

## Artificial Metalloproteins Exploiting Vacant Space: Preparation, Structures, and Functions

Satoshi Abe, Takafumi Ueno, and Yoshihito Watanabe

**Abstract** Molecular design of artificial metalloproteins is one of the most attractive subjects in bioinorganic chemistry. Protein vacant space has been utilized to prepare artificial metalloproteins because it provides a unique chemical environment for application to catalysts and to biomaterials bearing electronic, magnetic, and medical properties. Recently, X-ray crystal structural analysis has increased in this research area because it is a powerful tool for understanding the interactions of metal complexes and protein scaffolds, and for providing rational design of these composites. This chapter reviews the recent studies on the preparation methods and X-ray crystal structural analyses of metal/protein composites, and their functions as catalysts, metal-drugs, etc.

**Key words** Catalytic reaction, Metal-drugs, Metal materials, Nanocage, Protein, X-ray crystal structure

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S. Abe and T. Ueno  
Department of Chemistry, Graduate School of Science, Nagoya University,  
Nagoya 464-8602, Japan  
*Current address:* Institute for Integrated Cell-Material Sciences (iCeMS), Kyoto University, Kyoto  
615-8510, Japan

T. Ueno  
PRESTO, Japan Science and Technology Agency (JST), Saitama 332-0012, Japan

Y. Watanabe(✉)  
Research Center for Materials Science, Nagoya University, Nagoya 464-8602, Japan  
e-mail: yoshi@nucc.cc.nagoya-u.ac.jp

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## 1 Introduction

Construction of artificial metalloproteins has been intensively studied and applied to catalysts and biomaterials bearing electronic, magnetic, and medical properties [1–6]. In particular, topics on the composites of metal complexes or materials in protein vacant space are becoming the most important subjects in the field of bioinspired materials. In early works of this research area, Akabori et al. reported that the artificial metalloenzyme constructed by the deposition of Pd particles on silk protein fibers was able to catalyze asymmetric hydrogenation of oximes and oxazolones [7]. Whiteside et al. prepared the first artificial organometalloenzyme, which catalyzed asymmetric olefin hydrogenations using an avidin–biotin interaction [8]. However, at that time, it was difficult to characterize and improve the catalytic activities of the composites due to the lack of structural information and the limitations on molecular design using computational methods.

Recently, protein mutagenesis and X-ray structural analysis of proteins have become familiar tools for inorganic chemists in constructing artificial metalloproteins. Thus, the number of reports on the preparation and crystal structures of metal complex/protein hybrids have been increasing since 2000 [1, 3, 5, 6, 9–20]. Proteins and metal complexes utilized intensively for artificial metalloproteins are listed in Table 1. In these studies, the main subject is conjugation of metal complex catalysts with protein scaffolds for catalytic reactions [1, 3, 5]. On the other hand, some researchers have utilized nanoscaled protein cages for deposition of metal nanoparticles and encapsulation of functional nanomaterials [4, 5]. For the last few years, the direction of this research has been shifting toward biomineralization, design of metal-drugs, and fine-tuning of artificial enzymes.

In this chapter, the key topics of artificial metalloproteins utilizing protein vacant space are reviewed, outlining recent studies since 2000. Section 3 shows the approaches for construction of the metal complex/protein composites and their crystal structures and, further, describes the interactions between metal-drugs and proteins. Section 4

**Table 1** Summary of proteins and metal complexes for the preparation of artificial metalloproteins and their functions

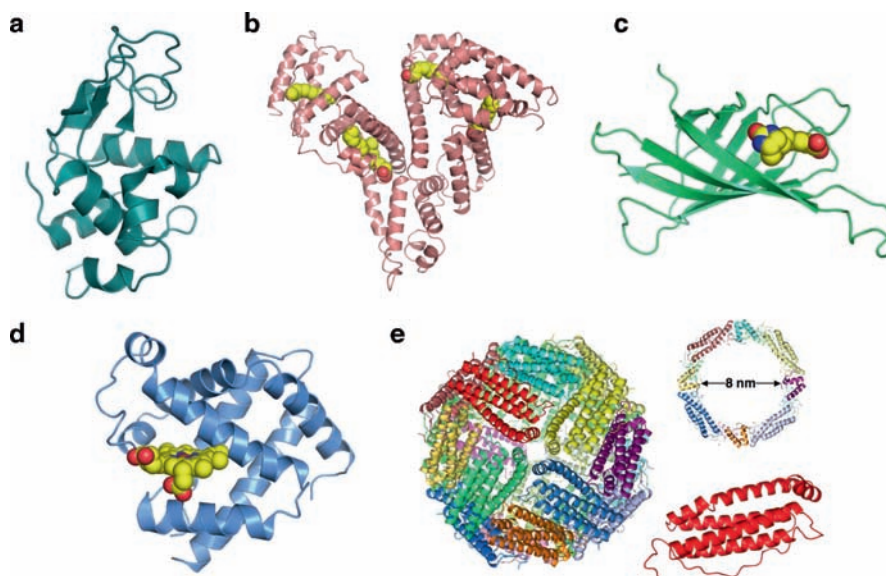
Protein	Metal complexes	Function	References
Myoglobin	Heme derivatives	Catalyst/O <sub>2</sub> storage/ electron transfer	[13, 21, 22]
	Cr and Mn Schiff base complexes	Catalyst	[17, 23, 24]
Avidin	Biotinated Rh and Ru complexes	Catalyst	[8, 25, 26]
Albumin	Heme, Cu, and Rh complexes	Catalyst/O <sub>2</sub> storage	[27–30]
Lysozyme	Ru and Cu complexes	Metal drug	[14, 31]
Kinase	Ru complex	Inhibitor	[12]
Ferritin	Pd, Ag, CdS, Fe <sub>3</sub> O <sub>4</sub> , ZnSe clusters and Gd complex	Catalyst/material	[32–37]
Virus	Au, CoPt, and FePt clusters	Material	[38–41]

provides the coordination structures of metal multinuclear clusters binding on protein scaffolds. Section 5 shows the direct observation of reaction intermediates in the active sites of some oxygenases. Section 6 describes how the self-assembled nano-protein cages are utilized for deposition of metal nanoclusters and metal complexes for their application to catalysts, and to magnetic and medical materials.

