Chem Soc Rev

REVIEW ARTICLE

View Article Online
View Journal | View Issue

Cite this: *Chem. Soc. Rev.*, 2015, 44, 5200

Peptide self-assembly triggered by metal ions

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Received 17th March 2015

DOI: 10.1039/c5cs00234f

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Through their unique and specific interactions with various metal ions, naturally occurring proteins control structures and functions of many biological processes and functions in organisms. Inspired by natural metallopeptides, chemists have developed artificial peptides which coordinate with metal ions through their functional groups either for introducing a special reactivity or for constructing nanostructures. However, the design of new coordination peptides requires a deep understanding of the structures, assembly properties, and dynamic behaviours of such peptides. This review briefly discusses strategies of peptide self-assembly induced by metal coordination to different natural and non-natural binding sites in the peptide. The structures and functions of the obtained aggregates are described as well. We also highlight some examples of a metal-induced peptide self-assembly with relevance to biotechnology applications.

1. Introduction

In nature, it has been estimated that approximately one-third of all proteins and enzymes require metal ions as cofactors for biological function. Metal ions often play a crucial structural, regulatory, and/or enzymatic role in the function of biological systems.[1] For decades, chemists have been trying to mimic natural metallopeptides by synthesizing artificial peptides with incorporated natural or non-natural metal binding sites.[2] On the other hand, coordination with metal ions can induce conformational changes in the peptide which can dramatically change the properties of the peptide.[3] For instance, the initial stage of Alzheimer's disease is mainly induced by α-helix/β-sheet transition of β-amyloid peptides by coordinating with metal ions.[4] On the other hand, peptides may self-assemble through metal–ligand interactions into nanostructures which are conformationally and functionally quite different from the original peptide.[5] Using natural examples as a guide, substantial advances have been achieved in the construction of nanostructures, including the fabrication of nanofiber materials for three-dimensional cell culture and tissue engineering,[6] the assembly of peptide nanotubes[7] and helical ribbons among others. The design of molecular building blocks that undergo spontaneous

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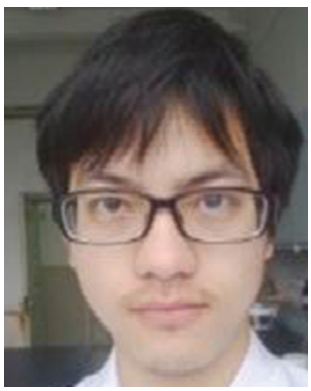

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organization into well-defined and stable macroscopic structure *via* covalent/noncovalent bonds is still a great challenge, especially if the formation of a specific secondary or even three-dimensional structure is desired. In many cases, specific three dimensional structures of peptide aggregates are achieved by the use of covalent connections between functional groups at different positions in the peptides (*e.g.* disulphide bridges). These linkages lead to constraints for the conformation of the peptide and thus fix its conformation or favour the assembly of individual peptides into other types of assemblies.[8] Furthermore, non-covalent interactions can also be involved in controlling the structure of such assemblies. For example, artificial collagen-based peptides synthesized by Chmielewski and co-workers exhibit a triple helix formation stabilized by an intermolecular hydrogen bond. Addition of metal ions then assembled these peptides into discs, spherical shells or fibrous structures.[9]

We present here a summary of the most frequently used functional groups for the construction of peptide building blocks which undergo a metal induced self-assembly. Albrecht *et al.* have provided a survey of the general approaches towards metallocyclopeptides either in the form of small cyclic peptide derivatives or as a part of bigger α-helix (or β-sheet) structures. We therefore do not discuss biological activities of such artificial protein–metal complexes. As metal-assembled modular proteins (MAMPs) have also been already reviewed,[10] we will not discuss such systems here either. The purpose of this article is rather to gain a deeper understanding of the influence of metal coordination on peptide self-assembly and the functions of such artificial metallopeptides. We will show that metal

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coordination is a powerful tool for constructing diverse molecular architectures. We focus on ligands and various modes of assembly of natural and artificial peptides with metal ions in order to introduce a special reactivity or to form different nanostructures and functionalities.

2. Common ligands for metal coordination

Peptide–metal assemblies are typically composed of three main parts, namely, a peptide with a given sequence containing metal binding residues and a specific metal ion. The peptide sequence mainly controls the shape, size, and monodispersity of the resulting self-assemblies.[11] Binding metals may not only determine the activity of the peptides but can also act as linkers between peptides,[12,13] especially when metal binding residues act as an anchor or a connector to fix a functional group or conjugate another binding motif through one or more metal ions. The type of metal binding site and its position in the peptide not only determine the structure of the assemblies but also significantly influence the properties of the assemblies.

Generally, metal binding sites within peptides can be classified into two types, artificial ligands and natural ligands (Table 1). Artificial metal–ligand residues have been synthesized to serve as triggers for higher-order or specific secondary structure assemblies.[14] Ligands such as nitrilotriacetic acid, pyridine, bipyridine, and terpyridine have been widely used in this context to regulate the structure and properties of peptides in the presence of metal ions.[15] In nature, histidine, cysteine, tryptophan and glutamic acid residues serve as metal-binding sites. Their interaction with metals induces conformational changes or the formation of supramolecular structures.[16] In terms of self-assembly, artificial ligands possess remarkable advantages over natural ligands mainly due to their tailor-made chemical structure. More specifically, the high strength and defined directionality of metal-binding by artificial residues allow precise control over the three-dimensional structure and stability of the final assembly.[17–19] Thus, in this review we emphasize artificial ligands (Table 1) which have been used in the self-assembly of peptides. However, in some cases, the individual metal binding residue acts only as low-affinity mono- or bidentate ligands in the absence of a protein scaffold.[20] In this situation, the specific peptide surrounding may also increase binding affinity.

3. Metal-triggered self-assembly of artificial ligands

Metal-triggered self-assembly of peptides is a promising direction in the synthesis of specific molecules with well-defined conformations or of nanostructures.[21–24] A wide range of artificial peptides have been synthesized in the last few years to broaden the range of functions and structures that can thus be achieved and to allow more precise control of the properties of these materials.[25]

In particular, peptides that utilize metal–ligand coordination as an additional binding interaction have been synthesized to overcome the limitations posed by the otherwise exclusive use of hydrogen bonding and hydrophobic interactions as binding forces to hold together different units of metal free peptide assemblies.[26–28] This additional binding motif takes advantage of the well-known features of the coordination bond. Its high strength, reversibility, and defined directionality allow precise control over the three-dimensional structure and stability of the final assembly.[29]

Efforts aimed at generating structured materials at the nanometer scale have already produced important results.[30] Ligands that bind metal ions have been anchored to peptides by using various groups. The most widely studied artificial coordination ligands in this context are pyridine, bipyridine, terpyridine, nitrilotriacetic acids, iminodiacetate, and crown ether moieties.

3.1 Self-assembly *via* pyridine coordination

Pyridines are heterocyclic compounds which possess good binding properties for metal ions. Adaptation of the metal–pyridine coordination strategy to the self-assembly of peptides should provide an even more tightly bound structure. Pyridine is a valuable building block in the design of different kinds of peptides which adopt different structures upon metal binding.

Table 1 Natural and artificial ligands and their binding metals

Ligands	Binding metals	Ref.
Part I: artificial ligands		
Pyridine	Fe^{2+}, Re^{+}, $^{99m}Tc^{+}$, Cu^{2+}, Pd^{2+}, Ag^{+}, Pt^{2+}	31–40
Bipyridine	Zn^{2+}, Fe^{2+}, Cu^{2+}, Ru^{2+}, Co^{2+}, Ni^{2+}	9, 32 and 42–50
Terpyridine	Fe^{2+}, Ni^{2+}, Cu^{2+}	58–63
Nitrilotriacetic acid	Ni^{2+}, Zn^{2+}, Cu^{2+}, Co^{2+}	65–70
Iminodiacetate	Cu^{2+}, Zn^{2+}, Ni^{2+}	81–84
Phosphane ligand	Rh^{+}, Mg^{2+}, Ca^{2+}, Ba^{2+}, Zn^{2+}, Gd^{3+}, Au^{+}, Pd^{2+}	7, 87 and 89–91
Crown ether	Cs^{+}, Zn^{2+}	92 and 93
Part II: natural ligands		
Histidine	Zn^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+}, Ag^{+}	100–107
Cysteine	Hg^{2+}, Pb^{2+}, Zn^{2+}, Cd^{2+}	111 and 112
Tryptophan	Cu^{2+}	114
Glutamic acid	La^{3+}, Yb^{3+}, Ca^{2+}	117–120
Catecholate	Ti^{4+}, Mo^{6+}	122

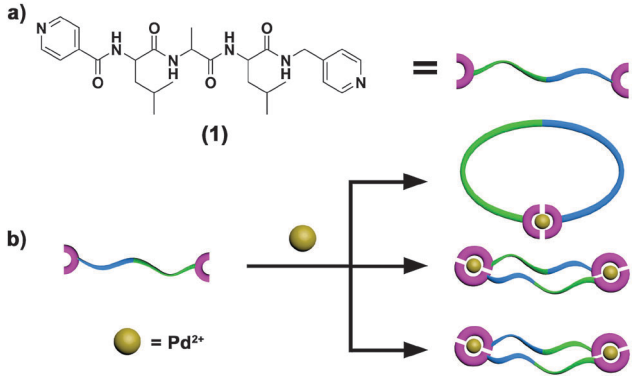

Fig. 1 (a) Structure of peptide **1** containing two pyridine ligands. (b) Upon interaction of **1** with Pd(en)$(NO_3)_2$ different types of self-assembled aggregates are formed. Reproduced with permission from ref. 31. Copyright 2004, Georg Thieme Verlag KG Stuttgart.

The resulting assembly is controlled by the amino acid sequence, the positions of the pyridine residues within this sequence, and the kind of metal ion or metal complex fragment.

For example, Albrecht *et al.* reported bis(pyridyl) terminated Leu–Ala–Leu derivative **1** (Fig. 1a) which could coordinate with Pd(en)$(NO_3)_2$ to form a mixture of 1:1 and 2:2 macrocycles [Pd(en)(**1**)$_n$] (n = 1, 2). Due to the directionality of the peptide strands, they obtained two different "regioisomers" of the 2:2 complexes (Fig. 1b). One in which the two peptides are aligned parallel and another one in which the two peptides are aligned antiparallel.[31]

Pyridine functionalized peptides could also coordinate with other metal complexes to form peptide-based hybrid materials. Utilization of metal complexation for molecular recognition in oligopeptides was reported by Gilmartin *et al.* They synthesized an artificial peptide with a pendant pyridine ligand and then incorporated the peptide into oligomeric strands (**2**) which are analogous to a peptide nucleic acid (Fig. 2a). Upon addition of transition metals (Cu^{2+}, Fe^{2+}), the oligomers could bind stoichiometric quantities of metals according to the number of pendant ligands (Fig. 2b).[32] Meanwhile, in order to mimic the structure of DNA (and RNA) by incorporation of the pyridyl ligands onto the peptide backbone, Ohr *et al.* developed a series of supramolecular structures containing 1, 4, 5, 6, 10 metal complexes tethered to pyridine-substituted oligopeptide scaffolds (peptides **3**, Fig. 2a). They examined the structural and functional properties of these single-strand pyridine oligopeptides depending on the peptide lengths. The pyridyl groups within the peptides will form metal complexes which induces specific folds into the peptide strand (Fig. 2b).[33]

In a related work, Gerhardt *et al.* reported the synthesis and controlled assembly of four tripeptides **4**. Each tripeptide **4** had either a central pyridyl glycyl or a pyridyl alanyl residue between two terminally protected glycines (Fig. 2a). All tripeptides were coordinated to a complementary recognition unit, a *p*-methoxy SCS–Pd pincer complex. They found that metal coordination in all cases was fast and quantitative and that the peptide backbones did not interfere with self-assembly (Fig. 2b). As compared with other peptides, 4-pyridyl tripeptides were the most

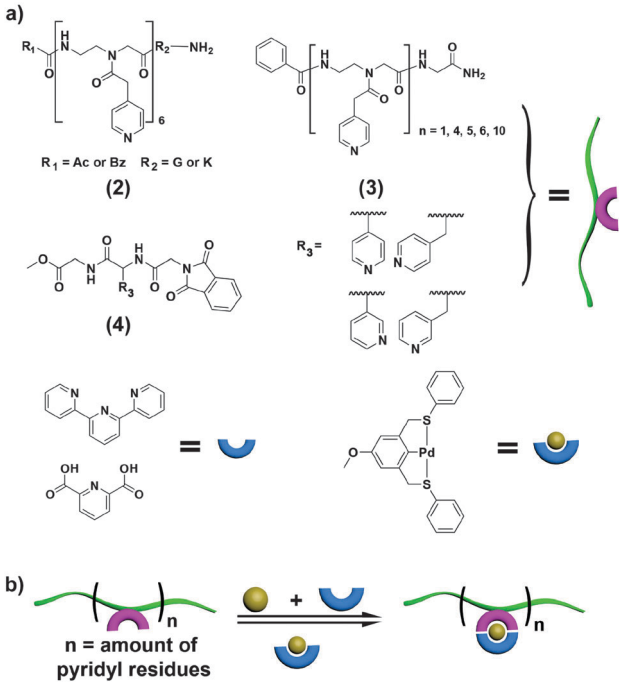

Fig. 2 (a) Structure of ligand–peptide monomers and oligomers **2**, **3**, **4**; Ac: acetyl; Bz: benzoyl; G: glycine; K: lysine. (b) Schematic shows pincer complexes formed with the pyridyl groups of the peptides. Reproduced with permission from ref. 32–34, Copyright 2005, 2005, 2006, American Chemical Society.

tightly binding ligands toward the palladated pincer complexes, with the alanyl derivative being the strongest overall. The high K_a value ($>1200\ M^{-1}$) suggests that the controlled coordination of the metalated pincer–pyridine interaction is an interesting biological synthon and that these peptide ligands can be used to assemble new and interesting biological scaffolds.[34]

The controlled coordination of radioactive metals by pyridine functionalized peptides opens up the way to interesting radiopharmaceutical applications. Jiang *et al.* used cysteine-derived peptide **5**, derived from the α-melanocyte stimulating hormone (α-MSH) (Fig. 3a), to incorporate a $[M^I(CO)_3]^+$ moiety (M = Re, ^{99m}Tc) (Fig. 3b). The peptide chelate with ^{99m}Tc showed excellent radiolabeling yields and *in vitro* stability during amino acid challenge and serum stability assays.[35]

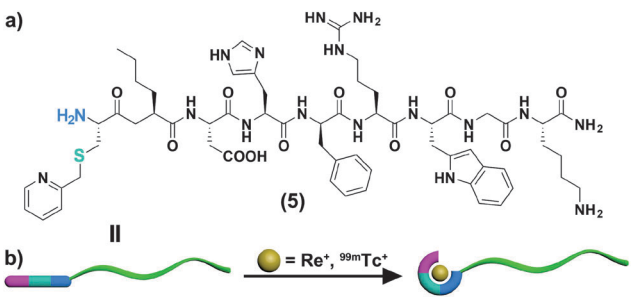

Fig. 3 (a) Structures of cysteine-based peptide **5**. (b) Schematic shows a radioactive metal coordinated with the N-S-NPy group of peptide **5**. Reproduced with permission from ref. 35, Copyright 2012, American Chemical Society.

