable large spherical particles (50 nm) (Fig. 4a).[99] This study demonstrates the possibility to use this template-driven co-assembly to generate emerging properties such as the acquisition of a native secondary structure and enhancement of DNA binding stability inside and outside of cells. This is a clear example of how a template could dramatically affect self-assembling precursors.

Supramolecular nanostructured templates

Supramolecular frameworks such as aggregates, nanowires, and nanofibres can also serve as templates for protein assembly, bringing additional advantages as a result of their more complex template structure.[100] The use of biomolecules is particularly interesting because of their high efficiency, high specificity and genetic programmability. For example, oligonucleotides have an innate ability to form metal-induced G-quadruplex complexes, which are able to direct peptide assembly in a precise manner. Gosh and Hamilton used "click" chemistry to synthesize a peptide–oligoguanosine conjugate capable of forming a G-quadruplex when metallized.[101] This G-quadruplex drives the assembly of the conjugate and thereby templates the peptide into a hierarchical multiloop architecture, making this oligonucleotide a unique molecular template. Another example where templated self-assembly can give rise to new properties relies on DNA templates to organize proteins into 3D crystals. While this approach was first proposed decades ago,[102] it is now a gold standard of increasingly functional systems.[103] Bearing this in mind, Stupp and co-workers designed a cationic coiled–coil peptide functionalized with PEG and spermin into a mushroom-shape nanostructure. Upon charge screening with a negatively charged double strand DNA molecule, the circular DNA was organised and served as a template to guide the peptide supercoiled aggregate into a 1D supramolecular architecture (Fig. 4b).[104] In another elegant example, Collier and co-workers reported that peptide nanofibres were able to arrange a mixture of different fluorescent β-tail fusion proteins in a predictable manner with gradated concentrations of the proteins along the nanofibres (Fig. 4c). This approach not only enabled the maintenance of the native protein functionality but also the emergence of tailorable multi-antigen immunogenic properties.[105]

Multicomponent self-assembly based on non-specific supramolecular interactions

In addition to the approaches described in previous sections, non-specific supramolecular interactions (*i.e.* electrostatic, hydrophobic, π–π, hydrogen bonds and van der Waals) have also been used to tap into the benefits of multicomponent self-assembly. The strength of each of these non-covalent interactions is weak (0.1–5 kcal mol^{-1}) compared to typical covalent interactions (40–100 kcal mol^{-1}). However, the cooperativity

and synergism of multiple non-covalent interactions over large areas of molecular surfaces in the building blocks are particularly essential for accessing thermodynamically stable nanostructures. More so, this interplay of non-covalent interactions underpinning multicomponent self-assembly must be more energetically favourable than interactions between individual components and the solvent and as such to be able to overcome the entropic advantages of disassembly.[106] While the lack of molecular specificity and selectivity can be seen as an engineering disadvantage, the non-specific nature of such interactions can actually open up new opportunities by enabling the emergence of more complex and adaptive processes. Here we describe how non-specific interactions facilitate simple and versatile self-assembling systems that enable the fabrication of materials with enhanced complexity.

