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FEATURE ARTICLE

Constructing multicomponent cooperative functional systems using metal complexes of short flexible peptides

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The construction of cooperative systems comprising several units is an essential challenge for artificial systems toward the development of sophisticated functions comparable to those found in biological systems. Flexible frameworks possessing various functional groups that can form weak intra/intermolecular interactions similar to those observed in biological systems have promising design features for artificial systems used to control cooperative systems. However, it is difficult to construct multiple component systems >1 nm using these flexible units by controlling the arrangement of functional units, beginning with the precise control of the cooperative switching of multiple units. In general, it is difficult for oligopeptides to form stable conformations by themselves, although they have designability and structural features suitable for the development of cooperative systems. Increasing the number of coordination bonds in peptides, which are stronger than hydrogen bonds, can be used to control the assembled peptide structures and stabilize their structures owing to the variety of coordination bonds and selective binding affinity. Thus, metal complexes of artificial short peptides have great potential for the development of multicomponent cooperative systems. Based on this concept, we have developed a series of novel metal complexes of flexible peptides and have achieved, to date, cooperative systems, the formation of giant structures, and precise control over the functional units that are the essential bases for designable multifunctional systems that can be regarded as artificial enzymes. In this feature article, we summarize these results and discuss the principal/essential design of artificial systems.

Introduction

Biological systems exhibit various functions such as recognition, reactivity, and transport by utilizing cooperative structural transformations between their functional units under a variety of conditions that are extremely difficult to achieve in artificial systems.[1] Such cooperative mechanisms are caused by the flexible frameworks constructed from biological molecules possessing functional groups that form weak interactions and support the structural transformations seen in peptides and DNA. The switching of these weak interactions induces conformational changes in the flexible frameworks and enables the existence of various metastable states exhibiting different properties that are switchable using external stimuli. The conformation of the flexible skeleton changes to maximize the sum of the various weak interactions so that the activation energy for structural transformation is increased even when the energy differences are small. This stabilization of metastable states is a key feature that leads to a wide variety of functions from a limited number of building blocks in biological systems. Therefore, to create multifunctional systems comparable to those of biological systems, one of the most promising approaches is the use of a flexible backbone possessing various functional groups that form reversible interactions.

However, using such a flexible backbone, it is extremely difficult to precisely control the arrangement of the functional groups in the assembled structure as well as the formation of giant structures >1 nm, even though they are essential for the artificial construction of multifunctional systems. Thus, to begin designing cooperative structural changes in artificial systems, it is essential to establish designable artificial motifs for flexible molecules.

Peptides provide suitable motifs for this purpose as they are used to construct multifunctional cooperative systems in nature (i.e., enzymes). In addition to their structural flexibility, the various functional groups in the main chain and/or side chains

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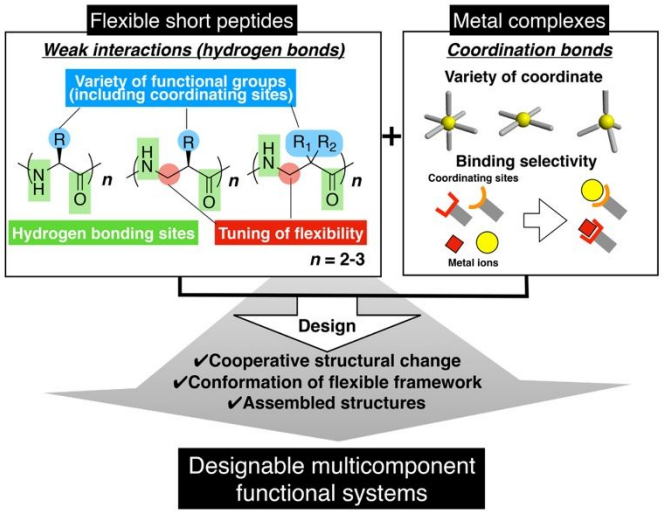


Figure 1. Conceptual illustration showing the merit of metal complexes of short peptides towards the construction of artificial multicomponent cooperative systems

of peptides, including their artificial derivatives, are suitable for controlling their conformation via the formation of various intra/intermolecular interactions (such as hydrogen, electrostatic, and π-π interactions). Furthermore, the structural flexibility of the peptide backbone can be further tuned by the type of amino acid backbone used. For example, β-amino acids are more flexible than α-amino acids because of the additional -CH_2- group. Despite their great potential for developing artificial multifunctional systems, the structural flexibility of peptides makes it difficult to artificially control their conformation or secondary structure from an oligopeptide. In addition, the multiple coupling steps used to prepare oligopeptides often prevent their large-scale synthesis as well as the design of the amino acid sequence. One solution to this problem is the use of short peptides bearing several amino acid residues assembled via reversible bond-forming processes such as hydrogen bonding[2,3] and coordination bonding.[4-23] These reversible bonds help peptides form stable structures in their assembled structures. In particular, coordination bonds are useful for the design of the assembled structure and conformation of short peptides because of the variety of coordinate structures and relatively high binding constants (compared to hydrogen bonds) determined by the combination of coordinating sites and metal ions (Figure 1). Various functions, such as selective inclusion[7,11,12,22,23], separations[9], and reactions[15], have been demonstrated using metal complexes of short peptides, based on the functional groups and/or chirality of the peptides used. In addition, cooperative behaviors have been recently reported using flexible peptides as the skeleton of metal complexes [6,8,11,20,23] suggesting their great potential for the formation of cooperative systems comparable to those observed in nature.