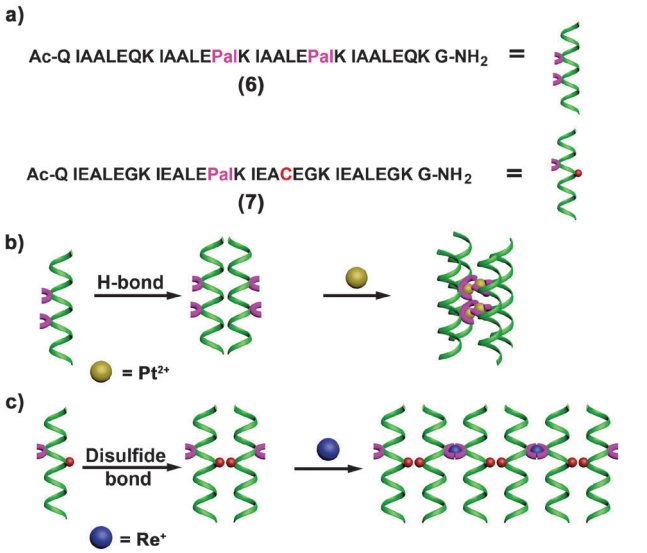

Fig. 4 (a) Structure of peptides **6** and **7**; (b) Four α-helical coiled coil peptides **6** connected by platinum ions; (c) metal–peptide nanoassemblies in which the directional bonding properties of an octahedral rhenium complex are used to induce the self-assembly of α-helical coiled-coils formed from two peptides **7** linked with a disulfide bond. Reproduced with permission from ref. 36 and 37. Copyright 2007, 2004, American Chemical Society & Royal Society of Chemistry.

Pyridyl groups have also been introduced into designed α-helical peptides to alter the oligomerization state of the peptides. Tsurkan *et al.* used an assembly of pyridine-modified 30-residue polypeptide **6** (Fig. 4a). The AQ-Pal14Pal21 polypeptide contains two metal-binding 4-pyridylalanine residues on its solvent-exposed surface and exists as a very stable, two-stranded α-helical coiled coil. Upon addition of $Pt(en)(NO_3)_2$, the peptide showed a significant conformational change into a metal-bridged, four-helix bundle (Fig. 4b).[36] Tsurkan *et al.* further utilized disulfide cross-linked coiled coil peptides as linear bridging ligands joining adjacent *fac*-$[Re(CO)_3]$ cores. The peptide sequence was based on the IEALEGK heptad repeat, which is a two-stranded α-helical coiled-coil peptide. The synthetic amino acid 4-pyridyl alanine (Pal) was placed at position 14 of this sequence, which is the most solvent-exposed position of the second heptad repeat. A cysteine residue was placed at residue 19, which occupies the d position in the hydrophobic core of peptide 7 (Fig. 4a). The Pal residues were introduced to incorporate a strong metal-binding site into the peptide and the cysteine residues were introduced to engineer an interchain disulfide bond crosslink to stabilize the resulting coiled-coil structure. Hence, firstly two coiled-coil peptides were conjugated with a disulfide bond. Upon coordination with metal ions, these two-stranded α-helical peptides formed higher-order metal–peptide conjugates (Fig. 4c).[37]

Pyridines have also been used to self-assemble cyclic peptides. Panciera *et al.* synthesized α, γ-cyclic peptide **8** bearing a nicotinic acid attached to a serine residue through its side chain (Fig. 5a). Directed by the pyridine moiety upon coordination with palladium ions, the cyclic peptide forms dimers and further self-organizes in a hierarchical process to form pom-pom-like

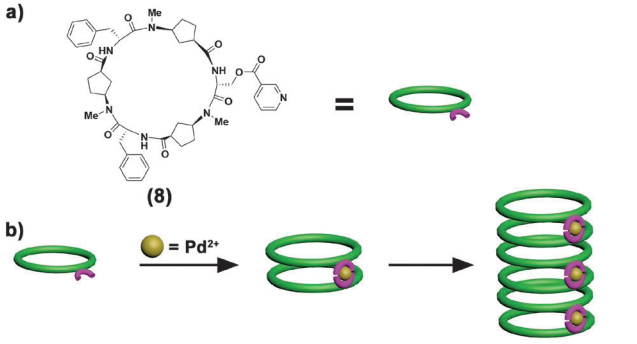

Fig. 5 (a) Structure of cyclic peptide **8** (CP3). (b) Proposed model for the formation of the molecular pom poms formed by self-assembly from this cyclic peptide. Reproduced with permission from ref. 38. Copyright 2013, Wiley-VCH Verlag GmbH & Co. KGaA.

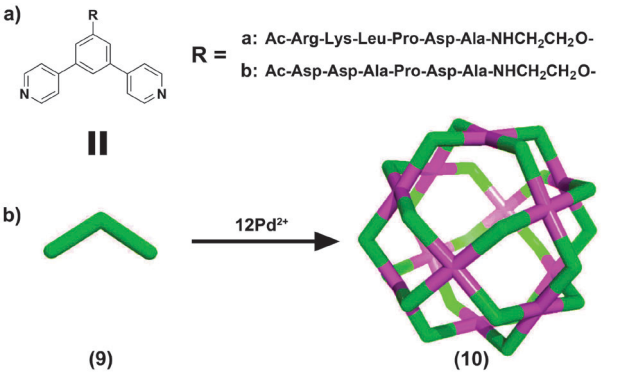

Fig. 6 (a) Molecular structure of peptides **9a**, **9b**. (b) Self-assembly of $M_{12}L_{24}$ spherical complexes **10a**, **10b**.

molecular structures (Fig. 5b). These spherical structures are porous materials that can encapsulate and subsequently liberate small molecules.[38]

The pyridyl groups have also been introduced into peptides to construct sphere nanostructures or nanometer-sized channels by metal coordination. Ikemi *et al.* synthesized pyridine containing peptide aptamers **9a**, **9b** which could coordinate with 12 Pd^{2+} ions to form a nano-sized $M_{12}L_{24}$ coordination sphere (Fig. 6a). Quantitative formation of the $M_{12}L_{24}$ spherical complex **10a** was obtained when ligand **9a** was reacted with $Pd(NO_3)_2$ in DMSO (Fig. 6b). Ligand **9b**, in which anionic Asp residues replace the cationic Arg and Lys residues, displayed similar behavior and properties to **9a**.[39]

Recently, Sawada *et al.* presented a new artificial nano-assembly of pyridine substituted short peptide helices into a protein-like two-nanometer-sized channel by metal coordination. The short peptide Gly–Pro–Pro was modified by the introduction of 3-pyridyl groups with amide bonds at both termini to yield peptide **11** (Fig. 7a). Folding and assembly of this short peptide ligand were induced by Ag^+ coordination in aqueous alcohol, and gave rise to a single crystal showing a polyproline II helical conformation for the peptide in the solid state. Furthermore, a three-dimensional network formed by coordination driven self-assembly was observed (Fig. 7b).[40]

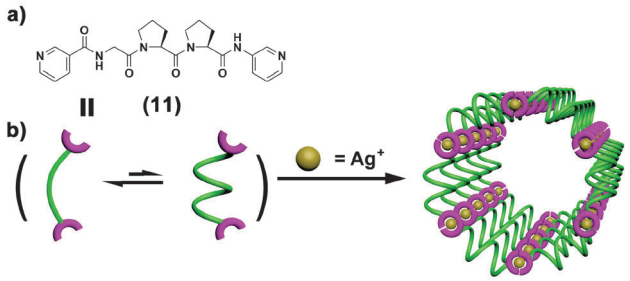

Fig. 7 (a) Molecular structure of tripeptide **11**. (b) Schematic shows how random-coil peptide ligands are concomitantly folded by metal-directed networking. Reproduced with permission from ref. 40. Copyright 2014, Wiley-VCH Verlag GmbH & Co. KGaA.

The presented examples show that pyridine residues can be introduced into peptides as "unnatural" metal binding sites to chelate transition metal ions for biomolecular recognition or radiolabeling metals for radiopharmaceutical applications. Moreover, as a monodentate linker pyridine can be used to connect two or more α-helical peptides to alter the oligomerization state of the designed peptide structure. Pyridine can also be used to self-assemble cyclic peptides or to coordinate with metal ions to form different types of nanostructures.

3.2 Self-assembly *via* bipyridine coordination

2,2′-Bipyridine (hereafter referred to as bipyridine or bpy), one of the most commonly used artificial ligands, is a well-characterized metal binding site which is utilized for preparing various supramolecular assemblies.[41] It has been introduced into peptides for both intra- and intermolecular folding of artificial peptides. By using bipyridine as a coordination ligand, the peptides usually form three-dimensional octupolar molecules with transition metals. Bipyridine can also be used as a chelating group to conjugate two or more peptides.

An example for the intramolecular self-assembly of a peptide based on bipyridine metal coordination has been reported by Imperiali *et al.* They synthesized short peptide **12** which contained two 2,2′-bipyridine units (Fig. 8a). Upon coordination with different metal ions (Zn^{2+}, Co^{2+}), the triglycine peptidic unit in between the two bipyridine units is forced into a loop type conformation (Fig. 8b). The compound formed a metallacyclopeptide which decreased the distance between the two termini as monitored by a fluorescence quenching between

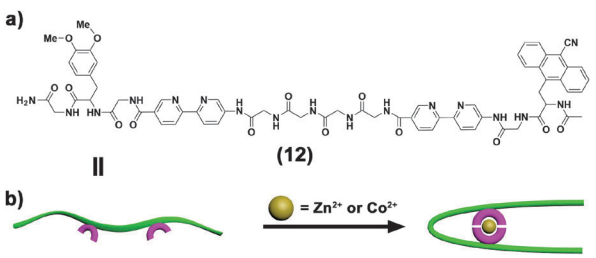

Fig. 8 (a) Chemical structure of peptide **12**. (b) Bipyridine metallacyclopeptides formed from peptide **12**. Reproduced with permission from ref. 42. Copyright 1996, American Chemical Society.

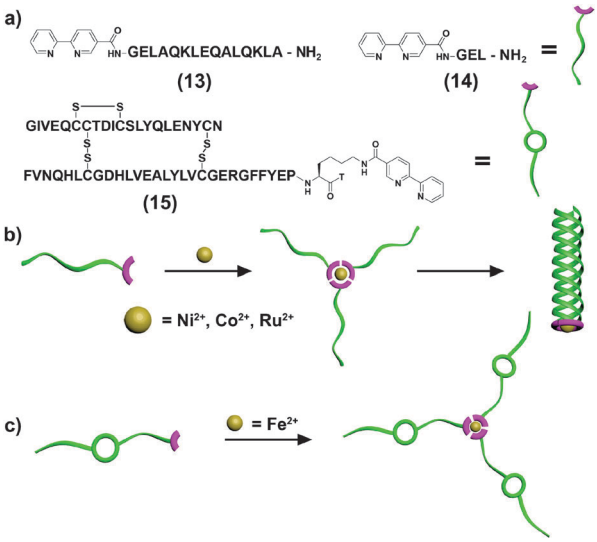

Fig. 9 (a) Chemical structure of peptides **13**, **14**, **15**. (b, c) Hypothetical folding pathway illustrated for the metal ion assisted self-assembly of such metalloproteins. Reproduced with permission from ref. 43–45. Copyright 1992, 1998, 2011, American Chemical Society & Wiley-VCH Verlag GmbH & Co. KGaA.

two attached reporter groups, a veratrole and anthracene group, respectively.[42]

For intermolecular self-assembly, bipyridine groups usually act as bidentate chelators to coordinate with metal ions. Ghadiri *et al.* first reported 15-residue amphiphilic peptide **13** with a 2,2′-bipyridine functionality at the N-terminus (Fig. 9a). The peptide could spontaneously self-assemble with transition metal ions to form a 45-residue triple-helical coiled-coil metalloprotein (Fig. 9b).[43] Similarly, Case *et al.* presented a 2,2′-bipyridyl substituted tripeptide (Bpy–Gly–Glu–Leu–amide, peptide **14**, Fig. 9a) which self-assembled into three-helix bundle metalloproteins.[44]

In another example, Munch *et al.* introduced an abiotic bipyridine ligand into insulin that complements the native metal-ion binding site of the peptide and enables the formation of novel types of self-assemblies from this re-engineered insulin variant **15** (Fig. 9a). Native insulin binds Zn^{2+} to form a hexamer. However, the use of Fe^{2+} to coordinate with the engineered insulin **15** resulted in chemoselective and reversible binding to the engineered bipyridine groups, forming a homotrimer (Fig. 9c). This approach provides the first well-defined insulin trimer and the first insulin variant for which self-assembly could be followed visually.[45]

Chmielewski and co-workers have done a series of work on incorporating bipyridines into small collagen peptides and used metal–ligand interactions to drive self-assembly. They designed two kinds of ways to self-assemble such peptides: incorporating metal-binding ligands within the center of the collagen peptide (radial design), and both at the termini and center of a collagen peptide (cross-linked design).