## 2 Protein Structures Utilized for Artificial Metalloproteins

For the construction of artificial metalloproteins, protein scaffolds should be stable, both over a wide range of pH and organic solvents, and at high temperature. In addition, crystal structures of protein scaffolds are crucial for their rational design. The proteins reported so far for the conjugation of metal complexes are listed in Fig. 1. Lysozyme (Ly) is a small enzyme that catalyzes hydrolysis of polysaccharides and is well known as a protein easily crystallized (Fig. 1a). Thus, lysozyme has been used as a model protein for studying interactions between metal compounds and proteins [13, 14, 42, 43]. For example, [Ru(*p*-cymene)]<sup>2+</sup>, [Mn(CO)<sub>3</sub>]<sup>+</sup>, and cisplatin are regiospecifically coordinated to the N<sup>ε</sup> atom of His15 in hen egg white lysozyme [14, 42, 43]. Serum albumin (SA) is one of the most abundant blood proteins, and exhibits an ability to accommodate a variety of hydrophobic compounds such as fatty acids, bilirubin, and heme (Fig. 1b). Thus, SA has been used to bind several metal complexes such as Rh(*acac*)(CO)<sub>2</sub>, Fe- and Mn-corroles, and Cu-phthalocyanine and the composites applied to asymmetric catalytic reactions [20, 28–30].

Avidin is a glycoprotein and consists of four identical subunits. Avidin shows a very strong affinity to biotin with a  $K_a$  of approximately  $10^{15} \text{ M}^{-1}$  (Fig. 1c). The affinity of avidin for biotin can be utilized to introduce metal complexes into the avidin cavity by a covalent bond with biotin. In fact, hybrids of avidin and biotin conjugated with Rh diphosphine and Ru diamine moieties have been shown to allow asymmetric hydrogenations of olefin and ketone substrates [8, 25, 26].



**Fig. 1** Ribbon diagrams of protein architectures: **a** lysozyme, **b** serum albumin, **c** avidin, **d** myoglobin, and **e** ferritin taken from PDB ID: 2VB1, 1BJ5, 1AVD, 4MBN, and 1DAT, respectively

Myoglobin (Mb) is a small hemoprotein that functions as an oxygen storage, and has been used as a model for many heme enzymes by modifying the heme prosthetic group and/or replacing some amino acids near the heme (Fig. 1d) [44–46]. Thus, Mb is a good candidate for a host protein scaffold to incorporate synthetic metal complexes such as  $M^{III}(\text{Schiff base})$  ( $M = \text{Fe, Cr, and Mn}$ ) and iron porphycene [22–24]. Ly, SA, avidin, and Mb have been used to fix a single metal complex in their cavities. On the other hand, self-assembled protein cages are capable of accommodating many metallic compounds and inorganic materials [4, 5, 47, 48].

Ferritin (Fr) is a spherical protein composed of 24 subunits with a cavity where iron atoms are accumulated as a cluster of ferric oxyhydroxide (Fig. 1e) [49]. Some metal ions and organic molecules are able to penetrate through the hydrophilic channels of the threefold axis to the inside of Fr, which has an internal diameter of 8 nm [50]. Fr is stable both at high temperature ( $<80^{\circ}\text{C}$ ) and in a pH range of 3–11. Fr has been used for the deposition of monodisperse metal particles such as FeS, CdS, CdSe, Pd, and Ag in the cage [35, 37, 40, 48, 51–55]. Thus, it is possible to use the Fr cage to incorporate and fix many metal complexes [32, 56, 57]. Self-assembled supermolecular proteins have different size of cages, such as cowpea chlorotic mottle virus (CCMV) with 28 nm diameter cages, small heat shock protein (sHsp) with 12 nm, and DNA binding protein (Dps) with 9 nm. Huge supermolecular assemblies such as tobacco mosaic virus, M-13 bacteriophage, and chaperonins have also been utilized as well-defined spaces for the encapsulation of metal compounds and organic materials [4, 33, 47, 58–65].

### 3 Functions and Structures of Metal Complexes in Protein Cavities

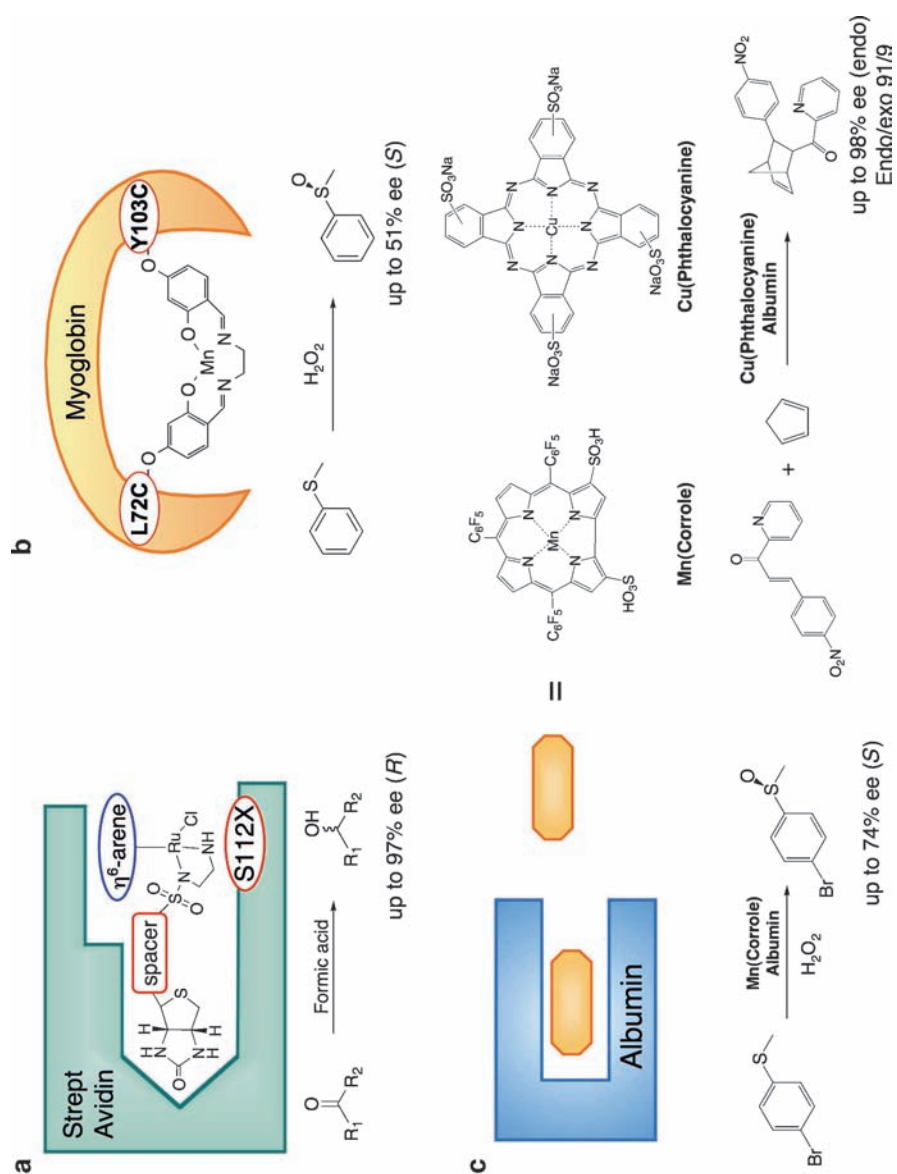
#### 3.1 Preparation of Artificial Metalloproteins

There have been many reports that described protein composites containing metal complexes [1–3, 5, 6]. Three different approaches for the incorporation of synthetic metal complexes into protein cavities have been reported: (i) modification of natural substrates, (ii) covalent anchoring, and (iii) non-covalent insertion. For example, Whiteside et al. constructed artificial metalloenzymes by the conjugation of a Rh diphosphine complex with biotin, which strongly binds to avidin [8]. Ward et al. have improved this method to increase the reaction activities [25]. They optimized the reaction conditions by screening the structures of metal complexes and protein environments. Finally, optimized composites catalyzed asymmetric hydrogenation with up to 97% ee (Fig. 2a) [2, 3, 26].