In this feature article, our efforts to develop metal complexes of short peptides suitable for the construction of cooperative systems are described and include the development of the basic design for the formation of giant structures and control over the arrangement of the functional units (such as metal centers),

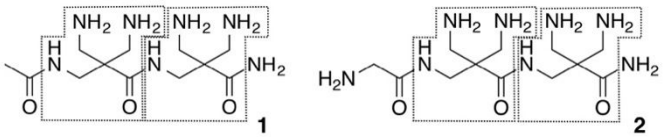


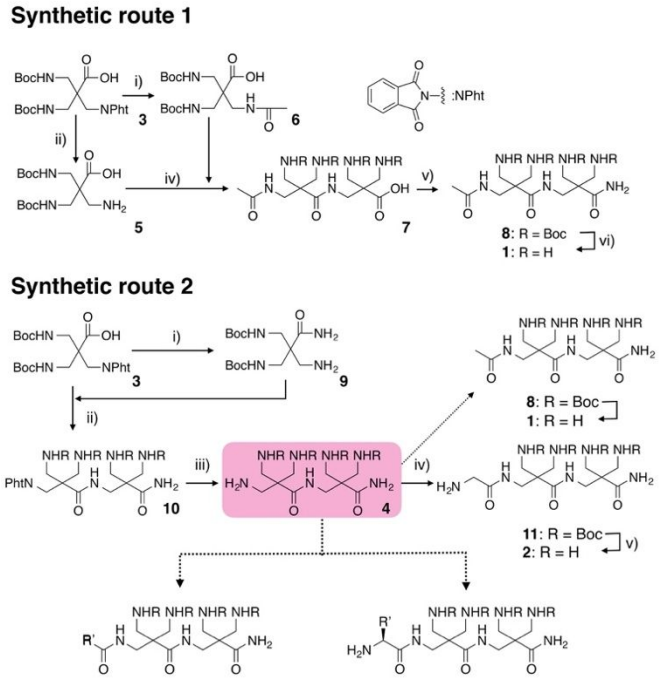
Figure 2. Structures of dipeptide **1**[18] and tripeptide **2**[19] bearing artificial coordinating sites (shown using dashed lines)

which are essential for the development of artificial cooperative systems consisting of multiple functional units.

Design and synthesis of artificial peptide ligands suitable for cooperative structural changes

As described in the introduction, metal complexes of peptides have suitable structural flexibility and designability. However, control over the structures of peptide complexes via selective binding of the metal ions to the target binding site with the desired coordination bond is required. For this selective binding, it is essential for the metal binding sites to be designed with a higher affinity toward the metal ions when compared to the other functional groups in peptides that can also act as metal coordination binding sites. Multidentate metal-binding sites are useful for the formation of coordination bonds to target metal ions.

We designed artificial β-amino-acid units possessing tridentate binding groups as the metal binding units (i.e., propanediamine (two aminomethyl groups)) in the side chain and amide carbonyl groups in the peptide backbone indicated by the dashed line in Figure 2). This β-amino acid unit is suitable for structural transformations because of the additional methylene group in the peptide backbone. This unit can be easily prepared from *N*-protected 2,2-bis(*tert*-butoxycarbonylaminomethyl)-3-phthalimidopropanoic acid (**3**) reported by Heinoen et al.[24] By connecting these β-amino acid units, we synthesized the simplest bridging ligand, dipeptide **1**,[18] in which the amino group at the N-terminus and carboxyl groups at the C-terminus were capped with an acetyl and amide group, respectively, to reduce their coordination abilities (Figure 2a). We also designed the simplest tripeptide (**2**)[19] possessing a natural amino acid (glycine) to determine the design potential of our artificial peptides (Figure 2b). The structure of these short peptides is tunable by replacing the functional groups or amino acids in their amino acid sequences. To further synthesize various short peptides, we established two synthetic routes (Scheme 1). Synthetic route 1[18] could be used to prepare artificial peptides that possess additional amino acids between the artificial β-amino acids or at the C-terminus. Utilizing this route, we accomplished the synthesis of **1** via amidation of the carboxylic group at the C-terminus, followed by a coupling reaction between an acetyl-capped β-amino acid (**5**) and free β-amino acid (**6**). We also established synthetic route 2[19] using dipeptide intermediate **4**, which possesses a free amino group at the N-terminus and is suitable for the further elongation of a peptide, such as tripeptide **2**. Almost all of the possible peptide derivatives in this study could be synthesized using these two synthetic routes.



Scheme 1‡: Synthesis of dipeptide **1** (synthetic route 1 reported in ref. 18) and tripeptide **2** (synthetic route 2 reported in ref. 19). The peptide ligands that can be possibly synthesized from dipeptide intermediate **4** are indicated in synthetic route 2.

Heterogeneous cooperative binding systems in the crystalline state

In biological systems, cooperative structural changes between functional units are essentially controlled for multiple functions such as efficient transfer, accurate and sensitive switching, and selective reactions. Despite the great development of artificial multifunctional systems in the solution state,[25] it is still difficult to develop multiple functions at the molecular level in the solid state using the cooperative changes of several units. This can be attributed to the restriction of motion (structural changes) in the solid state compared with that in the solution state. However, structural changes are often cooperative for all structures in the solid state, particularly in the crystalline state. In some cases, it is possible to precisely control the molecular arrangement up to a micrometer scale using their packing structures.[26-28]

Many examples of the sharp on/off switching of molecular inclusion complexes have been reported using the cooperative opening and closing of crystalline nanocavities[29,30], which are well-known in metal-organic frameworks (MOFs) or porous coordination polymers (PCPs). This switching responds to slight changes in the surrounding environment similar to the sigmoidal behavior observed in biological systems. These facts suggest that crystalline materials possessing multicrystalline cavities may be suitable platforms for the design of multifunctional units at the molecular level. However, most previous reports have focused on selective molecular capture or molecular separation[31] and not on mimicking the heterotropic cooperative binding systems found in nature.

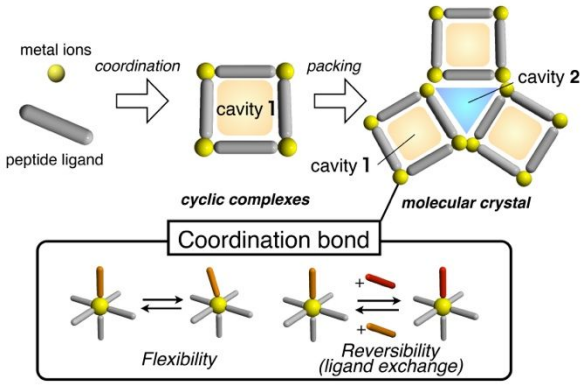


Figure 3. Schematic representation of formation of heterogeneous units that work cooperatively using the crystal packing structure of cyclic complexes