The first example of radial design was achieved by the synthesis of a centrally positioned, bipyridine-modified amino acid within $(Pro–Hyp–Gly)_9$ peptide **16** (Fig. 10a). Peptide **16**

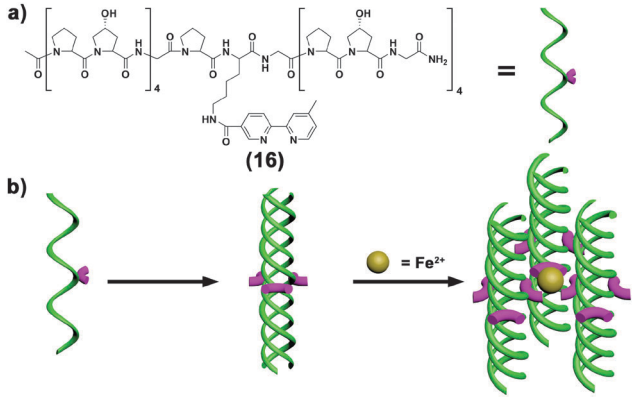

Fig. 10 (a) Chemical structure of peptide **16**. (b) Proposed model for the formation of the triple helix and the trimer of triple helice collagen peptide from **16**. Reproduced with permission from ref. 46. Copyright 2008, American Chemical Society.

firstly formed a triple helix by intermolecular H-bonds which positioned the three bipyridyl ligands on the surface of the midsection of the resulting triple helix fibres. Upon addition of Fe^{2+}, a trimer of such triple helices was formed held together with the aid of bipyridyl–Fe^{2+}-complexes (Fig. 10b).[46]

A much more complex radial design was further reported by Przybyla *et al.* Based on their previous research, they envisioned that the use of two bipyridine ligands along a collagen peptide could provide multiple sites for metal coordination within the triple helix and potentially would add rigidity to metal-promoted radial assemblies. Peptide **17** contains repeating POG sequences and two bipyridine ligands conjugated to lysines (Fig. 11a). The collagen peptide firstly formed a triple helix which led to two sets of three bipyridyl ligands each on the surface of the triple helix. The triple helix further assembled by coordination with transition metals (*e.g.* Fe^{2+}) and hierarchically assembled into collagen peptide discs (Fig. 11b). Assembly formation was reversed upon addition of ethylenediaminetetraacetic acid (EDTA) as a competing ligand.[47]

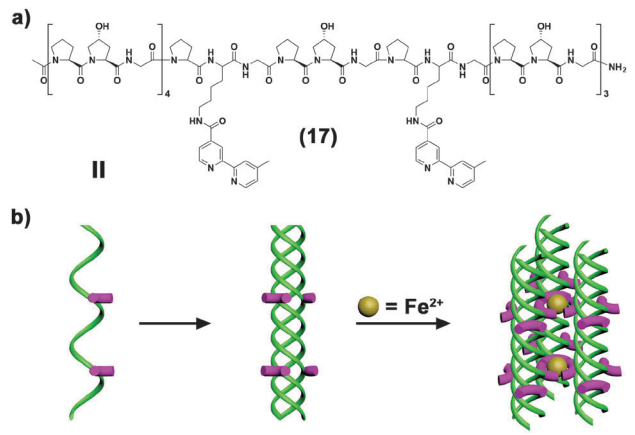

Fig. 11 (a) Structure of peptide **17**. (b) Triple helix formed from peptide **17**, with six bipyridine ligands mediating metal-promoted radial assembly. Reproduced with permission from ref. 47. Copyright 2010, American Chemical Society.

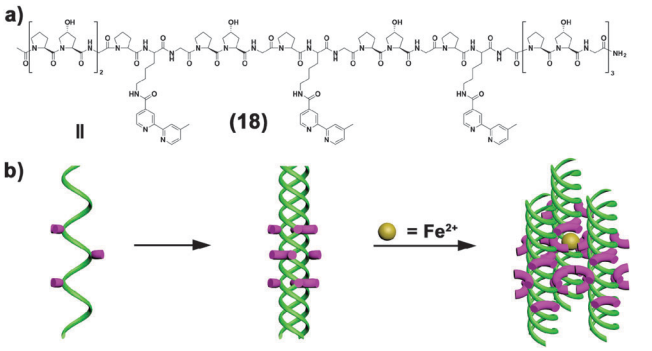

Fig. 12 (a) Sequence of collagen-based peptide **18** containing three central bipyridine ligands. (b) Schematic representation of the triple helix Hbyp3 and its self-assembly triggered by metal ions. Reproduced with permission from ref. 9. Copyright 2013, American Chemical Society.

Extending this concept further, now three bipyridine ligands were introduced in the collagen peptide. Accordingly, 27 amino acid collagen-based peptide **18** was designed to radially display nine bipyridine moieties from the resulting triple helical scaffold. To improve the structural integrity of the aggregates, the peptides was designed in such a way that it contained repeating units of Pro–Hyp–Gly (POG) with an increasing number of aromatic groups per triple helix (Fig. 12a). 27 amino acid peptide **18** first self-assembled into a triple helix. These helixes were then fused together in a metal-ion-dependent fashion into spherical shells that were about two orders of magnitude larger than related protein cages found in nature (Fig. 12b).[9]

Combining linear coordination ligands with their previous radial-growth collagen peptide, Chmielewski and coworkers also designed a new kind of cross-linked collagen peptide **19**. In this design, 2,4-nitrilotriacetic acid (NTA) as well as His_2 terminal moieties were combined with the central bipy ligand within one collagen peptide (Fig. 13a). The multidirectionality of the metal–ligand interactions provided opportunity for the formation of extensively cross-linked collagen peptide materials. Upon addition of metal ions (Ni^{2+}, Cu^{2+}, Co^{2+}, and Zn^{2+}), the triple helix formed from peptide **19** further assembled into

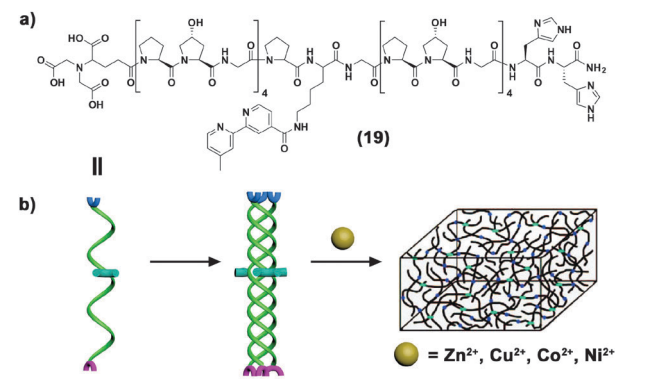

Fig. 13 (a) General structures of peptide **19**, (b) schematic representation of triple-helix formation and their assembly into a cross-linked three-dimensional scaffold following addition of metal ions. Reproduced with permission from ref. 48. Copyright 2009, Wiley-VCH Verlag GmbH & Co. KGaA.

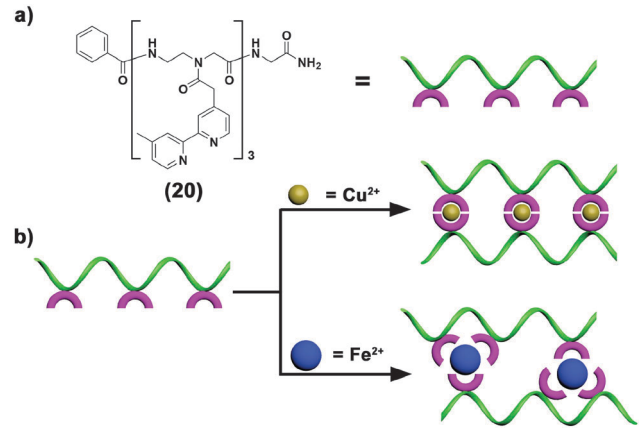

Fig. 14 (a) Structure of artificial peptide **20**. (b) Schematic diagram of the self-assembly of **20** triggered by metal ions. Reproduced with permission from ref. 32. Copyright 2005, American Chemical Society.

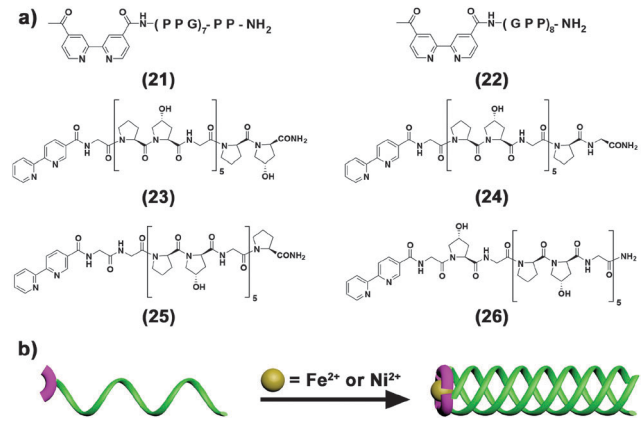

Fig. 15 (a) Structures of synthetic peptides **21–26**. (b) Schematic representation of the triple-helical collagenous peptide assembled from the N-terminal Fe^{2+}, Ni^{2+} complex. Reproduced with permission from ref. 49 and 50. Copyright 2002, 2011, American Chemical Society & Wiley-VCH Verlag GmbH & Co. KGaA.

highly cross-linked, fibrous structures due to additional intermolecular NTA–metal, His_2 and bipyridine–metal coordination (Fig. 13b).[48]

Gilmartin *et al.* have chosen bipyridine modified peptide nucleic acid (PNA) and studied its self-assembly with metal ions. PNA molecule **20** contained three bipyridine groups (Fig. 14a), which could coordinate with either two or three metal ions (Cu^{2+} and Fe^{2+} respectively) to form two different oligopeptide duplexes (Fig. 14b).[32]

In another example of the self-assembly of triple-helix peptides, Koide *et al.* designed two collagenous peptides **21** and **22** which contained a bipyridine group at the N-terminal (Fig. 15a). By coordination with Fe^{2+} ions, bpy-containing collagen peptides **21** and **22** were assembled with Fe^{2+} ions to form triple helix assemblies (Fig. 15b).[49] Similarly, LeBruin *et al.* designed and synthesized four peptides, **23–26** (Fig. 15a). These sequences consisted of five repeats of the POG tripeptide. Bidentate 2,2′-bipyridyl ligands were appended to the N-termini of the four peptides which were designed to be optimally placed for the formation of **23**, **25**, **26** heterotrimers upon addition of an octahedral-coordination metal ion Ni^{2+} (Fig. 15b).[50]

The present examples show that bipyridine units can be attached to peptides. In the presence of appropriate metal ions, the peptide could self-assemble to adopt specific conformations and form metallacyclopeptides or to form various nanostructures.

3.3 Self-assembly *via* terpyridine coordination

Terpyridine (2,2′:6′,2″-terpyridine, often abbreviated tpy) is a heterocyclic tridentate compound derived from pyridine.[51] The terpyridine moiety was shown to have outstanding complexation properties for a wide range of transition-metal ions.[52] Control of the geometries and spacing of functional complexes by using terpyridine is an important aspect in the design of bioinorganic supramolecular structures.[53,54] For example, terpyridine has been used for the self-assembly of nucleic acid. The use of 2,2′:6′,2″-terpyridine together with a monodentate, nitrogen-coordinating ligands was reported to create an alternative, metal-mediated base pair of [3+1] scaffold in nucleic acid.[55] Choi *et al.* used Fe^{2+} to coordinate with DNA duplexes that had terminal tpy ligands to form DNA triangles.[56] By metal-induced cyclization, Göritz *et al.* used Fe^{2+} to coordinate with terpyridine for the allosteric control of oligonucleotide hybridization.[57]

In combination with peptides, terpyridine is usually connected to the termini or in the middle of the peptide to form cyclic peptides and dimer assemblies. They can also be used in the self-assembly of cyclic peptides.

In one example, terpyridine has been used for cyclic peptide formation. Constable *et al.* reported a metallamacrocycle peptide $[(\mathbf{27})Fe]^{2+}$ which consists of two terpyridines as terminal coordination groups (Fig. 16a). The proline was introduced into the peptide as a central amino acid to support turn formation upon metal complexation. By reaction with Fe^{2+}, peptide **27** spontaneously formed a $[(\mathbf{27})Fe]^{2+}$ cyclic peptide in solution (Fig. 16b). However, the formed metalla-macrocycle soon decomposed upon prolonged standing.[58]

For intermolecular self-assembly, terpyridine moieties could be appended to the termini of the peptides or in the middle of the peptides. Vandermeulen *et al.* described the supramolecular organization of a metalloprotein that consisted of two N-terminal terpyridine-modified coiled-coil proteins **28** held together by Fe^{2+} ions (Fig. 17a). Self-assembly of the

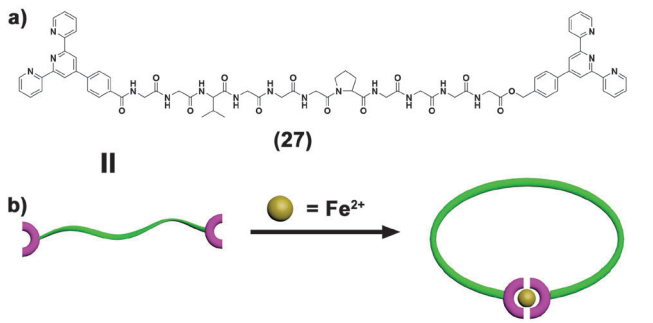

Fig. 16 (a) Structure of terpyridine moidified peptides **27**. (b) Schematic representation of the cyclicpeptide formed from **27** coordinate with Fe^{2+}.

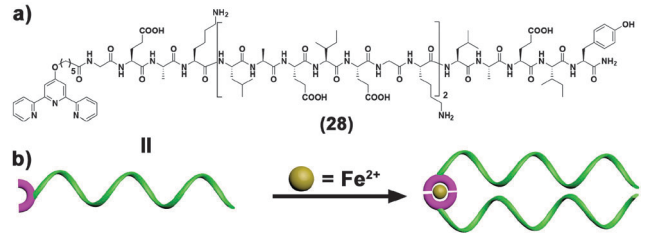

Fig. 17 (a) Structure of terpyridine modified peptide **28**. (b) Schematic representation of the dimer formed from **28** upon coordination with Fe^{2+}. Reproduced with permission from ref. 59. Copyright 2004, CSIRO Publishing.

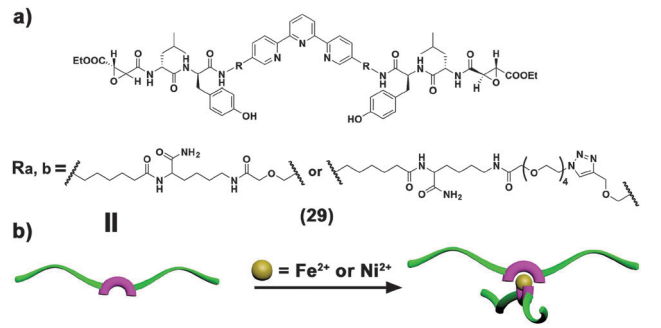

Fig. 18 (a) Molecular structure of triskelion peptides **29a**, **29b**. (b) General principle of the construction of protein–inorganic hybrid compounds. Reproduced with permission from ref. 60. Copyright 2010, Wiley-VCH Verlag GmbH & Co. KGaA.

metalloprotein was the result of the interplay between metal-ion complexation and protein folding. At high Fe^{2+} ion concentrations, folding and organization is dominated by the formation of octahedral $[Fe^{II}(tpy)_2]$ complexes by Fe^{2+} coordination with two terminal terpyridine moieties (Fig. 17b).[59]

The terpyridine residues can also be positioned in the middle of the peptide. Bogdan *et al.* designed and synthesized a series of such peptides for the directed construction of inorganic–hybrid-protein frameworks. The synthesized ligands were composed of a terpyridine moiety conjugated to two epoxysuccinyl peptides on either side (peptides **29a**, **29b**, Fig. 18a). Complexation of the synthesized peptides with Fe^{2+} and Ni^{2+} ions was found to proceed similar to that of the parent terpyridine leading to cross-linked peptide dimers (Fig. 18b).[60]

Terpyridine moieties were also combined with bipyridine and pyridine ligands in one peptide ions. Coppock *et al.* synthesized heterofunctional artificial tripeptide **30** that self-assembled into an antiparallel duplex by coordination of three Cu^{2+} ions. The tripeptide contains three pendant ligands, pyridine, methyl bipyridine, and terpyridine, along an amino-ethylglycine backbone (Fig. 19a). These ligands chelate three Cu^{2+} ions, forming two $[Cu(tpy)(py)]^{2+}$ complexes and one $[Cu(bpy)_2]^{2+}$ complex which cross-linked two strands to give a trimetallic supramolecular structure (Fig. 19b).[61]

As an example for terpyridine used in peptide nucleic acid (PNA) self-assembly, Bezer *et al.* reported the self-assembly of ss or ds PNA to interduplex complexes. Consequently, PNA

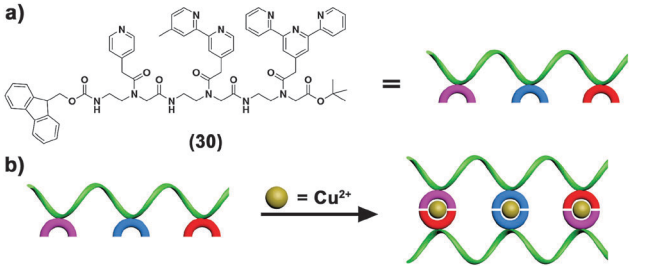

Fig. 19 (a) Molecular structure of tripeptide **30**. (b) Molecular model of $[Cu_3(\mathbf{30})_2]^{6+}$ formed from tripeptide **30**. Reproduced with permission from ref. 61. Copyright 2011, American Chemical Society.