Covalent anchoring is an attractive approach for conjugation of metal complexes directly to specific sites of protein scaffolds. Several metal complexes such as Cu, Pd, and Rh complexes have been incorporated into protein cavities by covalent anchoring of the thiol group of Cys or the amine group of Lys with metal complexes [66–68]. On the other hand, Lu et al. have succeeded in dual covalent attachment of a Mn(salen) complex bearing two thiosulfonate moieties to apo-myoglobin (apo-Mb) mutant L72C/Y103C Mb to restrict the conformational freedom of the metal complex in the cavity [23]. The dual covalent attachment also improved the enantioselectivity of thioanisole sulfoxidation up to 51% ee while a single attachment exhibited only 12% ee (Fig. 2b) [23].

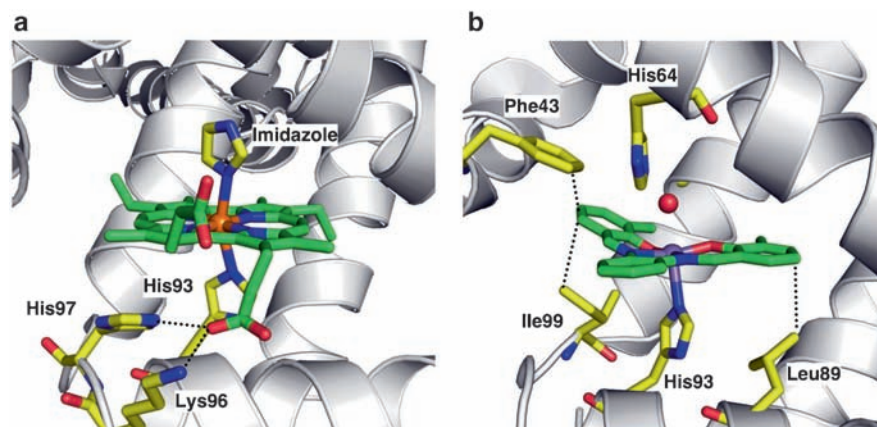
Non-covalent insertion of several modified metal cofactors and synthetic metal complexes into protein cavities such as serum albumin (SA) and Mb has been reported [5, 24, 28, 30, 69]. If synthetic metal complexes, whose structures are very different from native cofactors, can be introduced into protein cages, the bioconjugation of metal complexes will be applicable to many proteins and metal complexes. Mn(corrole) and Cu(phthalocyanine) are inserted into SA by non-covalent interactions and the composites catalyze asymmetric sulfoxidation and Diels-Alder reactions with up to 74 and 98% ee, respectively (Fig. 2c) [28, 30]. Since the heme is coordinated to Tyr161 in the albumin cavity, determined by X-ray crystal structure [20], it is expected that both Mn(corrole) and Cu(phthalocyanine) are also bound to albumin with the same coordination. The incorporation of synthetic metal complexes in protein cavities using these methods is a powerful approach for asymmetric catalytic reactions. However, there are still some difficulties in further design of the composites for improving reactivities and understanding reaction mechanisms because detailed structural analyses are not available for most of the composites.

Successful structural analyses of artificial metalloproteins have been reported [5, 6, 17, 22]. Hayashi et al. have determined the crystal structure of a reconstituted apo-Mb with the iron porphycene derivative 13,16-dicarboxyethyl-2,7-diethyl-3,6,12,17-tetramethylporphycenato-Fe<sup>III</sup> (iron porphycene) [22]. The structure shows



**Fig. 2** Artificial metalloenzymes and asymmetric reactions catalyzed by them. Metal cofactors are introduced by **a** covalent modification of biotin, **b** double anchoring to myoglobin, and **c** non-covalent insertion to serum albumin



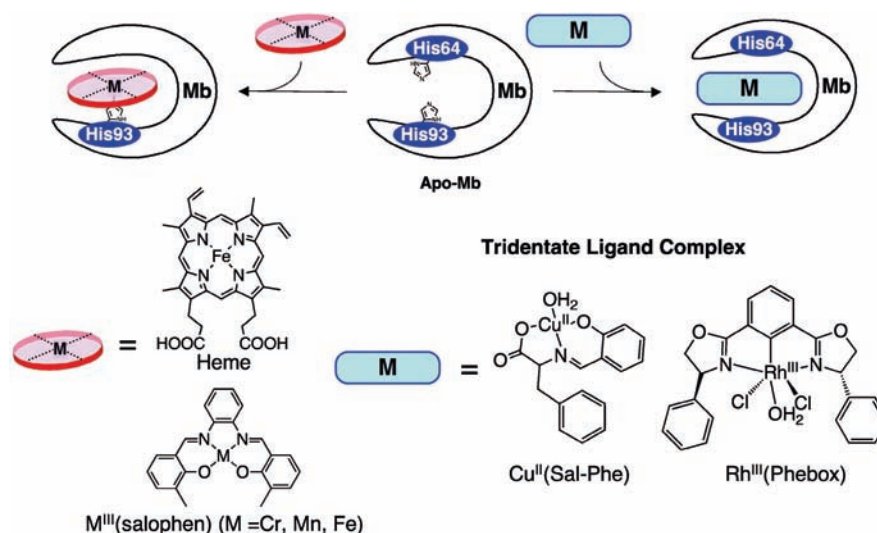


**Fig. 3** Crystal structures of active site of artificial metalloproteins: **a** Fe(Porphycene)•apo-Mb, and **b** Cr(Schiffbase)•apo-A71G Mb taken from PDB ID: 2D6C, and 1V9Q, respectively