Because the packing structure is useful for generating multiple positions, crystalline materials are a suitable platform for the design of multiple systems using simple molecules. For example, it is easy to form multiple systems that work cooperatively if the cavity inside the molecule and the cavity surrounded by the molecules are regarded as different units in the crystal structure.[32] Thus, we envisioned that heterotropic cooperative systems can be developed using molecular crystals of cyclic peptide complexes (Figure 3) that possess at least two types of cavities (one inside the cyclic framework and the other surrounded by the cyclic framework). We expected that the metal center in the complexes would drive and control the structural transformation of the flexible peptides via ligand exchange. In this study, we applied cyclic complexes of artificial β-dipeptide **1**, a bridging ligand possessing two tridentate binding sites (i.e., diaminopropane in the side chains and O atoms in the amide groups of the main chain). Artificial β-dipeptide **1** forms tetra-nuclear cyclic complexes ($[\mathbf{1}_4Ni_4]^{8+}$)[18,20,21] upon mixing with an equivalent mixture of **1** and Ni(II) ions. In this complex, the N-terminal tridentate binding site of the dipeptide ligand (shown in red in Figure 4) is connected to the C-terminus tridentate binding site of another dipeptide ligand (shown in blue) via an octahedral Ni(II) center to form a cyclic framework (Figure 4b). As expected, two kinds of crystalline nanocavities are formed in the crystal of its NO_3 salt: cavity 1 in the cyclic complex and cavity 2 as a gap surrounded by the cyclic complexes (Figure 4a).[20,21] Water molecules exist in both cavities of the as-synthesized crystal which are removed in a stepwise manner during the drying process. In this stepwise drying process, all of the structures can be clarified using single-crystal X-ray crystallography, revealing that the water molecules were first removed from cavity 2 and then from cavity 1 accompanied by the cooperative opening/closing of cavity 2. In each peptide complex, the cyclic framework (open form) was transformed into a framework without a cavity (closed form) (Figure 4c). This structural change was accompanied by ligand exchange at the Ni(II) metal center (between the N-terminus amide and NO_3^- ions) and supported by the switching of the hydrogen-bonded network formed in the peptide framework (Figure 5). The water molecules are located near the terminal amide groups in **1** and

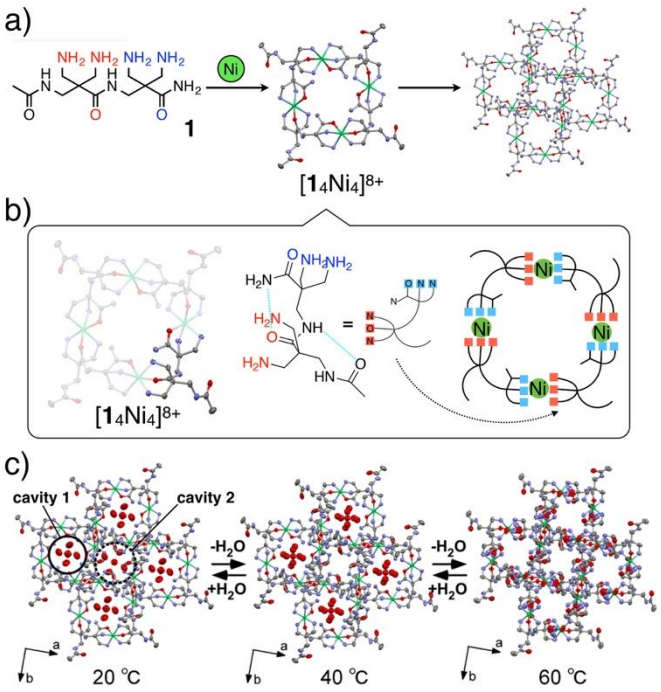


Figure 4. (a) Crystal packing structure in the NO_3 salt of a cyclic complex of **1** ([**1**$_4$Ni$_4$]$^{8+}$) with (b) schematic representation of the conformation of **1** in the cyclic complex and (c) heterogeneous crystalline cavities in the NO_3 salt and its crystal structural transformation accompanying the release/inclusion of water molecules (sky-blue dash lines in (b) represent possible hydrogen bonds). Color code: C, gray; N, blue; O, red; Ni, green. Reproduced from ref. 20.

NO_3^- ions, which are connected via hydrogen bonds because of the distance between the O atoms in the water molecules and the N atom in the terminal amide group or O atom in the NO_3^- ion. Therefore, water molecules act as stoppers for the cooperative opening/closing of the cavities via switching of the hydrogen-bonding network.[20] Since this ligand exchange process at the metal center is induced by non-coordinated water molecules, the structural transformations occur smoothly at low temperatures. Most reports of large crystalline transformations induced via ligand exchange at the metal centers require high-temperature conditions.[33] These results suggest that flexible frameworks possessing a network of weak interactions such as hydrogen bonds are useful for the development of smooth cooperative structural changes in the crystalline state.

This structural transformation does not occur when the counter anions are BF_4^- ions, while the crystal packing structure is almost identical (pseudo-isostructural).[21] The BF_4 salt of the cyclic complexes of **1** ([**1**$_4$Ni$_4$]$^{8+}$) maintained the same packing structure after the complete removal of the crystalline water molecules, while the framework shrank. The water inclusion behavior clarified by the isotherm curve obtained for the water molecules was almost the same for the open form of the NO_3 and BF_4 salts, while those for the closed form of the NO_3 salt were completely different from those of the BF_4 salt. Although the BF_4 salt selectively uptakes CO_2 gas into its very narrow cavities,[22] the NO_3 salt uptakes a negligible amount of CO_2 gas because of its closed structure in its dehydrated form. These results suggest that the cooperative switching of NO_3 salt may be used in heterotropic cooperative binding systems.