Peptide–peptide

In the past decades, the use of *N*-fluorenylmethoxycarbonyl (Fmoc) modified peptides as building blocks for self-assembled nanostructures has attracted a lot of attention. The widespread use of Fmoc-peptides in material design could be attributed to the intrinsic propensity of the Fmoc moiety to rapidly self-assemble through π–π and hydrophobic interactions. In the multicomponent self-assembly arena, mixtures of Fmoc-peptides have been extensively used by the laboratories of Adams and Hamachi to address fundamental questions about multicomponent self-assembly pathways – particularly, the concept of co-assembly *versus* self-sorting.[31b,107] It is important to note that we will not discriminate between self-sorting and co-assembly in this review. Going beyond the fundamentals of multicomponent self-assembly driven by non-specific supramolecular interactions, mixtures of Fmoc-peptides have been used in developed materials with remarkable properties. For example, Gough and co-workers demonstrated co-assembly of Fmoc-diphenylalanine (Fmoc-FF) and Fmoc-L-arginyl-glycyl-L-aspartyl-L-serine (Fmoc-RGDS) interlocked β-sheets that give rise to a bioactive gel through π–π interactions between the Fmoc fluorenyl groups.[108] By simply modulating the concentration of both peptide components, the mechanical properties can be tuned. Similarly Adam and co-workers demonstrated that co-assembly of peptide-based building blocks can facilitate synergistic enhancement of hydrogel stiffness.[109] The authors also highlighted the possibility of disruptive self-sorting when the distinct individual building blocks self-assemble orthogonally. Besides using π–π interactions to drive multicomponent self-assembly of peptides into hydrogels with tunable mechanical properties, Xu and co-workers demonstrated that such interactions can also transform secondary structures of peptides from α-helix to β-sheet by mixing two complementary pentapeptides.[110] Nature contains many examples of multicomponent systems (*e.g.* living cells) having various distinct components (*e.g.* actin filaments and microtubules) exhibiting orthogonal functionalities in response to stimuli. Therefore, using this inspiration as a guide, it should be possible to fabricate artificial systems with different supramolecular architectures that exhibit orthogonal functionalities. Such materials should exhibit innovative properties necessary for practical applications. Bearing this in mind, Hamachi and co-workers recently took advantage of the cooperativity and synergism that multiple non-covalent interactions provide to design multicomponent hydrogels comprising orthogonally self-assembled peptide and lipid-based molecules.[111] Interestingly, these supramolecular assemblies result in a self-sorting double network of nanofibres that independently respond to different external stimuli. In a similar study by Kar and Gosh radiation was used instead of chemical agents to selectively disassemble one of the nanofibre assemblies in a two-component hydrogel.[112] Taking advantage of the co-assembling propensity of self-complementary oligopeptides, Yu *et al.* co-assembled oppositely charged decapeptides based on either VK or VE to create hydrogels.[113] In this case, co-assembly enables the capacity to tune the β-sheet propensity of the generated nanostructures, which consequently leads to controlled viscoelasticity and self-healing properties. Given the currently intense interest in molecular electronic devices and most importantly the development of conductive nanostructures,[114] multicomponent self-assembly holds great potential as a facile approach for fabricating nanomaterials that are capable of directional and long-range electron transport. Polymeric and oligomeric p-conjugated systems including oligo/polythiophenes,[115] phthalocyanines[116] and tetrathiafulvalenes[117] are among the commonly used candidates to create electronic devices. However, the ability of these molecular species to facilitate energy transfer processes does not only depend on the electronic structures, but also the spatial orientation of one component with respect to another within nanostructures with well-defined geometries that multicomponent self-assembly provides. Taking advantage of the strength and synergism of multiple supramolecular interactions, Guler and co-workers developed a co-assembling system based on electrostatic interactions, hydrogen bonding, and charge-transfer complexes between electron rich n-type and electron deficient p-type peptides, which generate β-sheet forming peptide-chromophore conjugates (Fig. 5a).[118] Due to the stacking of the π-electron donor–acceptor peptides, the co-assembled nanostructures, and consequently the macroscopic materials, exhibit the capacity to conduct electricity, which is not observed in materials made from the single components. In another example, using peptides comprising different π-electron units [oligo(*p*-phenylenevinylene), quaterthiophene, and napthalene diimide], Tovar and co-workers generated co-assembled peptide-based nanostructures combining both photonic and electron donor–acceptor pairs.[119] The resulting co-assembled structures enable the creation of electric fields and localized energy gradients, opening the possibility to generate hydrogel materials with controlled energy transport properties. These examples demonstrate how co-assembly between simple peptide components can generate well-defined nanostructures that display new or enhanced properties. However, a higher level of complexity may be attained by co-assembling with more complex molecules.

Peptide–macromolecule

A system developed by Stupp and co-workers based on negatively charged hyaluronic acid (HA) and positively charged peptide amphiphiles (PAs) serves as a good example of such possibility.[120] Here, the authors trigger co-assembly at the