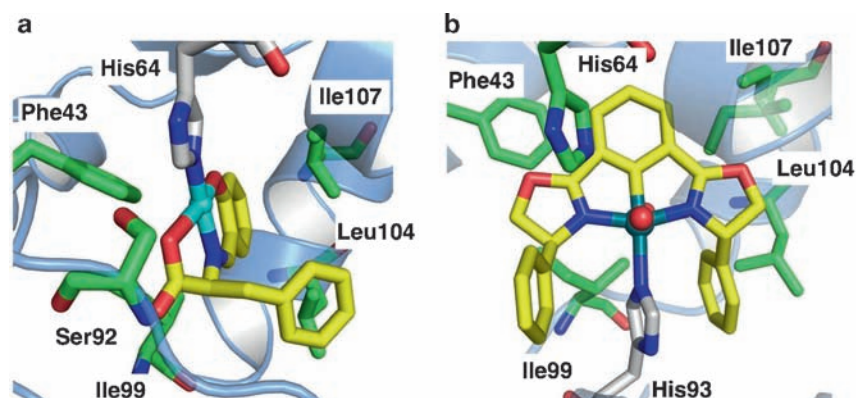
that the iron porphycene is located in the apo-Mb cavity by coordinating to N<sup>ε</sup> of His93 and an external imidazole ligand. The specific interactions such as hydrogen bonding with Lys96 and His97 are also observed (Fig. 3a). The composite showed higher peroxidase and peroxygenase activities than native Mb due to stronger coordination of iron porphycene to His93 than that of heme.

Crystal structures of a series of metal Schiff base complexes in the apo-Mb cavity have been determined by Watanabe et al. [16, 17]. The crystal structures of apo-Mb reconstituted with M<sup>III</sup>(3,3'-salophen) (salophen = *N,N'*-bis(salicylidene)-1,2-phenylenediamine, M = Cr, Mn, Fe) complexes show that the metal salophen complexes are tightly ligated to the N<sup>ε</sup> atom of His93 in the apo-Mb cavity with the same coordination geometry as that of heme, i.e., planar four coordinate ligands and the use of proximal histidine as an axial ligand (Figs. 3b and Fig. 4) [16, 17]. The metal salophen complexes fixed in the apo-Mb cavity are further stabilized by several  $\pi$ - $\pi$  and CH- $\pi$  interactions with surrounding amino acid residues (Fig. 3b). Ueno et al. also succeeded in controlling the enantioselectivity of sulfoxidation by design of metal complexes based on the crystal structures [17, 69]. They have extended this method to other metal complexes with different coordination geometries from heme and salophen complexes.

The group reported two novel coordination structures of Cu<sup>II</sup>(Sal-X) (Sal-X = *N*-salicylideneaminoacidato) and organometallic rhodium 2,6-bis(2-oxazoliny)-phenyl (Rh•Phebox) complexes, whose structures are completely different from heme, in the Mb cavity (Fig. 4). The crystal structure of Cu<sup>II</sup>(Sal-Phe)•apo-Mb shows that the Cu<sup>II</sup> complexes are coordinated to the nitrogen of His64 with a square-planar coordination geometry with the assistance of CH- $\pi$  and  $\pi$ - $\pi$  interactions between a benzene ring in the salicylidene moiety of the ligand and the surrounding hydrophobic amino acid



**Fig. 4** Various binding geometries of incorporated metal complexes in the apo-Mb cavity



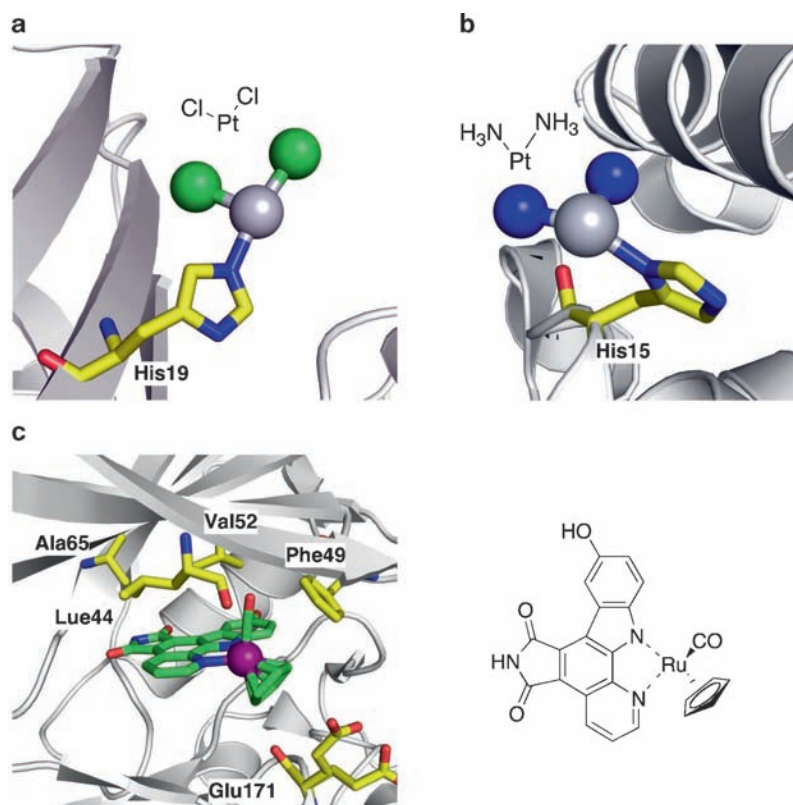
**Fig. 5** Crystal structures of active site of artificial metalloproteins: **a**  $\text{Cu}(\text{Sal-Phe}) \cdot \text{apo-Mb}$ , and **b**  $\text{Rh} \cdot \text{Phebox} \cdot \text{apo-A71G Mb}$  taken from PDB ID: 2EB8, and 2EF2, respectively

residues (Fig. 5a). The crystal structure of  $\text{Rh}^{III} \cdot \text{Phebox} \cdot \text{apo-A71G Mb}$  reveals that the  $\text{Rh} \cdot \text{Phebox}$  complex is fixed in the apo-Mb cavity with an unprecedented arrangement that is almost perpendicular to the heme (Fig. 5b). These results suggest that apo-Mb is capable of accommodating various metal complexes having different coordination structures and functions from the native metal cofactors.