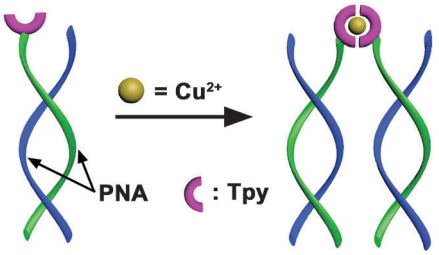

Fig. 20 Structure of a metalloprotein consisting of two folding motif sequences of a N-terminal terpyridine-modified coiled-coil protein. Reproduced with permission from ref. 62. Copyright 2011, American Chemical Society.

duplexes that contained one or two terpyridines instead of a nucleobase formed $[Cu(tpy)_2]^{2+}$ complexes in the presence of Cu^{2+} (Fig. 20).[62]

Fujimura *et al.* further applied terpyridine ligands in a self-assembling cyclic peptide. They designed cyclic tri-β-peptide **31** which has three terpyridine metal ligand binding sites (Fig. 21a).

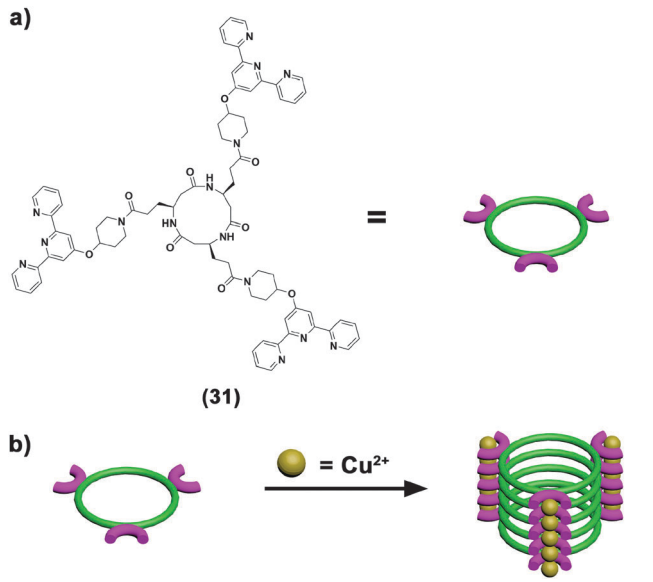

Fig. 21 (a) Chemical structure of peptide **31**. (b) Schematic shows the self-assembly process of this cyclic peptide with Cu^{2+}. Reproduced with permission from ref. 63. Copyright 2007, American Chemical Society.

The terpyridine ligands covered the surface of the assemblies, keeping the terpyridine plane parallel to the ring plane of the cyclic tri-β-peptides. The cyclic peptides formed a rod-shaped molecular assembly by coordinating with Cu^{2+} (Fig. 21b).[63]

Different from bipyridine, the self-assembly of terpyridine modified peptides often induces dimer assemblies by coordination with metal ions. This depends on the properties of the tridentate chelating terpyridine–ligand.

3.4 Self-assembly *via* nitrilotriacetic acid coordination

Nitrilotriacetic acid (NTA) is an aminopolycarboxylic acid that forms coordination compounds with metal ions-such as Ca^{2+}, Cu^{2+}, and Fe^{3+}.[64] The uses of NTA are similar to those of ethylene diamine tetraacetic acid (EDTA), as both are chelating agents. In contrast to EDTA, NTA is highly biodegradable. NTA confers great advantages when incorporated into peptides for self-assembly with metals. First, NTA-modified peptides are capable of self-assembly upon addition of an external stimulus such as metal ions. Second, the trigger is compatible with living cells (as it uses Ca^{2+} instead of rather toxic transition metals) and could cause self-assembly under physiological conditions. Third, the assembly of the scaffold may be fully reversible under mild conditions. In many cases, NTA residues act as an anchor to bind with metal ions of histidine tagged proteins in order to introduce a fluorescent quencher or to immobilize histidine-tagged proteins on biosensor surfaces.

Histidine tagged proteins (often used tags are hexahistidine: His_6 or decahistidine: His_{10}) could interact with multivalent NTA chelator heads (bis-, tris-, and tetrakis-NTA for example). Many researchers used this property to design new fluorescent probes for variety of applications. Wruss *et al.* used His_6 tagged protein self-assembly with tris-NTA to detect viruses. V33333 is one of the highest affinity very-low-density lipoprotein receptors for viruses. Wruss *et al.* substituted the N-terminus of V33333-His_6 with a fluorescein residue and attached a quencher dye molecule at the NTA moiety (Fig. 22a). Upon addition of 1–2 μM free Ni^{2+},

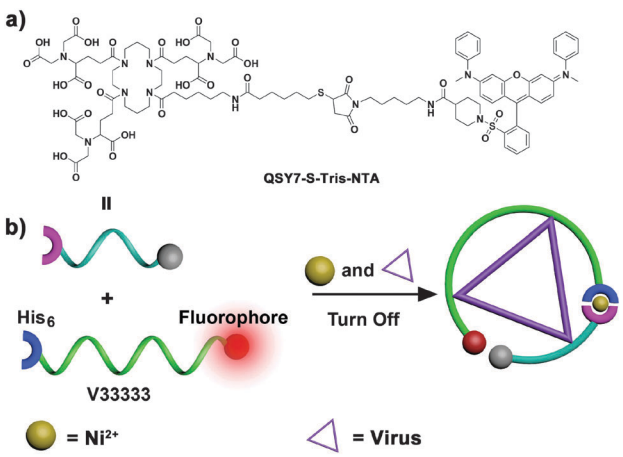

Fig. 22 (a) Structure of QSY7-S-Tris-NTA. (b) The very-low-density lipoprotein-receptor derivative V33333-His_6 was labelled at its N-terminus with 6-carboxyfluorescein and QSY7-S-tris-NTA was bound to the C-terminal His_6 Tag for detection of the virus. Reproduced with permission from ref. 65. Copyright 2009, American Chemical Society.

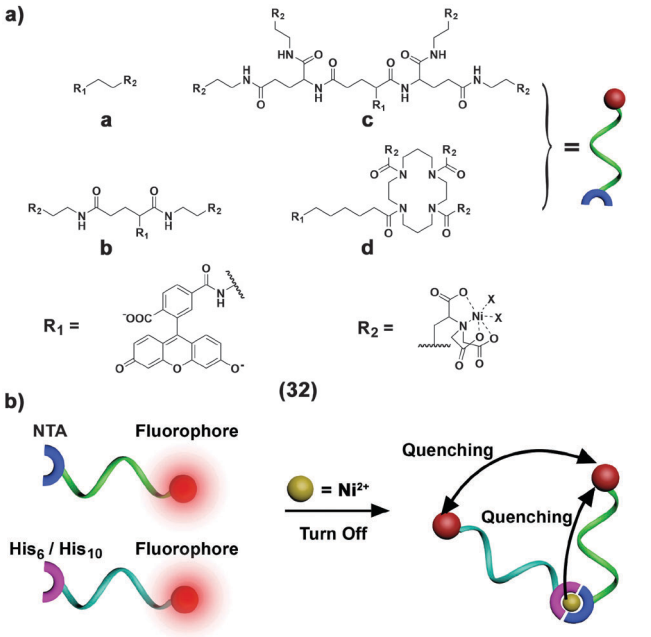

Fig. 23 (a) Chemical structure of the carboxyfluorescein-conjugated chelator heads **32a–d**: mono/bis/tris/tetrakis-NTA-fluo. (b) Schematic representation of the NTA-fluo complex with fluorescein-labeled His_6 in the presence of Ni^{2+}. Quenching processes leading to fluorescence decay upon complex formation are indicated by arrows. Reproduced with permission from ref. 66. Copyright 2005, American Chemical Society.

they found that the tris-NTA peptide was conjugated with the His_6 tagged protein (Fig. 22b). The interaction of this conjugate with a virus leads to a fluorescence resonance energy transfer from the fluorescein residue to the quencher, which can be used to detect virus particles.[65]

Later, Lata *et al.* created chemical recognition units that bind oligohistidine tags with high affinity for use as tools for selectively attaching spectroscopic probes and other functional elements to recombinant proteins. Compounds **32a–d** contained 1–4-NTA moieties, which additionally contained an amino group to which fluorescein, a sensitive reporter probe, was coupled (Fig. 23a). These multivalent chelator heads (MCH) (bis-, tris-NTA for example) could interact with hexahistidine (His_6)- and decahistidine (His_{10})-tagged proteins. Upon complex formation of these proteins with Ni^{2+}-loaded chelator–carboxyfluorescein conjugates, strong fluorescence quenching was observed (Fig. 23b).[66]

By using a NTA moiety to coordinate a His_6-tagged protein, Tinazli *et al.* successfully immobilized His_6-tagged proteins on chip surfaces. Bis-NTA **33** was linked through an oligoethylene glycol linker to alkyl thiols for the formation of mixed self-assembled monolayers on gold (Fig. 24a). The surface immobilized bis-NTA then conjugates with His_6-tagged proteins by metal coordination (Fig. 24b). These multivalent chelator chips allow specific, high-affinity, reversible, long-term immobilization of His_6-tagged proteins.[67] As an alternative to thiols to connect with the gold template, Heeres *et al.* envisioned D-biotin–tris-NTA hybrid compound **34** as the linkage between the biosensor surface (functionalized with streptavidin which

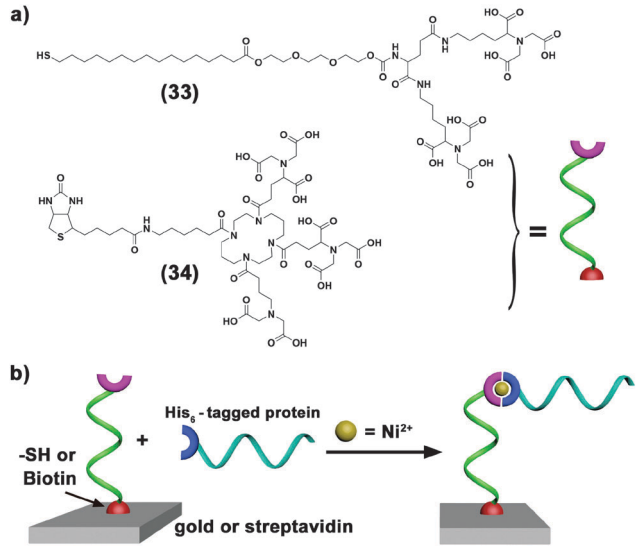

Fig. 24 (a) Chemical structure of compounds **33**, **34**; (b) schematic representation of thiol and biotin functionalized NTA compounds fixed on the chip surface and immobilize His_6-tagged proteins. Reproduced with permission from ref. 67 and 68. Copyright 2005, 2009, Wiley-VCH Verlag GmbH & Co. KGaA & American Chemical Society.

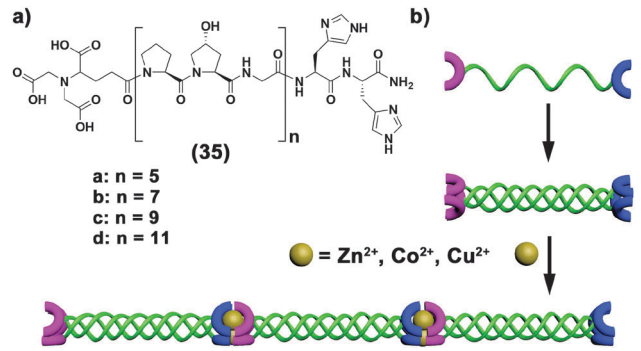

Fig. 25 (a) Structure of collagen-based peptides containing metal-binding ligands and various numbers of repeating POG units. All four peptides contain a NTA unit at the N-terminus (red) and a His_2 unit at the C-terminus (blue). (b) Schematic representation of the metal-promoted assembly of the resulting triple-helical collagen-based peptides. Reproduced with permission from ref. 69 and 70, Copyright 2009, 2012 American Chemical Society.

assembles with biotin residues) and a His_6-tagged protein of interest (Fig. 24a and b).[68]

When incorporating both natural and artificial coordination ligands into a peptide, researchers could obtain some interesting nanostructures. For example, Pires *et al.* reported a series of artificial peptides composed of histidine moieties and functional NTA/IDA groups. In the first case, they designed central collagen-based peptide **35c** (NCoH9) composed of nine repeating units of the tripeptide POG and two distinct metal-binding ligands (NTA, histidine) at each terminus (Fig. 25a). Individual strands **35c** firstly formed triple helices which clustered six histidines at one end and three NTAs at the other end. The addition of metal ions was found to promote a linear assembly of **35c** by the head to tail conjugation of such triple helices by the metal ions (Fig. 25b).[69]

Later, they demonstrated the metal-ion-promoted supramolecular assembly of collagen-based peptide triple helices into distinct morphologies that were controlled by the number of POG repeating units. In this study, they synthesized four collagen-based peptides **35a–d** that incorporated 5, 7, 9, and 11 POG repeating units (Fig. 25a). The number of repeating units effectively influences the stability of the collagen triple helix thus changing the structure of assemblies formed from **35a–d**. Similar to their previous research, collagen peptides **35b** (NCoH7), **35c** (NCoH9), **35d** (NCoH11) formed stable triple helices and further assembled into microsaddle structures by metal-promoted assembly (Fig. 25b). In contrast collagen peptides **35a** (NCoH5) neither formed a stable triple helix nor participated in supramolecular assembly with added metal ions.[70]

3.5 Self-assembly *via* iminodiacetate acid coordination

The iminodiacetate acid (IDA) acts as a tridentate ligand that forms a metal complex with different groups like histidine. Application of iminodiacetate has great advantages in biochemistry.[71] For example, the ability of Cu^{2+} conjugated to iminodiacetic acid (IDA–Cu^{2+}) to interact preferentially with the histidine residues of proteins has been utilized in two-dimensional crystallization of proteins at lipid bilayers,[72,73] purification of peptides and proteins,[74–76] probing the accessibility of histidine residues,[77] mapping the distribution of the histidine residues on the protein surface,[78] and design of inhibitors.[79,80] Moreover, IDA has also been used in metal-responsive hydrogelation systems in which it acts as an intramolecular chelating ligand.