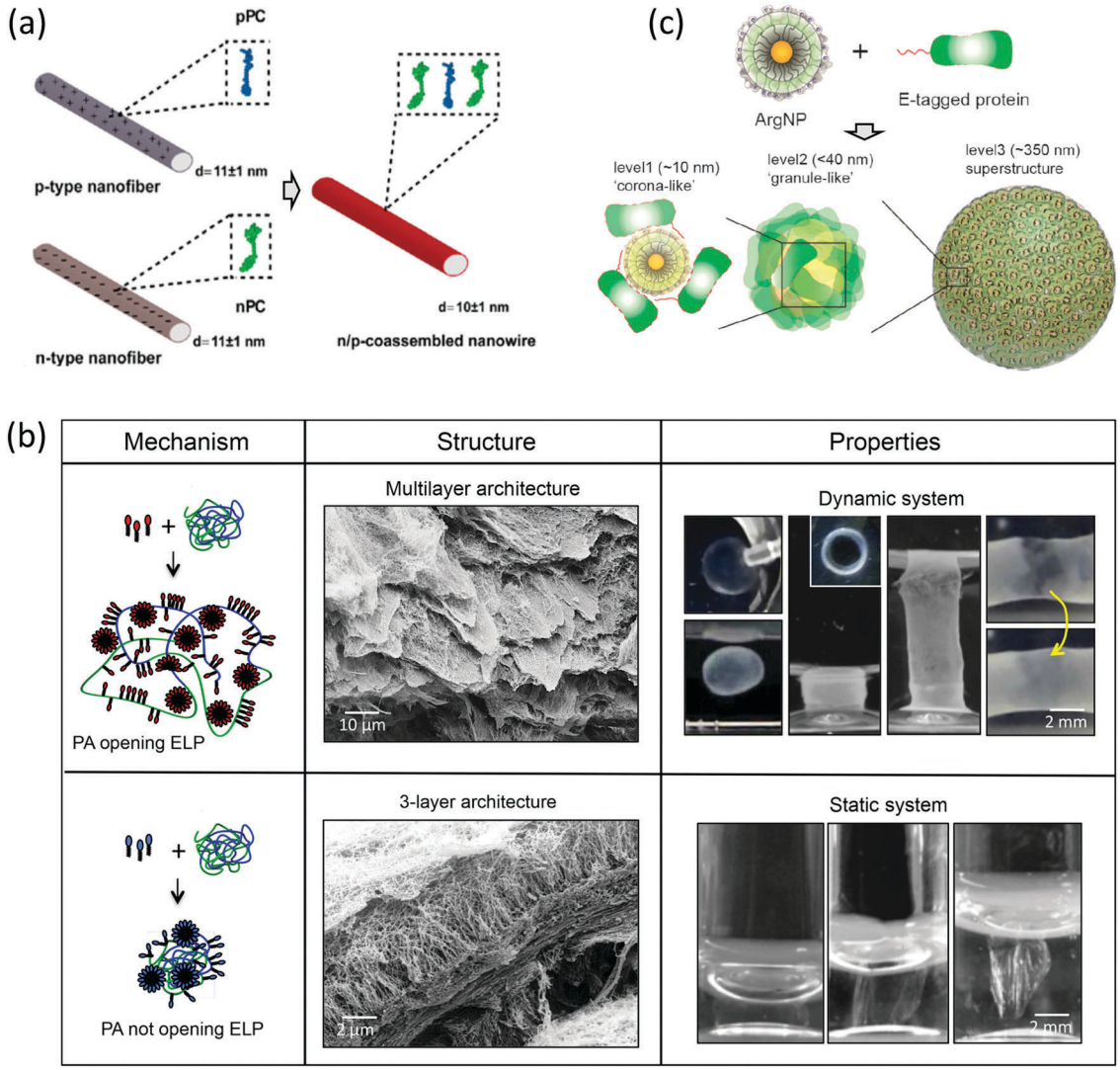

Fig. 5 Schematic representation of co-assembly of (a) p- and n-type nanofibers into supramolecular n/p conductive nanowires. Adapted with permission from ref. 118. Copyright 2017 American Chemical Society. (b) Co-assembly of ELP and positively charged PAs into hierarchical architectures that exhibit dynamic properties. Adapted with permission from ref. 24*a*. Copyright 2015 Springer Nature. (c) Schematic representation of the hierarchical organization of engineered proteins and metal nanoparticles into complex superstructures. Adapted with permission from ref. 124. Copyright 2017 American Chemical Society.

interface between solutions of each component, giving rise to the formation of a diffusion barrier that prevents chaotic mixing and enables compartmentalization. This then results in a dynamic synergy between osmotic pressure of the ions and static self-assembly, directing the formation of hierarchical, permeable, and self-healing structures with potential biomedical applications. In this case, co-assembly enables access to more complex processes such as the generation of compartments, concentration gradients, and controlled ionic transport, which are critical to produce the resulting material properties. In another example, we reported the use of PAs to co-assemble with elastin-like proteins (ELPs) while modulating their conformation.[24a] ELPs are disordered proteins with the capacity to acquire aggregated β-turn-rich conformations above a transition temperature. Upon mixing a solution of ELP with another solution of PA above the transition temperature, co-assembly triggers a diffusion–reaction mechanism that results in a multi-layered membrane with the capacity to disassemble controllably, seal to interfaces, self-heal, and undergo controlled morphogenesis into complex tubular networks. Interestingly, these properties emerge because of the possibility to modify the conformation of the ELP upon co-assembly with the PA. However, by introducing minor modifications in the amino acid sequence of the PA, different ELP conformations can be generated, which results in different co-assembling processes, structures, and material properties (Fig. 5b). This example demonstrates how small self-assembling peptides can be used as ''manipulators'' of complex proteins and how their co-assembly can generate exciting material properties. These approaches may be combined with top-down techniques such as microfluidics[121] and inkjet printing[122] to modulate further the co-assembly process through interfacial fluid-forces.