### 3.2 Interaction of Metal-Drugs with Proteins

There is increasing importance of metal-based drugs in the field of medicinal chemistry. Structural study of metal-drug/protein interactions is of utmost importance for understanding the molecular fragments interacting with proteins, their locations, and their binding affinities. There have been an increasing number of reports describing the X-ray crystal structures of metal-drug/protein composites in recent years [11–13, 42]. For example, the interactions of cisplatin, known as an anticancer drug, with proteins such as copper–zinc superoxide dismutase (SOD) and lysozyme have been reported [11, 42]. The crystal structure of SOD interacting with cisplatin shows that the Pt atom is selectively bound to N<sup>ε</sup> of His19. Two chloride atoms also ligate to the Pt atom with a distorted square-planar geometry (Fig. 6a) [11]. The crystal structure of cisplatin and lysozyme adducts reveals selective platination of N<sup>δ</sup> of His15 [42], which is also found to ligate to the organometallic complexes Ru(*p*-cymene) and Mn(CO)<sub>3</sub> (Fig. 6b) [14, 43]. Meggers et al. have designed an organometallic RuCp(CO) complex as a protein



**Fig. 6** Crystal structures of metal-drug/protein composites. **a** cisplatin/SOD, **b** cisplatin/lysozyme, and **c** Ru complex/Pim-1 taken from PDB ID: 2AEO, 2I6Z, and 2BZI, respectively

kinase inhibitor on the basis of the structure of staurosporine, a well-known organic inhibitor of protein kinases. The RuCp(CO) complex exhibits more than two orders of magnitude higher activity than that of staurosporine for Pim-1 (Human serine/threonine protein kinase), which is one of the protein kinases. The crystal structure of the RuCp(CO) complex and Pim-1 adduct shows the binding mode of the Ru complex to be completely identical to that of staurosporine (Fig. 6c) [12].

#### 4 Direct Observation of Metal Accumulation on Protein Surfaces

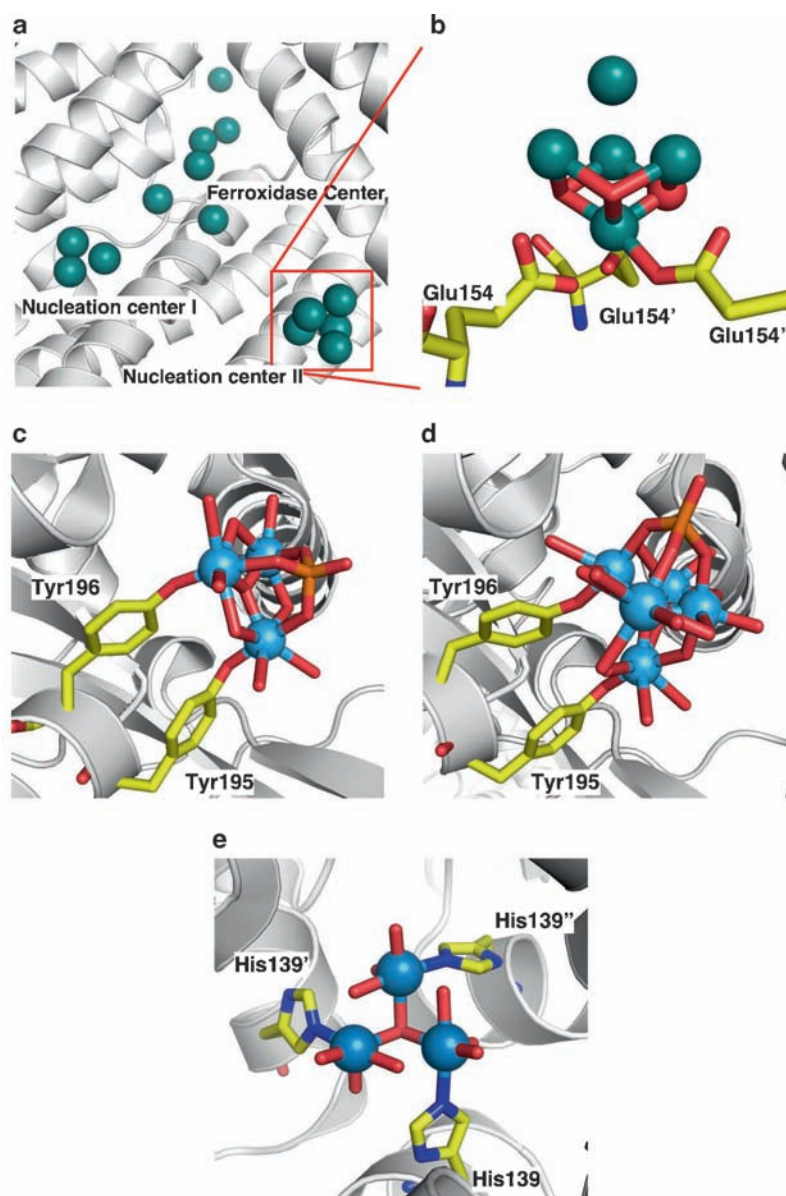
To understand the molecular mechanism of biomineralization, it is very important to study the initial metal binding process as well as the process of metal cluster formation. However, little is known so far on the interactions between amino acids and metal clusters. Ferritin and other ferritin-like spherical proteins are known to catalyze biomineralization in the protein cages [4, 49]. For example, Fe<sup>II</sup> ions incorporated in these protein cages are oxidized to Fe<sup>III</sup> at the ferroxidase center and deposited in the protein large cavities as iron oxides [49].

Zeth et al. have reported biomineralization processes by crystallographic analysis of Dps-like (Dps, DNA-protecting protein during starvation) ferritin proteins containing various amounts of Fe<sup>II</sup> ions [65]. Fe<sup>II</sup> ions penetrate channels composed of acidic amino acid residues (Glu13, Glu15, Asp18, Glu167, Glu171, and Asp173), which are expected to act as an electrostatic guide for incorporating Fe<sup>II</sup>, and travel to three translocation sites within the DpsA pore (Fig. 7a). Fe<sup>II</sup> is oxidized to Fe<sup>III</sup> at the ferroxidase center and iron oxide clusters are formed at two nucleation sites. Five iron atoms are accommodated at one of the nucleation sites and [4Fe–3O] clusters are created using glutamic acid (Fig. 7b).

Sadler et al. have shown multinuclear Hf and Zr clusters formed in a ferric-ion-binding protein, Fbp [10, 19]. The crystal structure of Fbp containing Hf atoms shows that three types of Hf clusters are formed by utilizing a di-tyrosyl cluster nucleation motif (Tyr195-Tyr196) in an interdomain cleft. Two types of trinuclear clusters and one pentanuclear oxo-Hf cluster are generated at this site (Fig. 7c, Fig. 7d) [10]. These results show that a Tyr–Tyr motif is very important for the metal mineralization.

Müller and Ermler et al. have observed various types of polynuclear tungsten oxide clusters in the cavity of a Mo/W-storage (Mo/WSto) protein [70], i.e., five types of polyoxotungstates such as W<sub>3</sub>, W<sub>6</sub>, W<sub>7</sub>, W<sub>2</sub>, and, W<sub>7+x</sub> are formed in the pockets of Mo/WSto protein. The W<sub>3</sub> cluster shown in Fig. 7e consists of ten O atoms and N atoms of three His139 in different subunits, described as W<sub>3</sub>O<sub>10</sub>H<sub>3</sub>N<sub>3</sub>.