By using the IDA functional group, Banerjee *et al.* devised a novel strategy to enhance the binding affinity of an active-site-directed inhibitor for carbonic anhydrase. They attached IDA–Cu^{2+} (*via* triethylene glycol as a spacer) to a known enzyme inhibitor and synthesized a new inhibitor **36** (Fig. 26a). The benzenesulfonamide group in the inhibitor is well known to interact with the active site of carbonic anhydrase. By connecting it with an IDA–Cu^{2+} moiety, IDA–Cu^{2+} could further interact with one of the surface-exposed histidine (His-4) residues thus increasing the binding affinity of the new inhibitor relative to the benzenesulfonamide group by *ca.* 40-fold (Fig. 26b).[81]

Based on their previous work, Banerjee *et al.* also attached IDA–Cu^{2+} to benzenesulfonamide *via* spacers of various chain lengths. They synthesized two ligands, **38** and **37**, in which the distances between the Cu^{2+} and NH_2 groups of sulfonamide are 29 and 22 Å (Fig. 26a), respectively. The binding affinity of **38** for recombinant human carbonic anhydrase I (hCA-I) was similar to that of benzenesulfonamide alone, whereas that of **37** was *ca.* two orders of magnitude higher, making **37** a potential ligand or an inhibitor of hCA-1 (Fig. 26c).[82]

Micklitsch *et al.* reported a metal-responsive peptide-based hydrogel in which gel formation is triggered by zinc binding. Peptide **39** is a 20-residue β-hairpin peptide (BHP) composed of two amphiphilic β strands (Fig. 27a). In aqueous solution and in the absence of metal ions, the peptide is unfolded and soluble. Addition of Zn^{2+} ions results in chelation of the metal by a synthetic ligand in the peptide, which in turn triggers

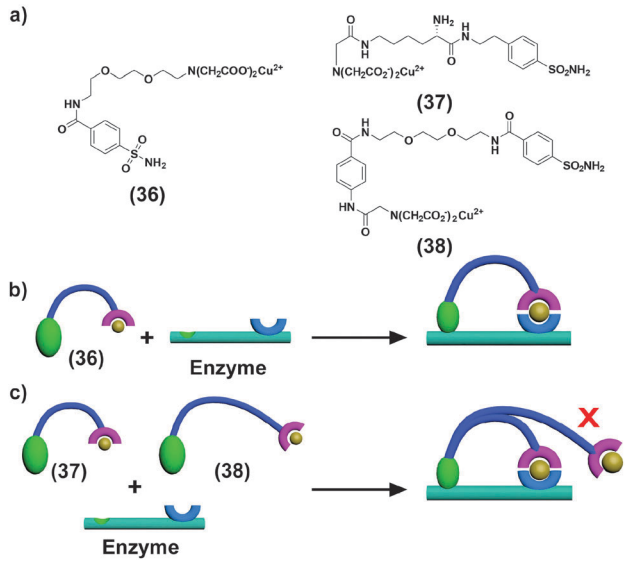

Fig. 26 (a) Structure of compounds **36–38**. The active-site-directed inhibitor benzenesulfonamide and the histidine-binding residue IDA–Cu^{2+} were connected *via* a triethylene glycol spacer. (b) Surface-assisted enhancement of the binding affinity of the inhibitor. The inhibitor is bound both to the active-site pocket and a surface-exposed histidine residue of the enzyme at the same time. (c) Binding affinity depends on the linker length. Whereas the second binding site of the ligand with a shorter linker can easily loop around and interact with a complementary peripheral site, the ligand containing a longer spacer fails to find a complementary interacting site on the surface enzyme. Reproduced with permission from ref. 81 and 82. Copyright 2004, 2005 American Chemical Society.

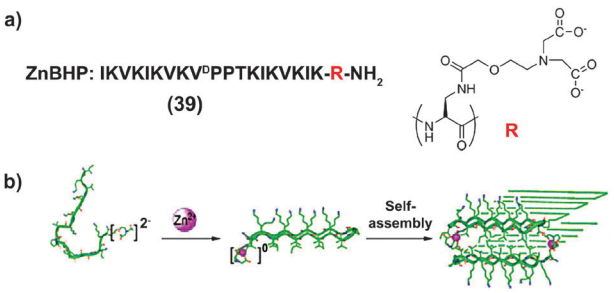

Fig. 27 (a) Primary sequence of β-hairpin peptide **39** which contains the synthetic amino acid 3-amidoethoxyaminodiacetoxy-2-amino propionic acid as a metal binding site. (b) Proposed mechanism of metal-triggered folding and the self-assembly of peptide **39**. Reproduced with permission from ref. 83. Copyright 2011, Wiley-VCH Verlag GmbH & Co. KGaA.

peptide folding and its subsequent self-assembly into a β-sheet-rich moderately rigid, fibrillar hydrogel (Fig. 27b).[83]

The controlled assembly of peptide-based copolymers into novel functional biomaterials may potentially lead to significant technological advances. Pires *et al.* used a block-like assembly process on tandem repeats of collagen peptides to expand the repertoire of accessible architectures based on collagen peptides. In this case, they synthesized two collagen blocks **40** and **41** which contained mono-functional chelating groups (with one IDA, or one histidine at each terminus, Fig. 28a). When the collagen peptides assembled in the presence of metal ions (Zn^{2+}, Cu^{2+}, and Ni^{2+}), the triple helices

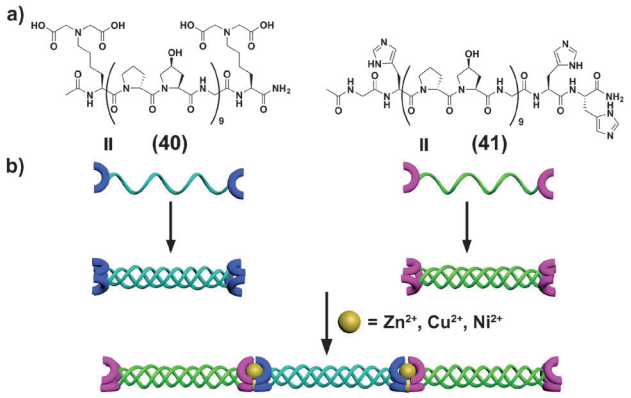

Fig. 28 (a) Sequences of collagen-based peptides **40** (IdaCol) and **41** (HisCol); (b) schematic representation of the metal-promoted assembly of triple-helical collagen-based peptides. Reproduced with permission from ref. 84. Copyright 2011, American Chemical Society.

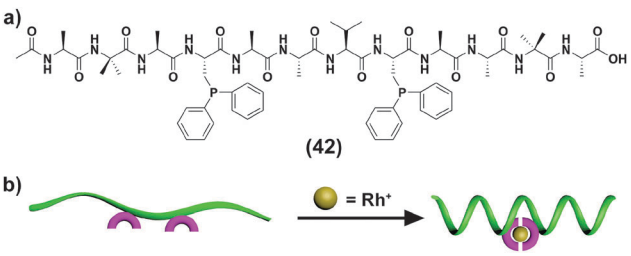

Fig. 29 (a) Chemical structure of **42**. (b) Induction of an α-helix by binding of rhodium(I) to phosphanoserine residues. Reproduced with permission from ref. 87. Copyright 1996, American Chemical Society.

were connected by metal ions at each terminus and formed periodically banded microstructures (Fig. 28b). This exquisite control of higher-order co-assembly mimics the banding observed in natural collagen fibers.[84]

3.6 Self-assembly *via* phosphane ligand coordination

Phosphorus is a good donor atom for the formation of coordination compounds. The coordination chemistry of phosphane ligands was pioneered by Mann, Chatt, and others beginning in the 1930s.[85,86] Phosphane ligands can act as intramolecular and intermolecular linkers to conjugate peptides to macrocyclic peptides or peptide nanostructures. Furthermore, phosphine moieties have been introduced into cyclic peptides for the precise construction of homo- and hetero-dimeric cyclic peptides.

For intramolecular self-assembly, Gilbertson *et al.* synthesized an artificial amino acid which contained two diphenylphosphano serine units in i and $i + 4$ positions (Fig. 29a). The two diphenylphosphano moieties of the peptide could bind to Rh^{+} to induce an α-helical conformation in the peptide (Fig. 29b).[87]

Stupp *et al.* introduced a series of artificial peptides which also self-assemble with the aid of phosphane ligands. They introduced peptide amphiphiles (PAs) which may self-assemble into supermolecules in response to various stimuli, including the presence of metal ions.[88] PAs **43–45** are composed of a peptide segment containing 6–12 amino acids coupled *via* an

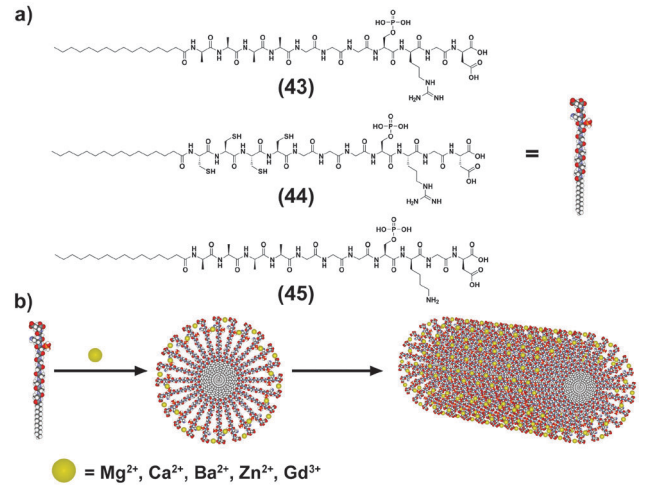

Fig. 30 (a) Structure of PA-**43–45**. (b) Scheme of PA-**43–45** nanofiber self-assembly. Reproduced with permission from ref. 90 and 91. Copyright 2005, 2006, Elsevier Ltd & Wiley-VCH Verlag GmbH & Co. KGaA.

amide bond to a fatty acid chain (Fig. 30a). They found that treatment of a solution of these PA with divalent Ca^{2+} immediately caused gelation of the solution by metals coordinating with the phosphane groups of the PA (Fig. 30b).[89] Later, they improved the self-assembly of PA with polyvalent metal ions. In this case, PA molecules can assemble into robust nanofibrillar networks at physiological pH upon the addition of polyvalent metal ions such as Mg^{2+}, Ca^{2+}, Ba^{2+}, Zn^{2+}, Gd^{3+}. These nanostructured networks even form in the presence of tissue fluids or cell culture media that contain these ions.[90,91]

Selective coordination properties of the appropriate metal with phosphine in a cyclic peptide allow us to precisely control the formation of homo- or heterodimers between two such peptides. Panciera *et al.* designed cyclic peptides **46**, **47** based on 3-aminocyclohexane-carboxylic acid (γ-Ach) for this purpose which contained phosphine or alkyne moieties attached to an amino acid side chain (Fig. 31a). In the presence of metals such as Pd^{2+} or Au^{+}, metal coordination leads to the formation of dimers (Fig. 31b). Selective heterodimer formation can be achieved by forming gold acetylide complexes. Homodimers can be formed by coordination with bidentate phosphines.[7]

3.7 Self-assembly *via* crown-ether coordination

Crown ethers are cyclic compounds that consist of a ring containing several ether groups. They can strongly bind certain cations. The oxygen atoms are well situated to coordinate with a cation located at the interior of the ring, whereas the exterior of the ring is hydrophobic. Crown ethers have been used to stabilize the conformation of peptides.

Voyer *et al.* described a crown ether functionalized peptide **48** (Fig. 32a). In the absence of metal ions the peptide adopts a β-sheet type structure which is not affected upon addition of sodium or potassium cations. However, Cs^{+} is able to form a sandwich complex with the crown ether units and thus leads to macrocyclization (Fig. 32b). In order to form the complex,

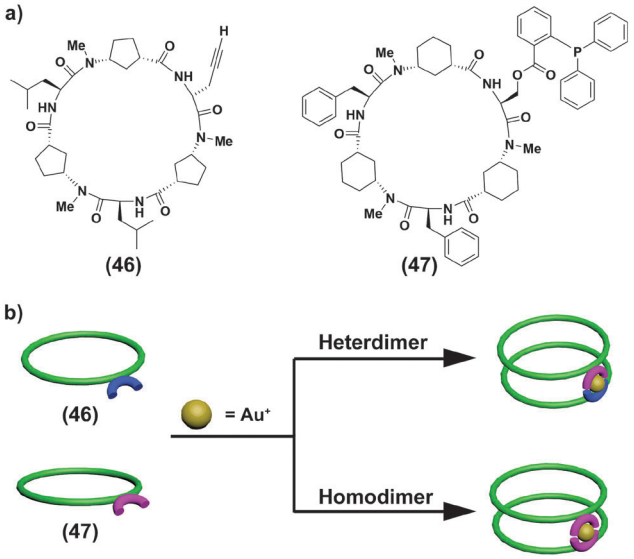

Fig. 31 (a) Cyclic peptides **46**, **47** containing 3-aminocyclohexane-carboxylic acid γ-Ach and γ-Acp residues. (b) Structure of heterodimer **D(*S*)47–47[Au]** and the observed homodimer **D(*S*)46–47[Au]** resulting from mixing **46** and **47** upon interaction with gold(I). Reproduced with permission from ref. 7. Copyright 2014, Wiley-VCH Verlag GmbH & Co. KGaA.

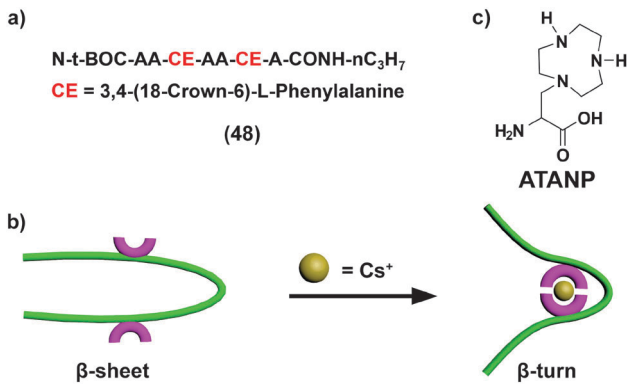

Fig. 32 (a) Schematic structure of peptide **48**. (b) Schematic representation of the β-sheet to β-turn conformational change induced by addition of metal ions. (c) Chemical structure of triazanonane (ATANP) residue used in this helix–loop–helix assembly.

peptide **48** had to change from a β-sheet conformation to a β-turn conformation.[92]

Rossi *et al.* reported three helix–loop–helix peptides which bear the triazanonane (ATANP) unit (Fig. 32c). Upon addition of Zn^{2+}, the α-helix conformation of the peptides was stabilized. They further used the Zn^{2+} complexes of the three peptides to successfully catalyze the transesterification of the RNA model substrate 2-hydroxypropyl-*p*-nitrophenyl phosphate (HPNP).[93]

4. Metal-triggered self-assembly using natural ligands

Metal-triggered self-assembly of natural peptides pertain to natural coordinating ligands, including histidine, cysteine,

tryptophan and glutamic acid *etc.* (Table 1). There has been increasing interest in the metal-triggered self-assembly of artificial peptides using natural coordination ligands, as synthetic biology is used to understand nature through mimicry.[94] This approach has provided us with the opportunity to develop novel biological systems and new functions.