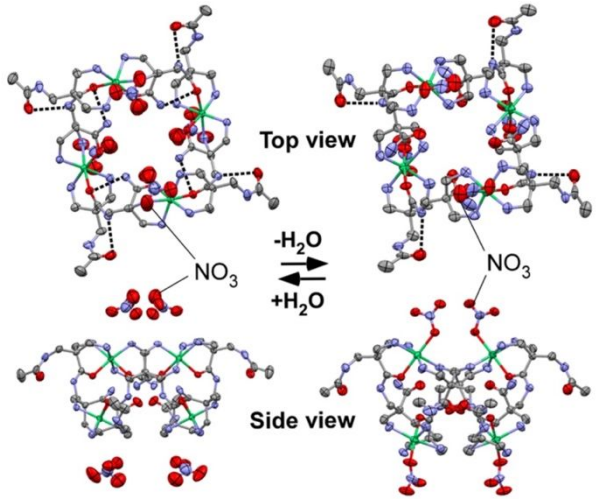


Figure 5. Comparison of the cyclic framework and hydrogen bond network in the NO_3 salt of the cyclic complex of **1** ([**1**$_4$Ni$_4$]$^{8+}$) before and after the removal of water molecules (dashed lines represent possible hydrogen bonds). Color code: C, gray; N, blue; O, red; Ni, green. Reproduced from ref. 20.

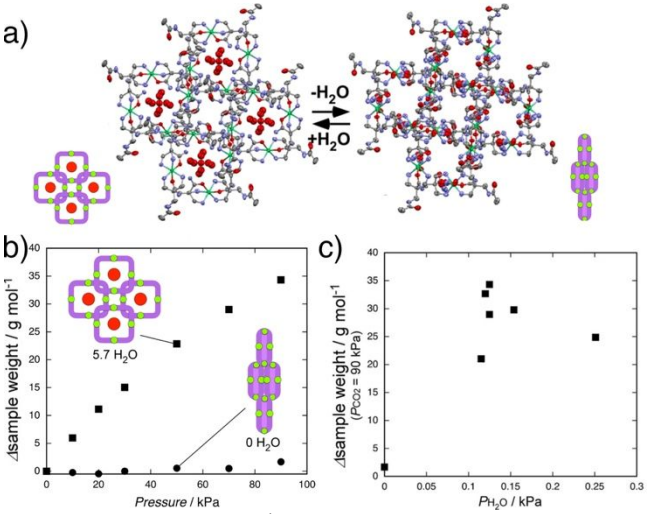


Figure 6. (a) Cooperative opening/closing of crystalline cavities in NO_3 salt of [**1**$_4$Ni$_4$]$^{8+}$ upon the recognition of water molecules in cavity 1, (b) plot of sample weight changes versus partial pressure of CO_2 gas for hydrated sample and dehydrated sample, and (c) plot of sample weight changes under 90-kPa CO_2 versus water vapor pressure at 297 K. Reproduced with permission from ref. 23. Copyright 2018 John Wiley&Sons, Inc.

By using the cooperative opening/closing of the crystalline cavities in the NO_3 salt, we successfully demonstrated the on/off switching of CO_2 inclusion in response to the recognition of water molecules by cavity 1 (Figures 6a and b).[23] The inclusion amount of CO_2 gas in the crystal suddenly increased at a very narrow range of humidity; i.e., the inclusion state switched sharply in response to a humid environment (Figure 6c). The switching of the CO_2 inclusion was reversible, and the included CO_2 gas was automatically released under dry conditions.[23] DSC measurements revealed that the included water molecules were stabilized under a CO_2 environment when compared to those under N_2, suggesting that the cooperativity between CO_2 inclusion and water inclusion was stabilized by the included water molecules.[23]

Similar switching of the inclusion state using the cooperative structural changes of peptides has also been reported by

Rosseinsky et al.[11] Fine tuning of the binding affinity of the nanocavities in peptide MOFs was demonstrated by the co-included molecules via the cooperative structural changes in the peptide framework. These results clearly suggest the great potential of flexible peptide complexes for the development of artificial cooperative systems, although it is still difficult to design such behavior because of the difficulty in designing the crystal packing structure, which is normally designed empirically.

Formation of giant structures using flexible short peptides

The abovementioned cooperative systems were demonstrated using the cooperation of small units (cavities) so that they can be applied to small molecules such as gaseous molecules. To develop our metal complexes of flexible peptides as multiple functional systems, it was essential to establish a breakthrough design for constructing giant structures/systems >1 nm. One of the most powerful approaches to form giant structures from small units is the use of the self-assembly processes using coordination bonds.[34] Fujita et al. reported their pioneering work using metal assemblies of well-designed rigid ligands up to 6 nm (cavity size) in scale.[35] In contrast to the assembly of "rigid" units, the assembly of flexible units is still a significant challenge for synthetic chemists because of their entropic disadvantage. In particular, the formation of structures with a giant void is extremely difficult because nature prefers packed structures. Recently, several studies have addressed this challenge using peptide moieties possessing natural peptides and artificial metal binding sites (such as pyridyl groups) at the N-and C-termini. In 2014, Sawada and Fujita reported the formation of a 2-nm cavity in a crystal using a metal complex of a tripeptide (Gly-Pro-Pro) derivative including proline, which is one of the most rigid amino acids.[12] They also achieved the formation of a 1-nm cavity by assembling three discrete complexes of a derivative of a short peptide (<9 amino acid residues), which preferred to form β-sheet structure in 2018 (Figure 7).[13] In contrast to their excellent works using natural amino acid sequences and their preferred structures, we focused on constructing giant structures by using artificial peptides including minimum required feature of natural peptides. In 2019, we succeeded in accomplishing the formation of cyclic complexes with giant cavities (diameter of ~2 nm) by using artificial peptide possessing coordinating sites over its main chain and side chain.[19] We envision that, as observed in nature, several inter/intramolecular interactions are useful for designing and controlling the conformation of a flexible backbone; the strongest interaction determines the conformation, and other weak interactions support the formation of other possible interactions in this conformation. In this study, we used tripeptide **2**, in which a tridentate metal-binding site provided a site to control the conformation of a flexible framework via metal coordination. **2** possesses three coordinating sites—an amino group at the N-terminus (green in Figure 8) and two tridentate binding sites (blue and red in Figure 8). Notably, the tridentate binding sites at the C-terminus (blue