Although we are able to observe coordination structures in metal accumulation processes on the protein surfaces, it is still difficult to design the coordination structures and functions of artificial metal clusters formed in protein scaffolds. If we are able to overcome the problems, we could construct metal clusters having desired coordination structures and functions. Furthermore, it may help us in understanding the formation processes of metal clusters prepared in natural proteins such as FeMoco, Fe–S clusters, and Mn clusters in nitrogenase, ferredoxins, and photosystem II [71–73].



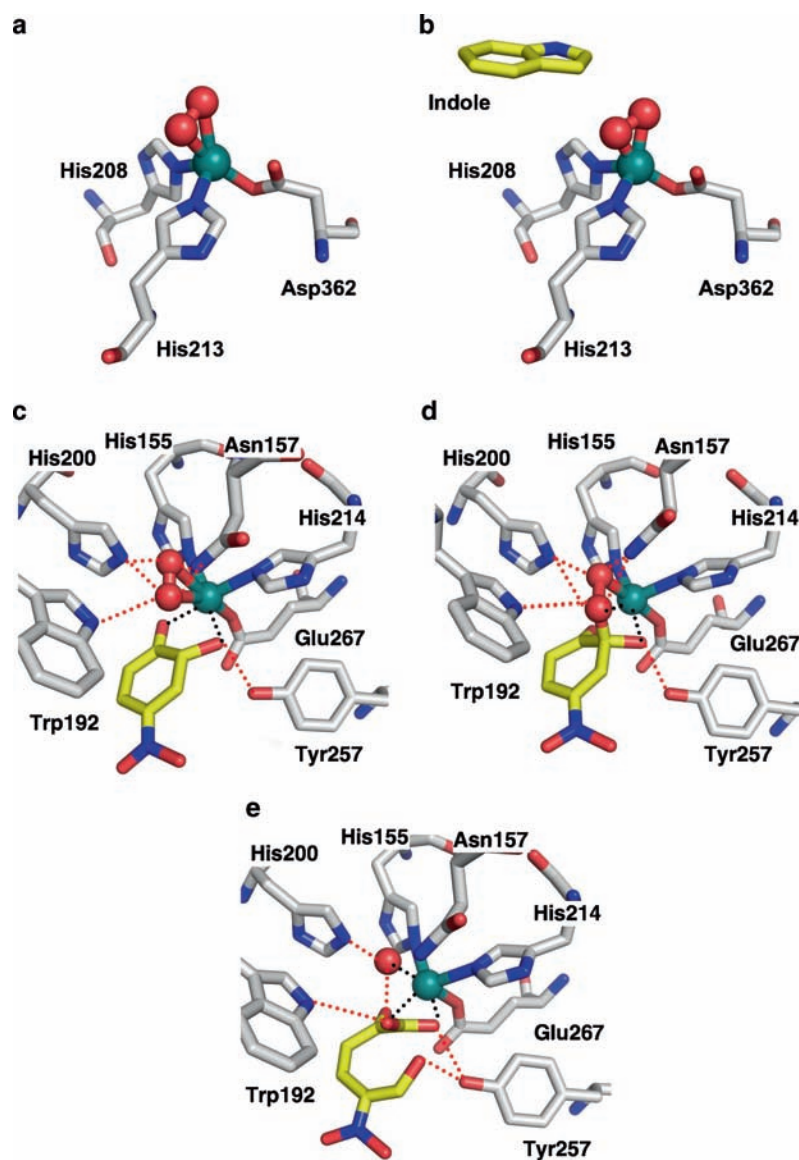
**Fig. 7** Crystal structures of proteins that interact with metal minerals. **a** iron-oxide clusters on the interior surface of DpsA, **b** close up view of the nucleation center II, **c** tri Hf-oxo cluster, and **d** penta Hf-oxo cluster at the di-tyrosyl nucleation motif of ferric-ion-binding protein, Fbp. **e** W<sub>3</sub> cluster at an intersection of subunits in Mo/WSto protein

## 5 Direct Observation of Enzymatic Reaction Processes

Direct observation of enzymatic reaction processes by X-ray crystallographic analysis is very important because the resulting structures are direct evidence for the intermediates of the reactions. There are a series of independent studies showing intermediates of enzymatic reactions of some oxygenases elucidated by X-ray crystallographic analyses [74–78]. For instance, the intermediate structures in hydroxylation of *d*-camphor by cytochrome P450cam show the ferrous dioxygen adduct and oxyferryl species [77]. The crystal structures of naphthalene dioxygenase (NDO) show that a molecular oxygen is bound to the mononuclear iron atom in a side-on fashion in the active site (Fig. 8a) [74]. The structure containing a substrate (indole) and molecular oxygen in the NDO active site shows that the dioxygen molecule bound to the iron atom is at an appropriate position to attack the double bond of the substrate. The structure clearly shows how the enzyme oxidizes the substrate with high stereospecificity (Fig. 8b). The crystal structures of  $\text{Fe}^{2+}$  containing 2,3-homoprotocatechuate dioxygenase show three intermediate states in the  $\text{O}_2$  activation and oxygen insertion reaction by the dioxygenase [76]. Figure 8c shows the dioxygen molecule bound to iron in a side-on fashion at the active site, having the substrate near to the oxygen. The dioxygen bound to the iron then attacks the substrate to afford an iron-peroxide-substrate intermediate (Fig. 8d) followed by formation of an aromatic ring-opened product (Fig. 8e). These crystal structures provide the actual reaction mechanisms, including the origin of high selectivity. By considering these structures, we could design artificial metalloenzymes having high activities.

## 6 Introduction of Functional Metal Materials in Protein Nanocages

Self-assembled protein cages have been utilized for the introduction of functional materials on interior or exterior surfaces of spherical proteins since they are thermally stable and it is easy for us to chemically and genetically modify the protein interior and exterior surfaces [4, 69]. For example, Fr, a small heat shock protein (sHsp), and cowpea chlorotic mottle virus (CCMV) are available for the deposition and modification of inorganic metal particles and organic materials [4, 35, 37, 39, 40, 47, 48, 51–55, 58, 64]. sHsp consists of 24 subunits and affords an interior cavity of 6.5 nm in diameter. sHsp has large pores (3 nm) at the intersections of subunits, which permit easy access into the cavity [40, 64]. CCMV is an RNA-containing plant virus composed of 180 coat protein subunits and provides an outer diameter of 28 nm and an interior cavity of 18–24 nm. CCMV exhibits an ability both to encapsulate organic polymers and metalloenzymes and to regulate metal incorporation in the cavity by controlling the pH-dependent gating behavior of its pores or dissociation of the viral assembly [47, 58]. Tobacco mosaic virus (TMV) [59, 63],



**Fig. 8** Active site structures of  $O_2$  adducts of NDO and HPCD. The  $O_2$  adducts of NDO in the absence (a) and in the presence (b) of indole. The  $O_2$  and 4-NC bound structures of HPCD are shown before the oxygen insertion (c), the C-O-O-Fe formation (d), and the product formation (e). These structures are taken from PDB ID: a 1O7M, b 1O7N, c-e 2IGA. Red dashed lines show hydrogen-bonds. Black lines indicate bonds or potential bonds to iron