Peptide–nanoparticle

A simpler component to modulate the organization of a more complex one can also be used with proteins. For example, Liu *et al.* used quantum dots (QDs) to electrostatically interact with SP1 proteins and direct protein assembly into sandwiched nano-rings that come together to form nano-wires with tuneable shape, size, and energy transfer properties.[123] Also, Rotello and co-workers reported arginine-functionalized gold nanoparticles capable of binding green fluorescent proteins with oligo(glutamate) sequences and direct their assembly into collapsible protein multilayers (Fig. 5c).[124] These structures are capable of responding to environmental conditions and controllably deliver proteins within the cytosol of cells.[125] Alternatively, co-assembling systems can also allow proteins to direct the organization of the smaller/simpler component. For example, Grigoryan and co-workers used a tyrosine-rich protein to organize buckminsterfullerene (C_{60}) into ordered structures.[126] Here, the two components self-organize into crystal structures comprising a protein lattice with fullerene groups occupying periodic sites localized between two helical segments of the protein. The resulting co-assembled structure exhibits high charge conductance even though each of the components is electrically insulating. In another example, Thery *et al.* takes advantage of the self-organizing properties of actin to co-assemble with and organize gold nanoparticles into well-defined 3D interconnections.[9] While the gold nanoparticles are non-conductive on their own, the templating effect of the actin complexes on the nanoparticles enables metallization and plating, which results in conductive hybrid filaments. These examples demonstrate how interactions between a simple building-block (*i.e.* peptide, nanoparticle) and a more complex molecule (*i.e.* proteins, polysaccharides) can be used to generate hybrid order and subsequent functionality.

Protein disorder opportunities

Another opportunity for enhanced bioinspired engineering arises from the growing recognition that both ordered and disordered regions of proteins play a fundamental role in their functionality. The possibility to create protein-based materials that can control the interplay between protein order and disorder would be a major step forward. Lu and colleagues reported a protein–protein co-assembly system capable of generating hierarchical nanofibres and films with high wet bonding strength, material robustness, enhanced stability to autooxidation, and intrinsic fluorescence.[127] In this study, the authors genetically fused the amyloid protein CsgA with the disordered DOPA-containing mussel foot proteins Mfp3 and Mfp5. These two complexes were then co-assembled by the extension of amyloidogenic fibril through self-polymerization and beta-strand lamination by lateral stacking. The key opportunity emerges thanks to the disordered nature of the Mfps, which, thanks to its flexible and adapting structure, allows both polymerization of the CsgA segment and large surface area contact with the CsgA fibril, enabling the material's strong adhesive properties. Our group is particularly interested in the opportunities that emerge by engineering materials incorporating protein order–disorder transitions as a design element. The previously mentioned study by Inostroza-Brito *et al.* demonstrates the possibility to trigger different co-assembling pathways and access different material properties by tuning the conformation of the protein thanks to its inherent disordered nature.[24a]

Conclusion

There is widespread agreement that peptides and proteins are some of the most exciting and promising building-blocks to create the next generation of advanced materials. Just in the last two decades, the number of yearly publications on peptide or protein-based materials has showed an exponential growth going from 1934 publications in 1997, to 4493 in 2007, and up to 9688 in 2017. Together with this mounting body of work, our growing capacity to design and fabricate from the molecular scale has also generated increasing expectations to deliver real-world practical solutions. However, while great progress has been made enabling a remarkable control and diversity of structure, translating the structure into functionality with practical applications continues to be a major challenge. Inspired by an increasing understanding of how peptides and proteins in nature work as multi-component ensembles, here we propose that multi-component self-assembly represents an attractive nanotechnology strategy to fabricate peptides and proteins in ways that can enhance complexity and functionality. We have demonstrated this possibility by highlighting recent studies that use specific, non-specific, covalent, and non-covalent interactions as part of multi-component self-assembling processes capable of developing materials with emergent properties. These approaches enhance our capacity to overcome major challenges in peptide and protein-based materials and open exciting new opportunities to bridge supramolecular complexity with material functionality.

Conflicts of interest

Q5

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