4.1 Self-assembly *via* histidine coordination

Histidine is an α-amino acid with an imidazole functional group. The imidazole ring of histidine bears a nitrogen lone pair which is able to coordinate with metal ions. This makes histidine a common group to control the structure of metal organic structures. In many metallopeptides, histidine moieties act as co-initiators to reconstruct the structure of the proteins. For example, the self-assembly of histidine rich amyloid-β peptides (Aβ peptides) with metal ions has been considered to be one of the most important causes in Alzheimer's disease. Many chemists have done a lot of work on the self-assembly of amyloid-β peptides with metal ions for a better knowledge of amyloid structures and mechanisms in Alzheimer's disease.[95–97] Besides, histidine residues were used as an anchor to bind metal ions or as a connector to assemble collagen peptides. Histidine moieties also act as stabilizers to stabilize helical coiled coil peptides in the presence of transition-metal ions.

In order to figure out the possible mechanism of Alzheimer's disease, Lynn and co-workers did a lot of work on the influence of metal ions to induce amyloid fibril formation.[98,99] For example, they described conditions which radically alter the relative rates of nucleation and propagation in amyloid formation. To emphasize the impact of these interactions on fibril formation, they focused on the most critical histidine residues at positions 13 and 14 of full length β-amyloid Aβ(1–42) (middle sequence YEVHHQKLVFFA; Fig. 33). Upon addition of Zn^{2+}, the nucleation and propagation rates of self-assembly for this peptide increased significantly.[100] Later, they found that by restricting the possible metal binding sites of the amyloid peptide, Zn^{2+} can specifically control the rate of self-assembly and dramatically regulate amyloid morphology *via* distinct coordination environments. Hence, their studies open up the possibility of better resolving the precise mechanism of amyloid self-assembly.[101]

Yang *et al.* used peptide **49** (Ac-AEAEAKAKAEAEAKAK-NH_2) as a model peptide to coordinate metal ions. The peptide was modified by adding a functional Cu^{2+}-binding group, GGH25 (G: glycine; H: histidine), to the C-terminus of **49**. The molecular structure of peptide **49**, known as EAK16 (II) GGH, is shown in Fig. 34a. The designed peptide may bind strongly to copper to form short or long fibres, leading to highly stable metal–peptide complexes (Fig. 34b).[102]

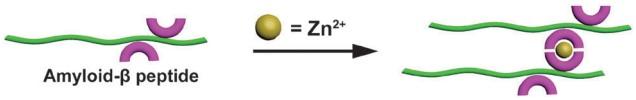

Fig. 33 Zn^{2+}-induced self-assembly of two β-amyloid strands. Reproduced with permission from ref. 100 and 101. Copyright 2002, 2006, American Chemical Society.

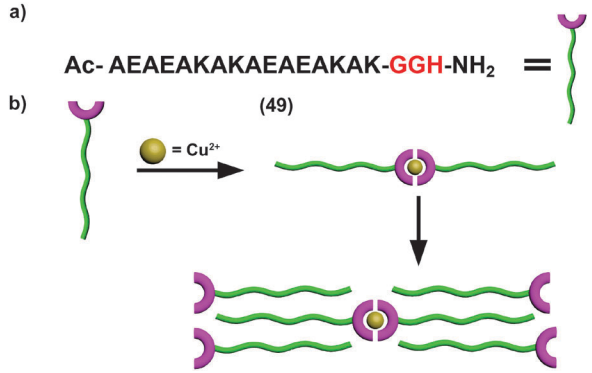

Fig. 34 (a) Schematic diagram of peptide **49**. (b) Possible self-assembly mode of peptide **49** coordinated to Cu^{2+}. Reproduced with permission from ref. 102, Copyright 2006, American Chemical Society.

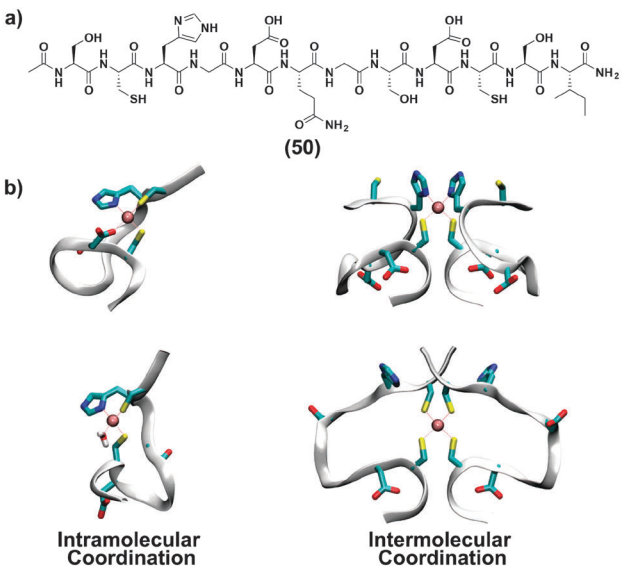

Fig. 35 (a) Schematic structure of Ac-SCHGDQGSDCSI-NH_2 **50**. (b) Models of peptide **50** coordinated to different equivalents of Cd^{2+} generated using molecular modeling.

Inspired by the metal binding loops of metal-responsive transcriptional activators, Jancsó *et al.* designed dodecapeptide **50** (Fig. 35a). In the absence of metal ions, the peptide mainly displays characteristics of a random coil. This protein forms different types of structures upon addition of various amounts of Cd^{2+}. One equivalent leads to folding of the peptide into a cyclic aggregate (intramolecular coordination of Cd^{2+}) whereas in the presence of only half an equivalent a cyclic dimer is formed (intermolecular coordination) (Fig. 35b).[103]

Hsu *et al.* reported highly effective and simple means to assemble small, synthetic collagen-related peptides into various higher-order structures by utilizing metal–histidine coordination. They synthesized two short, collagen-related peptides **51** and **52**, HG $(PPG)_9GH(X_9)$ (PPG refers to Pro–Pro–Gly) and $HG(PPG)_4(PHG)(PPG)_4GH$ (PHG), in which histidine residues are incorporated as metal-binding sites (Fig. 36a). The two peptides could form marginally stable collagen triple helices.

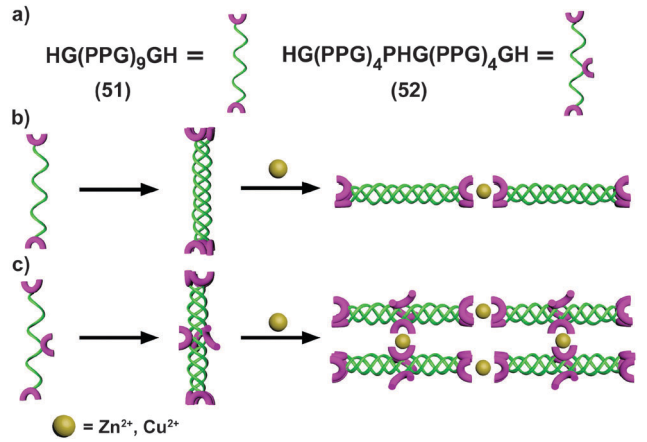

Fig. 36 (a) Chemical structure of peptides **51**, **52**. (b) Illustration of a PPG triplet and self-assembly of peptide **51** *via* metal–histidine coordination. (c) Illustration of a PPG triplet and self-assembly of peptides related to PPG repeat collagen **52** *via* metal–histidine coordination. Reproduced with permission from ref. 104. Copyright 2012, American Chemical Society.

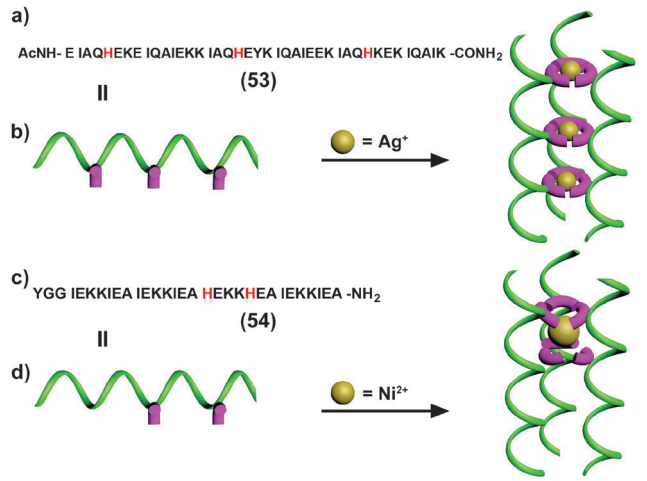

Fig. 37 (a, c) Amino acid sequence of **53**, **54**. (b, d) Model of **53**, **54** self-assembly in the presence of metal ions. Reproduced with permission from ref. 105 and 106. Copyright 2008, 1998, American Chemical Society.

Upon addition of Cu^{2+} and Zn^{2+}, the histidine groups at the end of peptide **51** could coordinate with metal ions by end-to-end assembly to form nanofibrils (Fig. 36b). However, histidine groups in the middle of **52** combined the end-to-end coordination with radial assembly and led to nanofibrils and microscale spherical aggregates (Fig. 36c).[104]

Histidine self-assembly with metal ions has also been applied to helical coiled coil peptides. Dublin *et al.* reported 41-residue peptide **53** comprising six heptad repeats of a coiled-coil structural motif (Fig. 37a). Three proximal histidine residues within the structure provide a potential metal-ion binding site. The peptide had the ability to self-assemble in the presence of silver(I). The process involved selective recognition and binding of Ag^{+} with histidine groups at a complementary site within the peptide sequence (Fig. 37b). Metal coordination induced a conformational transition that resulted in a specific mode of self-association.[105] Suzuki *et al.*

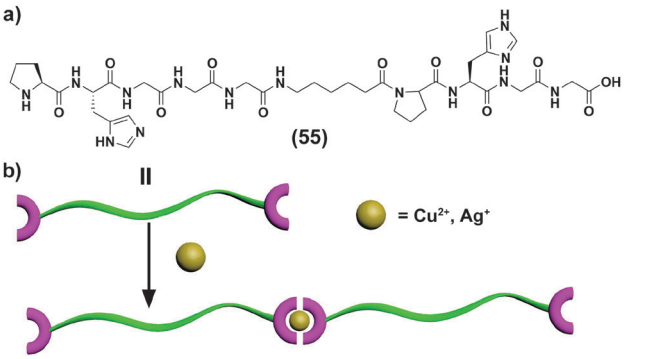

Fig. 38 (a) Molecular structure of peptide **55**. (b) Possible mode of **55** self-assembly in the presence of metal ions. Reproduced with permission from ref. 107. Copyright 2007, Elsevier Ltd.

designed a triple-stranded parallel α-helical coiled coil with the amino acid sequence $YGG(IEKKIEA)_4$ (Fig. 37c). A metal-binding site located in the hydrophobic core of this peptide was obtained upon replacing the two Ile residues of the third heptad with His residues. This peptide **54**, $YGG(IEKKIEA)_2(HEKKHEA)(IEKKIEA)$, was found to undergo metal-ion-induced self-assembly into a triple-stranded coiled coil (Fig. 37d). The peptide had a random structure in aqueous solution but exhibited an α-helical conformation in the presence of Ni^{2+}. Its very high affinity for Ni^{2+} suggests that its metal-binding site, the histidine residues, has a six-coordinated, octahedral geometry.[106]

Ghosh *et al.* reported bis-peptide conjugate **55**, which contained two histidine residues (Fig. 38a). The peptide could interact with copper or silver ions. FT-IR spectroscopy shows that the amide I band frequencies of peptide **55** shifted from 1656 cm^{-1} to 1635 cm^{-1} when incubated with Cu^{2+} while being slightly higher when coordinate with Ag(I). They proposed that Cu^{2+} with an intermediary soft character is likely to interact both with the histidine residues as well as the backbone amide groups, while Ag^{+} primarily coordinates to histidine preferentially in a linear Ag–N–Ag fashion, without interacting with the peptide backbone (Fig. 38b). Upon addition of metal ions, peptide **55** formed morphological and thermal stable peptide fibers (cross-section: 220–230 nm with Cu^{2+}; 15–40 nm with Ag^{+}) upon aging.[107]

4.2 Self-assembly *via* cysteine coordination

The thiol side chain in cysteine (Cys) often participates in enzymatic reactions by coordination with different metals,[108] for example with zinc in zinc finger proteins and in alcohol dehydrogenase, copper in the blue copper proteins, iron in cytochrome P450, and nickel in the [NiFe]-hydrogenases.[109] The thiol group also has a high affinity for heavy metals, so that proteins containing cysteine, such as metallothionein, will bind metals such as mercury, lead, and cadmium tightly.[110] Peptides (including α-helical peptides) which contained cysteine have been developed to induce a conformational change or enhance structural stability and to promote self-assembly upon binding to a variety of metal cations.

Knerr *et al.* designed self-assembling peptide **56** (Fig. 39a, metal-binding hairpin), which could undergo hydrogelation upon binding to heavy-metal ions such as Pb^{2+}, Zn^{2+}, Cd^{2+}

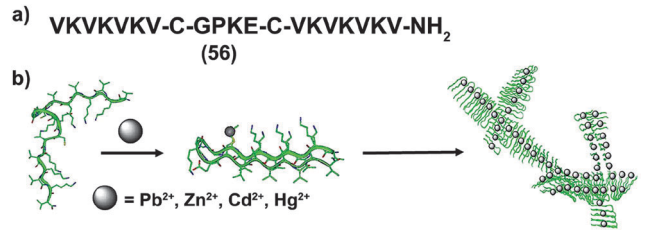

Fig. 39 (a) Primary sequence of metal-binding hairpin (MBHP) peptide **56**. (b) Proposed mechanism of metal-ion-triggered folding and self-assembly of **56**.