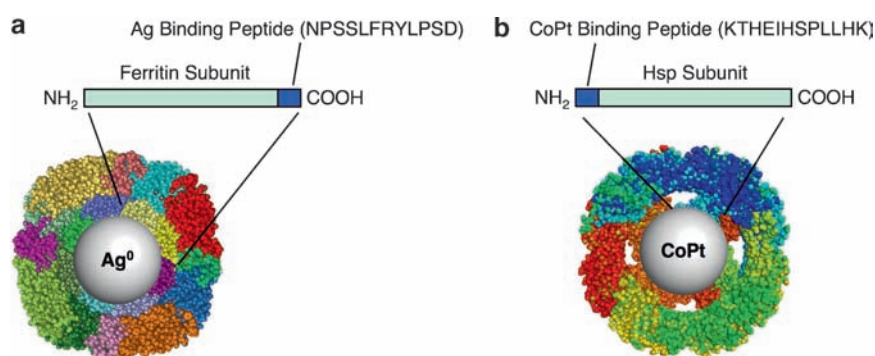
chaperonins composed of two stacked supermolecular protein cages [33, 61], and the gene product 27–gene product 5 component from bacteriophage T4 [79, 80] have also been utilized as biological templates for preparation, deposition, and encapsulation of metal nanoparticles in their vacant spaces.

### 6.1 Preparation of Metal Particles in Protein Cages

Naik et al. and Douglas et al. have prepared Ag and CoPt clusters having a precise crystal phase in the Fr and sHsp cages, respectively, by the introduction of metal binding peptides identified from phage-display screening on protein interior surfaces [35, 40]. The AG4 peptide introduced in the ferritin interior acts as a binding site of silver ions and helps to reduce them to  $\text{Ag}^0$  nanoparticles whose size and crystal phase are highly restricted (Fig. 9a) [35]. A CoPt bimetallic particle was also prepared by the reduction of Co and Pt ions incorporated in the sHsp interior cavity having the CoPt binding peptide. The peptide provided the growth of a tetragonal L10 phase of a CoPt particle in the Hsp cage (Fig. 9b). The particle shows a ferromagnetic property at room temperature [40].

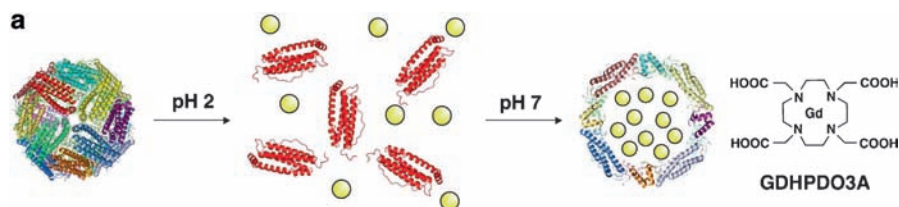
### 6.2 Application to Biomedicines

Protein nanocages are utilized not only for the deposition of metal nanoparticles, but also for the entrapment of chemotherapeutic agents. For example, Gd-HPDO3A (gadolinium-[10-(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid]), which is known to be a magnetic resonance imaging (MRI) contrast agent, is entrapped in the apo-ferritin (apo-Fr) cage by an acid-dissociation method (Fig. 10) [32]. The Gd complexes entrapped in the apo-Fr cage exhibit high relaxivity of water protons, thus, the complex is a potent candidate for its use in MRI.

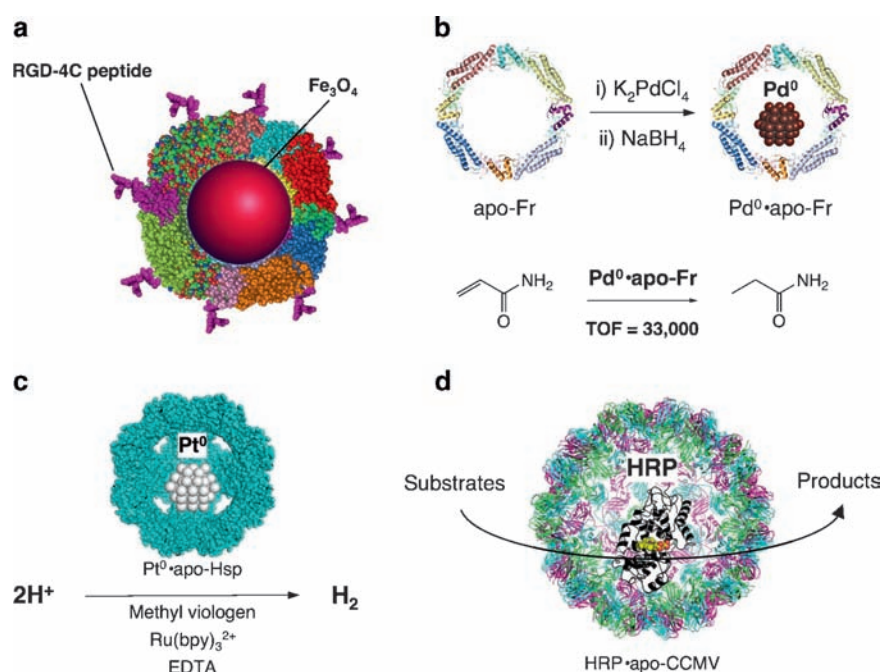


**Fig. 9** Representative structures of **a** Ag particle in L-ferritin captured by the Ag binding peptide, and **b** CoPt particle in Hsp on the CoPt binding peptide





**Fig. 10** Schematic drawing of incorporation of a GDHPDO3A complex into apo-ferritin by an acid-dissociation method



**Fig. 11** Schematic representation of **a** preparation of cell targeted ferritin with the binding RGD-4C peptide on the exterior surface of apo-Fr including  $\text{Fe}_3\text{O}_4$ , **b** preparation of a Pd nanoparticle in apo-Fr and olefin hydrogenation, **c** hydrogen production reaction using  $\text{Pt}^0 \cdot \text{apo-Hsp}$ , and **d** encapsulation of horseradish peroxidase (HRP) in the cavity of CCMV and an enzymatic reaction

Douglas et al. have reported the introduction of multifunctional molecules to apo-Fr. They introduced a cell-specific targeting peptide, RGD-4C on the exterior surface of apo-Fr, where the peptide is capable of binding particular tumor proteins [81]. Indeed, the transmission electron microscopic images of RGD-4C Fr containing a magnetite nanoparticle of  $\text{Fe}_3\text{O}_4$  show that electron-dense particles corresponding to  $\text{Fe}_3\text{O}_4$  were observed on the surface of the C32 melanoma cells (Fig. 11a). These results show how nanoscale protein containers serve to accommodate a variety of functional metal complexes and metal clusters.