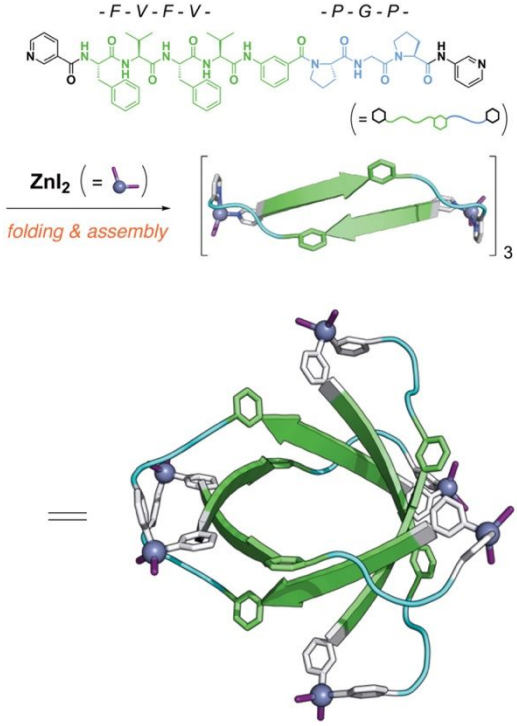


Figure 7. Giant cavities reported by Fujita and Sawada using a peptide possessing pyridine as the metal binding site and an amino acid sequence that formed relatively stable secondary structures. 1-nm cavity was formed by assembling three cyclic complexes via the formation of hydrogen bond as was seen in β-sheet structure. Reproduced with permission from ref 13. Copyright 2018 American Chemical Society.

in Figure 8) acted as a bidentate binding site depending on the coordination ability of the other metal binding sites.[20] Despite small changes in the molecular design of tripeptide **1** from dipeptide **2** (i.e., the introduction of amino groups), **2** formed a giant cyclic complex, $[\mathbf{2}_{14}Ni_{14}]^{28+}$ (cavity diameter of ~2 nm), as purple plate-like crystals upon mixing with an equivalent amount of Ni(II) ions, while **1** formed a small cyclic complex, $[\mathbf{1}_4Ni_4]^{8+}$ (cavity diameter of <0.2 nm) (Figure 8). Owing to the flexible framework of the peptide, **2** formed various types of giant complexes ($[\mathbf{2}_{12}Ni_{12}]^{24+}$ and $[\mathbf{2}_{28}Ni_{28}]^{56+}$) depending on the conditions used in the complexation reaction (Figures 8c). In particular, **2** formed [2]-catenane with 28 **2** and 28 Ni(II) ions,[19] which to the best of our knowledge is consistent with the largest number of units. Furthermore, the catenane was detected using CSI-TOF mass spectra in solution, indicating that this multicomponent giant structure also existed in the solution state. It is noteworthy that $[\mathbf{2}_{14}Ni_{14}]^{28+}$ was observed as an ellipsoid in the crystal structure, suggesting that the framework of cyclic complexes maintained flexibility. These results suggest that our approach is useful for constructing giant structures from flexible short peptide units.

A detailed study of the giant cyclic structure indicated the principal design for forming a giant structure from flexible units. The key structural feature of these giant structures is their mesh-like structure (Figure 8b). X-ray crystallography revealed that the giant framework formed a mesh-like structure upon

connecting three tripeptides at each Ni(II) center. At the Ni(II) center, the tridentate binding site of the tripeptide, bidentate

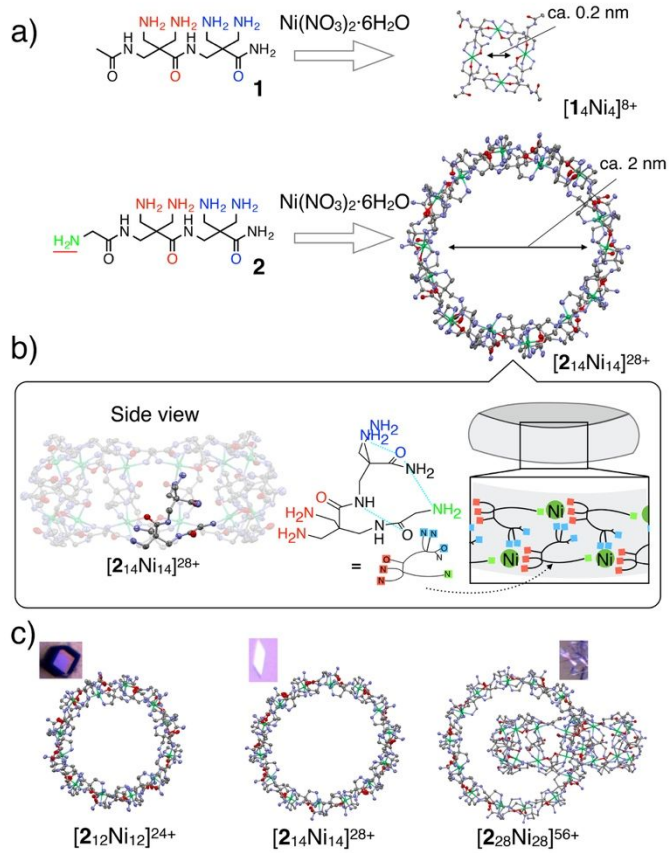


Figure 8. (a) Comparison of giant cyclic complex of **2** ($[\mathbf{2}_{14}Ni_{14}]^{28+}$) with cyclic complex of **1** ($[\mathbf{1}_4Ni_4]^{8+}$), (b) mesh-like structure and conformation of **2** in the giant complex, and (c) three kinds of giant complexes obtained from **2** depending on coordination conditions with their crystal images (sky-blue dash lines in (b) represent possible hydrogen bonds). Color code: C, gray; N, blue; O, red; Ni, green. Reproduced from ref. 19.

binding site of another tripeptide, and amino group of the other tripeptide formed an octahedral coordinate leading to a mesh-like structure that formed a giant cyclic structure supported by intramolecular hydrogen bonding (Figure 9). In the resulting structures, the tridentate binding sites at the C-terminus acted as bidentate binding sites (i.e., diaminopropane unit) because of the existence of the amino group in the glycine units.

Interconnected structures were also observed in the examples of giant discrete structures obtained from flexible peptide derivative reported by other groups.[14,16] Sawada and Fujita reported an interlocked catenane structure possessing 1-nm cavity prepared using a triglycine derivative in which two cyclic complexes was intertwined supported by the weak coordination bonds between the Ag(I) ions and O atoms in the amide groups and hydrogen bonding.[14] Therefore, the formation of interconnected structures using weak interactions is a useful approach for constructing giant structures from small flexible units.