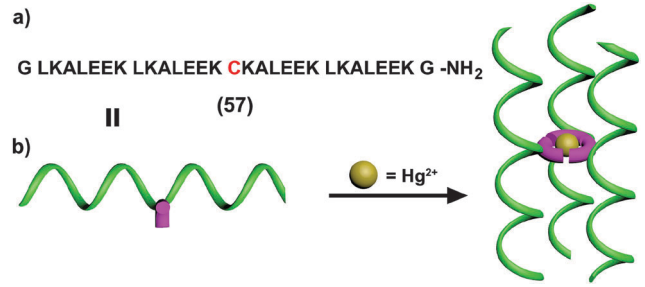

Fig. 40 (a) Amino acid sequence of *de novo* designed peptide **57**. (b) Model of peptide **57** self-assembly into three-stranded coiled coils conformation in the presence of metal ions. Reproduced with permission from ref. 112. Copyright 1998, Elsevier.

and Hg^{2+}. Its peptide sequence is composed of naturally occurring amino acids with strategically placed cysteine residues, which could chelate a number of hazardous metal ions. The peptide sequence contains two cysteine residues on opposing ends of a β-turn that can be employed as thiolate ligands for metal complexation. Upon coordination with metal ions, the peptide underwent an intramolecular β-turn folding and further self-assembled into a hydrogel network (Fig. 39b).[111]

Another example was reported by Dieckmann *et al.* They prepared peptide **57** that self-assembled in aqueous solution to form two- and three-stranded α-helical coiled coils (Fig. 40a). The peptide contains a single cysteine residue at an a position of the heptad repeat. The cysteine containing peptide could bind to Hg^{2+}. Cysteine at the a position forms three-coordinate Hg complexes at high pH, at which the trimeric aggregation state predominates whereas dimeric complexes formed at lower pH (Fig. 40b).[112]

4.3 Self-assembly *via* tryptophan coordination

Tryptophan contains a heterocyclic indole functional group, which is able to coordinate with metal ions. Tryptophan based self-assembly triggered by metal ions shows great advantages in biochemical processes.[113]

An interesting example is triskelion peptide **58** which has a tryptophan–tryptophan dipeptide unit on each of its three arms (Fig. 41a). Cu^{2+} could possibly coordinate with the amide groups and tryptophan residues of the peptide. Fig. 41b shows the possible mode of coordination for the peptide **58**–Cu^{2+} complex. Addition of Cu^{2+} altered the nanostructure

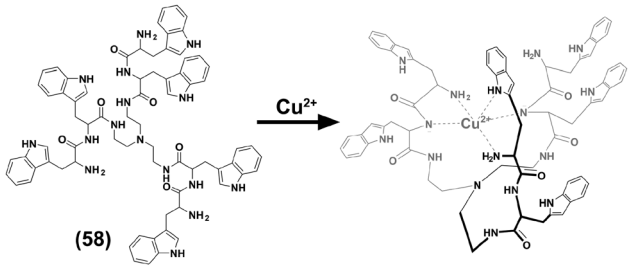

Fig. 41 Molecular structure of triskelion peptide conjugate **58** and its possible complexation mode with Cu^{2+}. Reproduced with permission from ref. 114. Copyright 2008, Elsevier.

of preformed vesicles obtained from **58** to short fibres or sheet structures.[114]

4.4 Self-assembly *via* glutamic acid coordination

Carboxyglutamic acid (Gla) is an uncommon amino acid introduced into proteins by a post-translational carboxylation of glutamic acid residues.[115] This modification is found in clotting factors and other proteins of the coagulation cascade.[116] This modification introduces an affinity for metal ions like calcium. By coordination with Ca^{2+}, carboxyglutamic acid moieties can stabilize the conformation of peptides or act as linkers to form dimeric coiled-coils.

Dai and co-workers reported Ca^{2+}-induced self-assembly in designed peptides **59a–d** with optimally spaced γ-carboxyglutamic acid residues. They firstly synthesized several carboxyglutamate (Gla)-containing neuroactive conantokin (con) peptides (**59a** and **59b**, Fig. 42). Ca^{2+} coordination induces dimer formation with strictly antiparallel chain orientation in both **59a** and **59b**

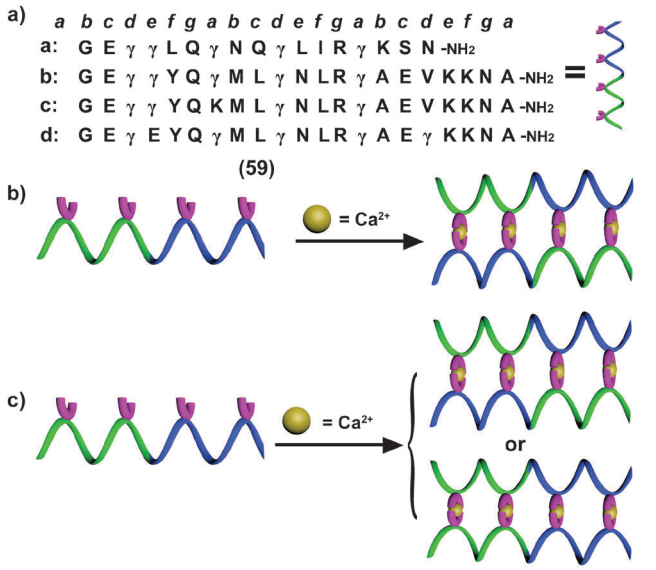

Fig. 42 (a) Primary sequences of conantokin (con) peptides. The helical heptad repeat for each peptide is denoted a–g. (b), (c) Possible mode of conantokin peptide self-assembly in the presence of metal ions. Reproduced with permission from ref. 117–119. Copyright 2004, 2009, 2011, Elsevier science Ltd & Elsevier Inc.

peptides in which the Gla residues are positioned at "*i*, *i* + 4, *i* + 7, *i* + 11" intervals (Fig. 42b).[117]

In order to probe the self-assembly in conantokin peptides with an extended Gla network, they further synthesized a **59c** (con-T) variant (**59d**) that contains five Gla residues spaced at "*i*, *i* + 4, *i* + 7, *i* + 11, *i* + 14" intervals (Fig. 42). Upon coordinating with Ca^{2+}, the peptide strands in the complex can orient in both parallel and antiparallel forms which is similar to the arrangement of a and d residues in typical heptads of coiled-coils (Fig. 42b).[118]

To further evaluate the role of Gla residues in peptide self-assembly, they extended the same Gla arrangement to designed peptides: NH_2-(γLSγEAK)$_3$-$CONH_2$ and NH_2-γLSγEAKγLSg-QANγLSγKAE-$CONH_2$. These peptides exhibit no helicity on their own, but undergo structural transitions to helical conformations in the presence of Ca^{2+}.[119]

Kohn *et al.* focused on the design of an α-helical coiled-coil peptide with the aid of Gla–metal coordination. The metal-binding coiled-coil **60** is composed of identical 35-residue polypeptide chains which contain a Cys residue allowing the formation of an interchain disulfide bridge (Fig. 43a). Upon binding with metal ions, the two disulfide-bridged peptides undergo transition from the random coil to the α-helix (Fig. 43b). The folded form is stabilized by metal binding because the metal is chelated by the Gla side chains.[120]

4.5 Self-assembly *via* catecholate coordination

Siderophores are small, high-affinity iron chelating compounds secreted by microorganisms such as bacteria, fungi and grasses. Hey contain catechols as metal-binding sites and are amongst the strongest soluble Fe^{3+} binding agents known.[121]

Albrecht *et al.* synthesized Val–Val–Val tripeptide **61** with two catechol units at each terminus (Fig. 44a). The tripeptide could coordinate to Ti^{4+} to form an interesting 3 : 2 loop-like macrocycle. One peptide acts a linker binding together two intramolecularly folded peptides to ultimately form loop-type macrocycles (Fig. 44b). If the tripeptide coordinates with Mo^{6+}, only single metallacyclopeptides are formed $[(\mathbf{61})MoO_2]^{2-}$ (Fig. 44b).[122]

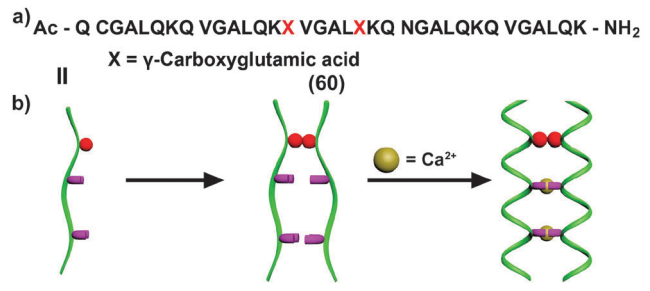

Fig. 43 (a) Sequences of native, 35-residue coiled-coil peptide **60**. (b) Possible mode of Gla2N$_x$ binding analog in **60** peptide self-assembly with metal ions. Reproduced with permission from ref. 120. Copyright 1998, American Chemical Society.

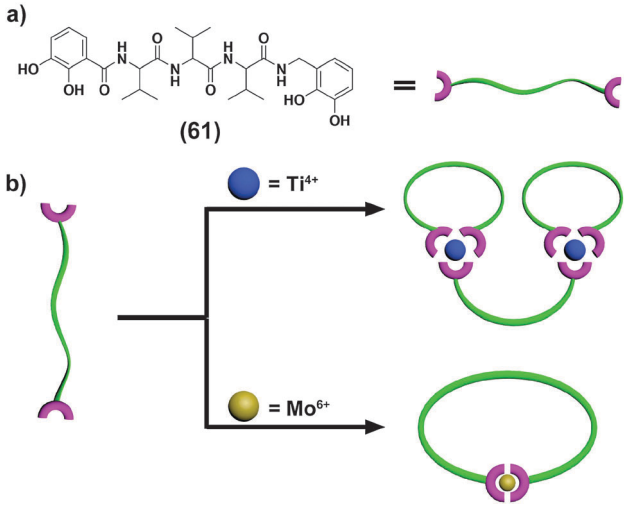

Fig. 44 (a) Chemical structure of tripeptide **61**. (b) Schematic representation of the loop-type macrocycle $[(\mathbf{61})_3Ti_2]^{4-}$ and of metallacyclopeptide $[(\mathbf{61})MoO_2]^{2-}$.

5. Conclusion and outlook

In this article, we have endeavoured to present examples for metal-ion-induced peptide self-assembly based on both natural and artificial ligands. Burgeoning biotechnological interest in this area has stimulated the discovery and development of new biomaterials that can undergo self-assembly into well-ordered and functional structures. Although the field is still in its infancy, the self-assembly of biological molecules ultimately holds promise in developing materials, devices, and technologies beyond our current imagination. Undoubtedly, future efforts will focus on the discovery, selection, and development of new, functional metal–peptide biomolecules from combinatorial peptide libraries for use in biofabrication. Furthermore, metallobiomolecules will remain highly attractive systems for further investigation in the foreseeable future, as they present diverse challenges in the structure, reactivity, kinetics, mechanism, and synthesis.

Acknowledgements

We thank the National Basic Research 973 Program (2013CB733700), the Fundamental Research Funds for the Central Universities (WJ1213007), the Program of Shanghai Pujiang (K100-2-1275), the Innovation Program of Shanghai Municipal Education Commission (J100-2-13104), and the National Natural Science Foundation of China (21341001) for financial support.

References

1 E. A. Permyakov, *Metalloproteomics*, John Wiley & Sons, Inc., 2009, pp. 7–19.
2 J. C. Lewis, *ACS Catal.*, 2013, **3**, 2954–2975.

3 M. Albrecht and P. Stortz, *Chem. Soc. Rev.*, 2005, **34**, 496–506.
4 B. Alies, C. Hureau and P. Faller, *Metallomics*, 2013, **5**, 183–192.
5 S. Zhang, *Nat. Biotechnol.*, 2003, **21**, 1171–1178.
6 D. E. Przybyla and J. Chmielewski, *Biochemistry*, 2010, **49**, 4411–4419.
7 M. Panciera, M. Amorín and J. R. Granja, *Chem. – Eur. J.*, 2014, **20**, 10260–10265.
8 J. H. Collier, J. S. Rudra, J. Z. Gasiorowski and J. P. Jung, *Chem. Soc. Rev.*, 2010, **39**, 3413–3424.
9 D. E. Przybyla, C. M. Rubert Pérez, J. Gleaton, V. Nandwana and J. Chmielewski, *J. Am. Chem. Soc.*, 2013, **135**, 3418–3422.
10 M. A. Case and G. L. McLendon, *Acc. Chem. Res.*, 2004, **37**, 754–762.
11 C. Tarabout, S. Roux, F. Gobeaux, N. Fay, E. Pouget, C. Meriadec, M. Ligeti, D. Thomas, M. IJsselstijn, F. Besselievre, D.-A. Buisson, J.-M. Verbavatz, M. Petitjean, C. Valéry, L. Perrin, B. Rousseau, F. Artzner, M. Paternostre and J.-C. Cintrat, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 7679–7684.
12 A. J. Thomson and H. B. Gray, *Curr. Opin. Chem. Biol.*, 1998, **2**, 155–158.
13 K. J. Waldron and N. J. Robinson, *Nat. Rev. Microbiol.*, 2009, **7**, 25–35.
14 C. M. Micklitsch, Q. Yu and J. P. Schneider, *Tetrahedron Lett.*, 2006, **47**, 6277–6280.
15 E. N. Salgado, R. J. Radford and F. A. Tezcan, *Acc. Chem. Res.*, 2010, **43**, 661–672.
16 G. Berthon, *Pure Appl. Chem.*, 2009, **67**, 1117–1240.
17 D. Vomasta, C. Högner, N. R. Branda and B. König, *Angew. Chem., Int. Ed.*, 2008, **47**, 7644–7647.
18 A. Winter, M. Gottschaldt, G. R. Newkome and U. S. Schubert, *Curr. Top. Med. Chem.*, 2012, **12**, 158–175.
19 C. Rodríguez-Rodríguez, M. Telpoukhovskaia and C. Orvig, *Coord. Chem. Rev.*, 2012, **256**, 2308–2332.
20 G. R. Lenz and A. E. Martell, *Biochemistry*, 1964, **3**, 745–750.
21 I. W. Hamley, *Angew. Chem., Int. Ed.*, 2007, **46**, 8128–8147.
22 T. P. Knowles, A. W. Fitzpatrick, S. Meehan, H. R. Mott, M. Vendruscolo, C. M. Dobson and M. E. Welland, *Science*, 2007, **318**, 1900–1903.
23 H. M. König and A. F. M. Kilbinger, *Angew. Chem., Int. Ed.*, 2007, **46**, 8334–8340.
24 O. Khakshoor and J. S. Nowick, *Curr. Opin. Chem. Biol.*, 2008, **12**, 722–729.
25 C. Tomasini and N. Castellucci, *Chem. Soc. Rev.*, 2012, **42**, 156–172.
26 A. Krebs, V. Ludwig, J. Pfizer, G. Dürner and M. W. Göbel, *Chem. – Eur. J.*, 2004, **10**, 544–553.
27 R. J. Brea, M. P. López-Deber, L. Castedo and J. R. Granja, *J. Org. Chem.*, 2006, **71**, 7870–7873.
28 S. J. Zuend, M. P. Coughlin, M. P. Lalonde and E. N. Jacobsen, *Nature*, 2009, **461**, 968–970.
29 R. P. Cheng, S. L. Fisher and B. Imperiali, *J. Am. Chem. Soc.*, 1996, **118**, 11349–11356.
30 E. Gazit, *Chem. Soc. Rev.*, 2007, **36**, 1263–1269.
31 M. Albrecht, P. Stortz, M. Engeser and C. A. Schalley, *Synlett*, 2004, 2821–2823.
32 B. P. Gilmartin, K. Ohr, R. L. McLaughlin, R. Koerner and M. E. Williams, *J. Am. Chem. Soc.*, 2005, **127**, 9546–9555.
33 K. Ohr, B. P. Gilmartin and M. E. Williams, *Inorg. Chem.*, 2005, **44**, 7876–7885.
34 W. W. Gerhardt and M. Weck, *J. Org. Chem.*, 2006, **71**, 6333–6341.
35 H. Jiang, B. B. Kasten, H. Liu, S. Qi, Y. Liu, M. Tian, C. L. Barnes, H. Zhang, Z. Cheng and P. D. Benny, *Bioconjugate Chem.*, 2012, **23**, 2300–2312.
36 M. V. Tsurkan and M. Y. Ogawa, *Inorg. Chem.*, 2007, **46**, 6849–6851.
37 M. V. Tsurkan and M. Y. Ogawa, *Chem. Commun.*, 2004, 2092–2093.
38 M. Panciera, M. Amorín, L. Castedo and J. R. Granja, *Chem. – Eur. J.*, 2013, **19**, 4826–4834.
39 M. Ikemi, T. Kikuchi, S. Matsumura, K. Shiba, S. Sato and M. Fujita, *Chem. Sci.*, 2010, **1**, 68–71.
40 T. Sawada, A. Matsumoto and M. Fujita, *Angew. Chem., Int. Ed.*, 2014, **53**, 7228–7232.
41 R. Chakrabarty, P. S. Mukherjee and P. J. Stang, *Chem. Rev.*, 2011, **111**, 6810–6918.
42 A. Torrado and B. Imperiali, *J. Org. Chem.*, 1996, **61**, 8940–8948.
43 M. R. Ghadiri, C. Soares and C. Choi, *J. Am. Chem. Soc.*, 1992, **114**, 825–831.
44 M. A. Case, M. R. Ghadiri, M. W. Mutz and G. L. McLendon, *Chirality*, 1998, **10**, 35–40.
45 H. K. Munch, S. T. Heide, N. J. Christensen, T. Hoeg-Jensen, P. W. Thulstrup and K. J. Jensen, *Chem. – Eur. J.*, 2011, **17**, 7198–7204.
46 D. E. Przybyla and J. Chmielewski, *J. Am. Chem. Soc.*, 2008, **130**, 12610–12611.
47 D. E. Przybyla and J. Chmielewski, *J. Am. Chem. Soc.*, 2010, **132**, 7866–7867.
48 M. M. Pires, D. E. Przybyla and J. Chmielewski, *Angew. Chem., Int. Ed.*, 2009, **48**, 7813–7817.
49 T. Koide, M. Yuguchi, M. Kawakita and H. Konno, *J. Am. Chem. Soc.*, 2002, **124**, 9388–9389.
50 L. T. LeBruin, S. Banerjee, B. D. O'Rourke and M. A. Case, *Biopolymers*, 2011, **95**, 792–800.
51 A. K. Pal and G. S. Hanan, *Chem. Soc. Rev.*, 2014, **43**, 6184–6197.
52 H. Hofmeier and U. S. Schubert, *Chem. Soc. Rev.*, 2004, **33**, 373–399.
53 U. S. Schubert and C. Eschbaumer, *Angew. Chem., Int. Ed.*, 2002, **41**, 2892–2926.
54 B. G. G. Lohmeijer and U. S. Schubert, *Angew. Chem., Int. Ed.*, 2002, **41**, 3825–3829.
55 J. Müller, *Eur. J. Inorg. Chem.*, 2008, 3749–3763.
56 J. S. Choi, C. W. Kang, K. Jung, J. W. Yang, Y.-G. Kim and H. Han, *J. Am. Chem. Soc.*, 2004, **126**, 8606–8607.
57 M. Göritz and R. Krämer, *J. Am. Chem. Soc.*, 2005, **127**, 18016–18017.