Although it is still extremely difficult to design interconnected giant structures based on molecular design, the metal-assisted interconnecting approach has great potential for the design of giant structures. Metal coordination bonds can be used as a design factor to determine the interconnected structures by placing multidentate binding sites at a location suitable for controlling the conformation of the flexible framework as demonstrated in our approach. In particular, it is important to control their conformations in short peptides such as dipeptides and tripeptides. Indeed, conformational tuning via the additional coordination of Ni(II) ions to the flexible framework causes the formation of a very small cyclic complex from **2** with a cavity whose diameter is <0.2 nm ($[(\mathbf{2}\text{-}3H^+)_4Ni_8]^{4+}$, Figure 10).[19] Furthermore, DFT calculations of the flexible framework in the giant complexes can only be reproduced when the distances between the atoms coordinated to the metal ions are fixed (Figure 11). Considering that metal coordinating sites and coordination structures at metal centers can be predicted in part empirically, these results suggest the great potential of metal coordination bonds to control and design the structure of giant structures. Unfortunately, using current calculation systems, giant molecules such as our complexes are difficult to

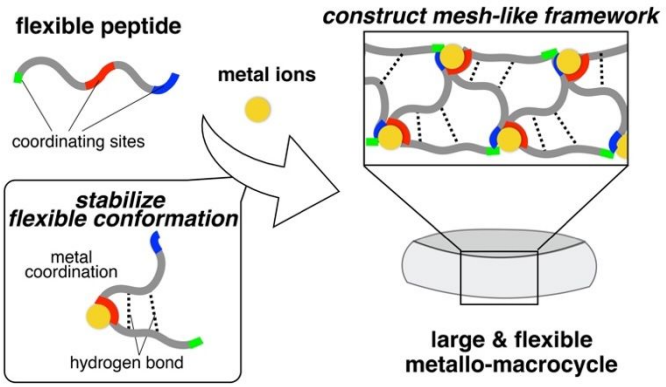


Figure 9. Schematic representation of formation mechanism of giant cyclic complexes prepared from a small flexible peptide via formation of coordination and hydrogen bonds. Reproduced from ref 19.

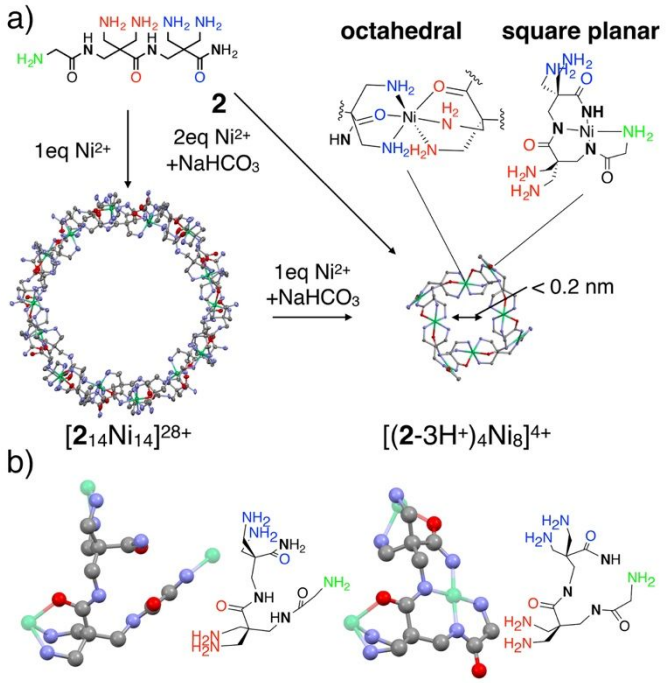


Figure 10. (a) Comparison of a giant cyclic complex ($[\mathbf{2}_{14}Ni_{14}]^{28+}$, left) and a small cyclic

complex ([(**2**-3H$^+$)$_4$Ni$_8$]$^{4+}$, right) of **2** and (b) conformational difference of their peptides. Color code: C, gray; N, blue; O, red; Ni, green. Reproduced from ref 19.

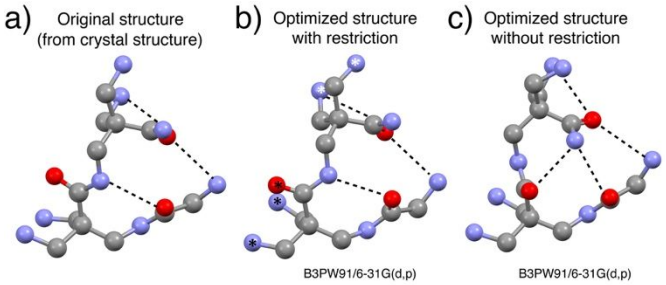


Figure 11. (a) Comparison of conformation of **2** in the crystalline state and energy-minimized structure optimized using DFT calculations starting from the crystalline structure with (b) and without (c) restriction of the distances between the O and N atoms in the same binding sites (black * for tridentate binding site and white * for bidentate binding site) in **2**. Color code: C, gray; N, blue; O, red. Reproduced from ref 19.

calculate precisely because of computer performance limitations, particularly around the metal centers. However, various calculative studies have demonstrated the dynamic behavior or conformational energy of peptide backbones in the metal complexes of short peptides.[6,8,11,36] If a suitable approximate calculation method is established, the prediction of giant structures with designable motifs (such as peptides) can be achieved in the future and provide a breakthrough toward design strategies for sophisticated multifunctions using the artificial systems observed in biological systems.

Control of the metal arrangement using flexible short peptides

It is well known that biological systems use metal centers as functional active sites in combination with other metal centers and/or functional groups. Therefore, the development of a method to design metal centers is an important basic technique for constructing multiple functional systems. To develop artificial cooperative multifunctional systems comparable to biological systems such as enzymes, it is important to establish approaches to control the arrangement of metal ions and functional groups using flexible peptides. One of the most promising approaches is to control the metal–metal interactions via the arrangement of metal ions. However, selective control over their arrangement is generally difficult for flexible molecules because selective binding often requires a fixed conformation.

Despite the disadvantages caused by their structural flexibility, the designability of peptides is useful for controlling the arrangement of metal ions using artificial amino acids possessing metal-coordination sites that selectively bind to the target metal ions.[38-40] Therefore, various heterometallic arrangements have been achieved using short peptides.[39,40] However, because of the lack of a conjugated moiety in the peptide backbone, the formation of through-bond metal–metal interactions has been limited,[40] although electronic interactions between the metal centers are key to precisely tuning their properties based on the combination of metal ions and the overlapping of the electron orbitals between the metal centers.

For the same reason, the formation of heterometallic interactions including metal centers possessing unsaturated sites available for extra coordination, such as square planar centers, has been rare for metal assembly using peptides. However, these unsaturated sites are useful for various functions (catalysis and molecular recognition) often observed in biologically active centers. In this section, we explain our efforts toward developing an approach to control the metal arrangement, including unsaturated metal centers, using flexible peptides.

To overcome the disadvantages of peptides, we used ligands possessing two different coordination sites that formed different types of coordinate bonds with different combinations of coordinated atoms in each coordinate (i.e., the HSAB rule). The different coordinating sites at the main chain and side chain were bridged via amide groups (i.e., both sites included the N or O atoms of the amide group) to connect the electron orbitals of each metal center (Figure 12). Based on this concept, we used tripeptide **2** because it forms a cyclic complex ([(**2**-3H$^+$)$_4$Ni$_8$]$^{4+}$) with two different Ni(II) centers (octahedral and square planar) connected by deprotonated amide groups (Figure 10).[19] Using **2**, we succeeded in selectively forming a cyclic Cu(II)–Ni(II) alternative arrangement.[41] Octanuclear complexes with a cyclic Cu(II)–Ni(II) arrangement were obtained as purple needle-like crystals upon mixing **2** with an equivalent amount of $Ni(ClO_4)_2{\cdot}6H_2O$ and $Cu(ClO_4)_2{\cdot}6H_2O$ under basic conditions to deprotonate the amide groups (Figure 13). The cyclic

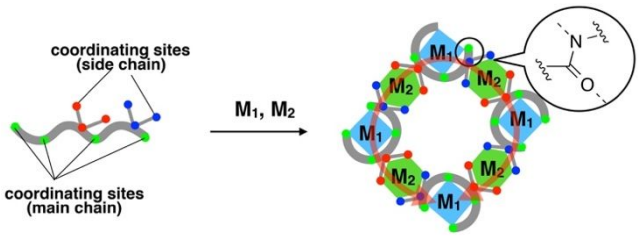


Figure 12. Schematic representation of formation of heterometallic cyclic metal–metal interactions through peptide amide groups. Reproduced with permission from ref. 41. Copyright 2021 John Wiley&Sons, Inc.

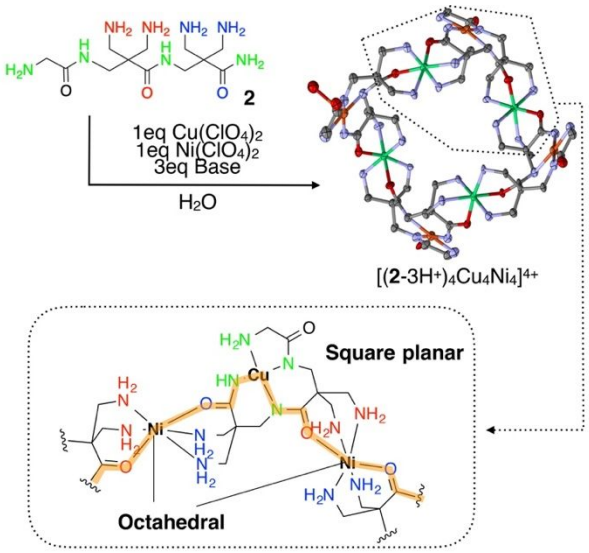


Figure 13. Cyclic Cu(II)–Ni(II) interactions formed in cyclic complexes of **2** ([(**2**-$3H^+)_4Cu_4Ni_4]^{4+}$). The possible pathway for the interactions formed between Cu(II) and Ni(II) are shown with an orange line. Color code: C, gray; N, blue; O, red; Ni, green; Cu, orange. Reproduced with permission from ref. 41. Copyright 2021 John Wiley&Sons, Inc.

framework consisted of four square planar Cu(II) centers formed by three deprotonated N atoms in the amide groups and the N-terminal amino group in the main chain, four octahedral Ni(II) centers formed by two tridentate binding sites (amino propane units and O atoms in the amide groups) connected to each other via the amide groups. A magnetic study of $[(\mathbf{2}\text{-}3H^+)_4Cu_4Ni_4]^{4+}$ revealed that efficient antiferromagnetic spin coupling existed in this arrangement (J/k_B = −25 K), leading to an S_{total} = 2 state (at 2 K) in one octanuclear cyclic arrangement. The results suggest an effective Cu(II)–Ni(II) antiferromagnetic coupling between the four square planar Cu(II) centers (S = 1/2) and four octahedral Ni(II) centers (S = 1) (Figure 14). This heterometallic interaction was selectively formed even when the mixing ratio of metal ions (Cu/Ni) was not 1. This was caused

by the selective formation of Cu(II) into a square planar center supported by the crystallization process. From another point of view, this result suggests that the design of square planar centers (e.g., tuning the functional groups at the N-terminus) efficiently controlled these arrangements. Scheme 1 shows that it is easy to synthesize peptide moieties with a sequence in which the N-terminus amino acid is replaced with other amino acids or carboxylic acids. This approach provides a novel general method for developing designable functional metal centers by tuning the heterometallic interactions, leading to designable catalytic centers or recognition sites.