58 E. C. Constable, C. E. Housecroft and S. Mundwiler, *Dalton Trans.*, 2003, 2112–2114.
59 G. W. M. Vandermeulen, C. Tziatzios, D. Schubert, P. R. Andres, A. Alexeev, U. S. Schubert and H.-A. Klok, *Aust. J. Chem.*, 2004, **57**, 33–39.
60 N. D. Bogdan, M. Matache, V. M. Meier, C. Dobrotă, I. Dumitru, G. D. Roiban and D. P. Funeriu, *Chem. – Eur. J.*, 2010, **16**, 2170–2180.
61 M. B. Coppock, J. R. Miller and M. E. Williams, *Inorg. Chem.*, 2011, **50**, 949–955.
62 S. Bezer, S. Rapireddy, Y. A. Skorik, D. H. Ly and C. Achim, *Inorg. Chem.*, 2011, **50**, 11929–11937.
63 F. Fujimura and S. Kimura, *Org. Lett.*, 2007, **9**, 793–796.
64 Y.-C. Li, Y.-S. Lin, P.-J. Tsai, C.-T. Chen, W.-Y. Chen and Y.-C. Chen, *Anal. Chem.*, 2007, **79**, 7519–7525.
65 J. Wruss, P. D. Pollheimer, I. Meindl, A. Reichel, K. Schulze, W. Schöfberger, J. Piehler, R. Tampé, D. Blaas and H. J. Gruber, *J. Am. Chem. Soc.*, 2009, **131**, 5478–5482.
66 S. Lata, A. Reichel, R. Brock, R. Tampé and J. Piehler, *J. Am. Chem. Soc.*, 2005, **127**, 10205–10215.
67 A. Tinazli, J. Tang, R. Valiokas, S. Picuric, S. Lata, J. Piehler, B. Liedberg and R. Tampé, *Chem. – Eur. J.*, 2005, **11**, 5249–5259.
68 J. T. Heeres, S.-H. Kim, B. J. Leslie, E. A. Lidstone, B. T. Cunningham and P. J. Hergenrother, *J. Am. Chem. Soc.*, 2009, **131**, 18202–18203.
69 M. M. Pires and J. Chmielewski, *J. Am. Chem. Soc.*, 2009, **131**, 2706–2712.
70 M. M. Pires, J. Lee, D. Ernenwein and J. Chmielewski, *Langmuir*, 2012, **28**, 1993–1997.
71 M. D. Loberg, M. Cooper, E. Harvey, P. Callery and W. Faith, *J. Nucl. Med.*, 1976, **17**, 633–638.
72 W. Frey, W. R. Schief, D. W. Pack, C. T. Chen, A. Chilkoti, P. Stayton, V. Vogel and F. H. Arnold, *Proc. Natl. Acad. Sci. U. S. A.*, 1996, **93**, 4937–4941.
73 D. W. Pack, G. Chen, K. M. Maloney, C.-T. Chen and F. H. Arnold, *J. Am. Chem. Soc.*, 1997, **119**, 2479–2487.
74 Y. Nakagawa, T.-T. Yip, M. Belew and J. Porath, *Anal. Biochem.*, 1988, **168**, 75–81.
75 N. S. Lamango, X. Zhu and I. Lindberg, *Arch. Biochem. Biophys.*, 1996, **330**, 238–250.
76 M. E. Kleinberg, D. Mital, D. Rotrosen and H. L. Malech, *Biochemistry*, 1992, **31**, 2686–2690.
77 P. P. Berna, N. T. Mrabet, J. Van Beeumen, B. Devreese, J. Porath and M. A. Vijayalakshmi, *Biochemistry*, 1997, **36**, 6896–6905.
78 M. A. Fazal, B. C. Roy, S. Sun, S. Mallik and K. R. Rodgers, *J. Am. Chem. Soc.*, 2001, **123**, 6283–6290.
79 C. T. Supuran, A. Scozzafava and A. Casini, *Med. Res. Rev.*, 2003, **23**, 146–189.
80 B. C. Roy, R. Hegge, T. Rosendahl, X. Jia, R. Lareau, S. Mallik and D. K. Srivastava, *Chem. Commun.*, 2003, 2328–2329.
81 A. L. Banerjee, M. Swanson, B. C. Roy, X. Jia, M. K. Haldar, S. Mallik and D. K. Srivastava, *J. Am. Chem. Soc.*, 2004, **126**, 10875–10883.
82 A. L. Banerjee, D. Eiler, B. C. Roy, X. Jia, M. K. Haldar, S. Mallik and D. K. Srivastava, *Biochemistry*, 2005, **44**, 3211–3224.
83 C. M. Micklitsch, P. J. Knerr, M. C. Branco, R. Nagarkar, D. J. Pochan and J. P. Schneider, *Angew. Chem., Int. Ed.*, 2011, **123**, 1615–1617.
84 M. M. Pires, D. E. Przybyla, C. M. Rubert Pérez and J. Chmielewski, *J. Am. Chem. Soc.*, 2011, **133**, 14469–14471.
85 G. Frenking, G. J. Leigh and N. Winterton, *Modern Coordination Chemistry: The Legacy of Joseph Chatt*, The Royal Society, London, 2002, p. 111.
86 W. Levason, *Organophosphorus Compounds*, John Wiley & Sons, Inc., 1990, pp. 567–641.
87 S. R. Gilbertson, X. Wang, G. S. Hoge, C. A. Klug and J. Schaefer, *Organometallics*, 1996, **15**, 4678–4680.
88 J. D. Hartgerink, E. R. Zubarev and S. I. Stupp, *Curr. Opin. Solid State Mater. Sci.*, 2001, **5**, 355–361.
89 J. D. Hartgerink, E. Beniash and S. I. Stupp, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 5133–5138.
90 E. Beniash, J. D. Hartgerink, H. Storrie, J. C. Stendahl and S. I. Stupp, *Acta Biomater.*, 2005, **1**, 387–397.
91 J. C. Stendahl, M. S. Rao, M. O. Guler and S. I. Stupp, *Adv. Funct. Mater.*, 2006, **16**, 499–508.
92 N. Voyer, J. Roby, D. Deschênes and J. Bernier, *Supramol. Chem.*, 1995, **5**, 61–69.
93 P. Rossi, P. Tecilla, L. Baltzer and P. Scrimin, *Chem. – Eur. J.*, 2004, **10**, 4163–4170.
94 M. S. Shoshan and E. Y. Tshuva, *Chem. Soc. Rev.*, 2011, **40**, 5282–5292.
95 C. J. Maynard, A. I. Bush, C. L. Masters, R. Cappai and Q.-X. Li, *Int. J. Exp. Pathol.*, 2005, **86**, 147–159.
96 K. Pagel, T. Seri, H. von Berlepsch, J. Griebel, R. Kirmse, C. Böttcher and B. Koksch, *ChemBioChem*, 2008, **9**, 531–536.
97 C. M. Rufo, Y. S. Moroz, O. V. Moroz, J. Stöhr, T. A. Smith, X. Hu, W. F. DeGrado and I. V. Korendovych, *Nat. Chem.*, 2014, **6**, 303–309.
98 J. Dong, J. M. Canfield, A. K. Mehta, J. E. Shokes, B. Tian, W. S. Childers, J. A. Simmons, Z. Mao, R. A. Scott, K. Warncke and D. G. Lynn, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 13313–13318.
99 J. Hernández-Guzmán, L. Sun, A. K. Mehta, J. Dong, D. G. Lynn and K. Warncke, *ChemBioChem*, 2013, **14**, 1762–1771.
100 D. M. Morgan, J. Dong, J. Jacob, K. Lu, R. P. Apkarian, P. Thiyagarajan and D. G. Lynn, *J. Am. Chem. Soc.*, 2002, **124**, 12644–12645.
101 J. Dong, J. E. Shokes, R. A. Scott and D. G. Lynn, *J. Am. Chem. Soc.*, 2006, **128**, 3540–3542.
102 H. Yang, M. Pritzker, S. Y. Fung, Y. Sheng, W. Wang and P. Chen, *Langmuir*, 2006, **22**, 8553–8562.
103 A. Jancsó, D. Szunyogh, F. H. Larsen, P. W. Thulstrup, N. J. Christensen, B. Gyurcsik and L. Hemmingsen, *Metallomics*, 2011, **3**, 1331–1339.
104 W. Hsu, Y.-L. Chen and J.-C. Horng, *Langmuir*, 2012, **28**, 3194–3199.

105 S. N. Dublin and V. P. Conticello, *J. Am. Chem. Soc.*, 2008, **130**, 49–51.
106 K. Suzuki, H. Hiroaki, D. Kohda, H. Nakamura and T. Tanaka, *J. Am. Chem. Soc.*, 1998, **120**, 13008–13015.
107 S. Ghosh and S. Verma, *Tetrahedron Lett.*, 2007, **48**, 2189–2192.
108 A. Dişbudak, S. Bektaş, S. Patır, Ö. Genç and A. Denizli, *Sep. Purif. Technol.*, 2002, **26**, 273–281.
109 S. J. Lippard and J. M. Berg, *Principles of bioinorganic chemistry*, University Science Books Mill Valley, CA, 1994, vol. 319.
110 D. H. Baker and G. L. Czarnecki-Maulden, *J. Nutr.*, 1987, **117**, 1003–1010.
111 P. J. Knerr, M. C. Branco, R. Nagarkar, D. J. Pochan and J. P. Schneider, *J. Mater. Chem.*, 2011, **22**, 1352–1357.
112 G. R. Dieckmann, D. K. McRorie, J. D. Lear, K. A. Sharp, W. F. DeGrado and V. L. Pecoraro, *J. Mol. Biol.*, 1998, **280**, 897–912.
113 E. U. Woehl and M. F. Dunn, *Biochemistry*, 1995, **34**, 9466–9476.
114 S. Ghosh, P. Singh and S. Verma, *Tetrahedron*, 2008, **64**, 1250–1256.
115 L. J. Sokoll and J. A. Sadowski, *Am. J. Clin. Nutr.*, 1996, **63**, 566–573.
116 E. W. Davie, K. Fujikawa and W. Kisiel, *Biochemistry*, 1991, **30**, 10363–10370.
117 Q. Dai, M. Prorok and F. J. Castellino, *J. Mol. Biol.*, 2004, **336**, 731–744.
118 Q. Dai, C. Xiao, M. Dong, Z. Liu, Z. Sheng, F. J. Castellino and M. Prorok, *Peptides*, 2009, **30**, 866–872.
119 Q. Dai, M. Dong, Z. Liu, M. Prorok and F. J. Castellino, *J. Inorg. Biochem.*, 2011, **105**, 52–57.
120 W. D. Kohn, C. M. Kay, B. D. Sykes and R. S. Hodges, *J. Am. Chem. Soc.*, 1998, **120**, 1124–1132.
121 A. E. Witter, D. A. Hutchins, A. Butler and G. W. Luther III, *Mar. Chem.*, 2000, **69**, 1–17.
122 M. Albrecht, O. Spieß, M. Schneider and P. Weis, *Chem. Commun.*, 2002, 786–787.