The chirality and/or direction of the peptides, which are important structural features, are useful for introducing chirality and/or anisotropy to the metal-metal interactions. For example, the chirality of peptides is essential for the chiral catalytic reactions of enzymes in biological systems. In artificial systems, various functions such as asymmetric catalysis[42] and selective separation of enantiomers[9] have been achieved using the chirality of peptide ligands in metal complexes. In addition to the direct use of chirality, it has also been useful for increasing the variety of metal–metal interactions. When the assembled structure of a chiral dipeptide is used as a template for metal arrangements, different types of metal–metal interactions can be formed from the metal ions. We succeeded in forming a 2D array of Ag(I) ions, including two types of Ag-Ag interactions showing different thermal elongation behaviors using an assembled chiral dipeptide as the template (Figure 15).[43] Although our complexes consisted of achiral peptides, they could be synthesized using chiral peptides. The various advantages of chirality will be useful for designing functions from metal–metal interactions in the future. The above

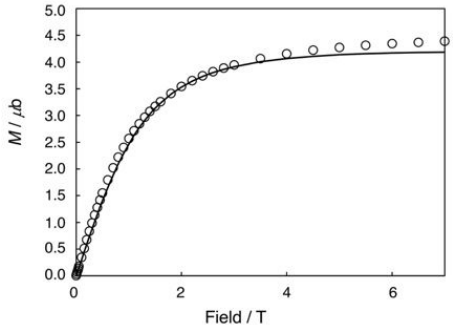


Figure 14. *M*–*H* curves obtained at 2 K for crystals of $[(\mathbf{2}\text{-}3H^+)_4Cu_4Ni_4]^{4+}$ (black line indicates Brillouin function for S = 2)

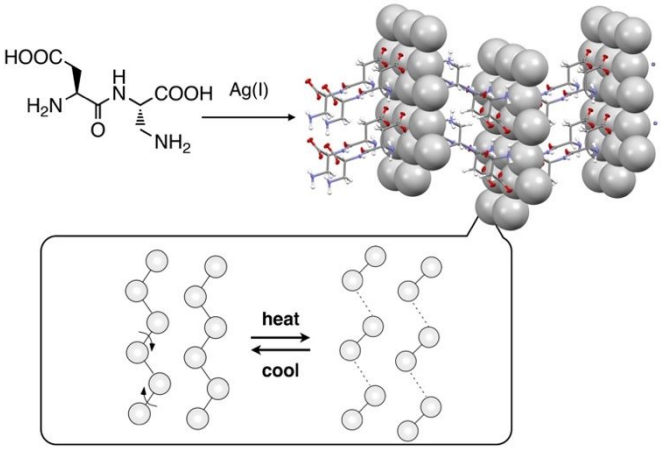


Figure 15. Two-dimensional Ag–Ag arrangement possessing two kinds of interactions showing different thermal expansions reflecting the anisotropy of the assembled peptide structures. Color code: C, gray; H, white; N, blue; O, red; Ag, gray (shown in space filling model). Reproduced with permission from ref. 43, Copyright, 2014, American Chemical Society.

mentioned peptide Ag(I) complexes formed spherical assemblies of nanocrystals that could be used for the preparation of hierarchical nano-silver structures.[44] This feature of the peptide backbone strongly indicates its great potential for building hierarchical structures from interacting metal centers.

Conclusions and perspectives

To form a completely artificial multifunctional system observed in biological systems, it is essential to develop a general design strategy for constructing giant structures with flexibility suitable for structural changes that possesses several functional centers (metal center, recognition site, and binding site). We developed artificial peptide complexes that are useful for demonstrating heterogeneous cooperative binding systems. We also succeeded in developing a new method to form giant structures and novel metal centers, which are essential for developing multifunctional systems using flexible molecules. The coordinating sites, including amide groups in the main chain, are useful for controlling the conformation of flexible peptides, leading to the formation of a giant structure assisted by the mesh-like assembly of the flexible units. The use of an amide group is also useful for forming heterogeneous metal–metal

interactions using peptides. Both the formation of giant structures and heterometallic cyclic interactions can be achieved using a single artificial tripeptide depending on the conditions of the metal coordination process. These results indicate the advantages of our design to mimic the cooperative functions observed in nature using artificial systems suitable for building structures from small flexible units by controlling their conformation, supported by weak interactions formed between the functional groups in their backbone. As described in the manuscript, this type of design will be useful for estimating possible conformations with the support of DFT calculations. Designing structures and properties based on peptide designability relies in most cases upon serendipity and is still the "big challenge" for synthetic chemists because biological systems develop their excellent systems via evolution over a long period of time as a result of trial and error. The combination of our design with predictions using DFT calculations has the potential to provide a breakthrough for this difficulty. Although giant molecular systems are difficult to predict (reproduce) using DFT calculations, we believe that further improvements in the calculation systems (both method and instrumentation), as well as the development of molecular design, will allow this basic design strategy to open up a way to develop cooperative functions in multicomponent systems that are currently impossible. Furthermore, since it is still unclear how such cooperative systems are designed in nature, we believe that exploring artificial cooperative systems is significant for studying such a design.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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‡Reagents and Conditions (Route 1)[18]: i) 1) 3.8 eq hydrazine monohydrate, 1,4-dioxane, rt; 2) 1.4 eq Ac_2O, 1.6 eq DIEA, CH_2Cl_2, rt, 79% (2 steps); ii) 3.7 eq hydrazine monohydrate, 1,4-dioxane, rt, 66%; iii) 1.7 eq EDCI·HCl, 1.6 eq hydroxysuccinimide, CH_2Cl_2, rt; iv) 1.2 eq 3, 1.0 eq DIEA, DMF, 50 °C, μW, 74% (2 steps); v) 1) 2.2 eq EDCI·HCl, 2.7 eq hydroxysuccinimide, CH_2Cl_2, rt; 2) 4 eq 0.5 M NH_3 in 1,4-dioxane, rt, 66% (2 steps); and vi) TFA, CH_2Cl_2, rt, 93%. (Route 2)[19]: i) 1) 1.2 eq EDCI·HCl, 1.2 eq hydroxysuccinimide, CH_2Cl_2, rt, then 4 eq NH_3 in dioxane, rt; 2) 4 eq hydrazine monohydrate, EtOH/dioxane, 80 °C, 80% (2 steps); ii) 1.0 eq 10, 1.0 eq 11, 1.2 eq HATU, 1.5 eq DIEA, CH_2Cl_2, rt, 99%; iii) 4 eq hydrazine monohydrate, EtOH/dioxane, 80 °C, 96%; iv) 1.5 eq Boc-Gly-OH, 1.2 eq HATU, 1.5 eq DIEA, CH_2Cl_2, rt, 88%; and v) 1) 30 eq TFA, CH_2Cl_2, rt; 2) ion exchange, 93 %

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