### 6.3 Application to Catalysts

Protein nanocages can be used as a catalytic reaction space of metal particles [54, 64]. For example, Ueno et al. have succeeded in the preparation of monodispersed Pd nanoparticles and the size-selective olefin hydrogenation catalyzed by the composite (Fig. 11b) [54]. Olefin substrates must penetrate the threefold channels of apo-Fr to react with the Pd particle, thus, larger substrates are less reactive than smaller substrates.

Varpness et al. prepared a biomimetic material, aiming for artificial hydrogenase, by employing small heat shock protein (sHsp) including Pt particles [64]. The composite catalyzes hydrogen production in the presence of EDTA, Ru(2,2-bioyridine)<sub>3</sub><sup>2+</sup>, methyl viologen as a reductant, photocatalyst, and electron-transfer mediator, respectively (Fig. 11c). The system in the protein cage architecture can provide a new concept for hydrogen production [64].

Engelkamp et al. have reported the incorporation of horseradish peroxidase in the interior cage of CCMV by using its disassembly/assembly property to elucidate a single molecule study of enzyme behaviors (Fig. 11d). They showed that CCMV permits easy access of substrates and products through the pores of CCMV and that this permeability can be controlled by pH [58]. These results show that protein nanocages are very useful templates for the entrapment of functional materials and provide chemical reaction space.

## 7 Summary

The recent examples described in this chapter demonstrate that the construction of artificial metalloproteins is an active field in bioinorganic chemistry. We are able to use small protein cavities as well as large protein cages such as ferritin and virus molecules as molecular templates for incorporating synthetic metal complexes and materials into the scaffolds. The metal complex/protein composites are able to function as asymmetric catalysts, electron transfer materials, and magnetic and electronic materials. It is possible to modify the functions of the composites by changing the metal complexes and/or protein cavities. This progress has been achieved by cooperation of molecular and structural biology and inorganic chemistry. In particular, X-ray crystal structure analyses of the composites provide coordination structures and non-covalent interactions between metal complexes and protein scaffolds. Structural information is very important for improving catalytic activities, to understand their reaction mechanisms, and to design novel metal-drugs and metal inhibitors. Furthermore, dynamic processes of mineralization and catalytic reactions on the metal binding sites through structural studies of intermediates have just started. We believe that these results will provide intriguing implications for their application in catalysts, sensors, electronics devices, and so on.

## References

1. Lu Y (2006) *Angew Chem Int Ed* 45:5588–5601
2. Steinreiber J, Ward TR (2008) *Coord Chem Rev* 252:751–766
3. Thomas CM, Ward TR (2005) *Chem Soc Rev* 34:337–346
4. Uchida M, Klem MT, Allen M, Suci P, Flenniken M, Gillitzer E, Varpness Z, Liepold LO, Young M, Douglas T (2007) *Adv Mater* 19:1025–1042
5. Ueno T, Abe S, Yokoi N, Watanabe Y (2007) *Coord Chem Rev* 251:2717
6. Ueno T, Yokoi N, Abe S, Watanabe Y (2007) *J Inorg Biochem* 101:1667
7. Akabori S, Sakurai S, Izumi Y, Fujii Y (1956) *Nature* 178:323–324
8. Wilson ME, Whitesides GM (1978) *J Am Chem Soc* 100:306–307
9. Abe S, Ueno T, Reddy PAN, Okazaki S, Hikage T, Suzuki A, Yamane T, Nakajima H, Watanabe Y (2007) *Inorg Chem* 46:5137–5139
10. Alexeev D, Zhu HZ, Guo ML, Zhong WQ, Hunter DJB, Yang WP, Campopiano DJ, Sadler PJ (2003) *Nat Struct Biol* 10:297–302
11. Calderone V, Casini A, Mangani S, Messori L, Orioli PL (2006) *Angew Chem Int Ed* 45:1267–1269
12. Debreczeni JE, Bullock AN, Atilla GE, Williams DS, Bregman H, Knapp S, Meggers E (2006) *Angew Chem Int Ed* 45:1580–1585
13. Hu YZ, Tsukiji S, Shinkai S, Oishi S, Hamachi I (2000) *J Am Chem Soc* 122:241–253
14. McNae IW, Fishburne K, Habtemariam A, Hunter TM, Melchart M, Wang FY, Walkinshaw MD, Sadler PJ (2004) *Chem Commun* 2004:1786–1787
15. Satake Y, Abe S, Okazaki S, Ban N, Hikage T, Ueno T, Nakajima H, Suzuki A, Yamane T, Nishiyama H, Watanabe Y (2007) *Organometallics* 26:4904–4908
16. Ueno T, Ohashi M, Kono M, Kondo K, Suzuki A, Yamane T, Watanabe Y (2004) *Inorg Chem* 43:2852–2858
17. Ueno T, Koshiyama T, Ohashi M, Kondo K, Kono M, Suzuki A, Yamane T, Watanabe Y (2005) *J Am Chem Soc* 127:6556–6562
18. Ueno T, Yokoi N, Unno M, Matsui T, Tokita Y, Yamada M, Ikeda-Saito M, Nakajima H, Watanabe Y (2006) *Proc Natl Acad Sci USA* 103:9416–9421
19. Zhong WQ, Alexeev D, Harvey I, Guo ML, Hunter DJB, Zhu HZ, Campopiano DJ, Sadler PJ (2004) *Angew Chem Int Ed* 43:5914–5918
20. Zunszain PA, Ghuman J, Komatsu T, Tsuchida E, Curry S (2003) *BMC Struct Biol* 3:6–14
21. Hayashi T, Hitomi Y, Ando T, Mizutani T, Hisaeda Y, Kitagawa S, Ogoshi H (1999) *J Am Chem Soc* 121:7747–7750
22. Hayashi T, Murata D, Makino M, Sugimoto H, Matsuo T, Sato H, Shiro Y, Hisaeda Y (2006) *Inorg Chem* 45:10530–10536
23. Carey JR, Ma SK, Pfister TD, Garner DK, Kim HK, Abramite JA, Wang Z, Guo Z, Lu Y (2004) *J Am Chem Soc* 126:10812–10813
24. Ohashi M, Koshiyama T, Ueno T, Yanase M, Fujii H, Watanabe Y (2003) *Angew Chem Int Ed* 42:1005–1008
25. Collot J, Gradinaru J, Humbert N, Skander M, Zocchi A, Ward TR (2003) *J Am Chem Soc* 125:9030–9031
26. Letondor C, Humbert N, Ward TR (2005) *Proc Natl Acad Sci USA* 102:4683–4687
27. Komatsu T, Ohmichi N, Zunszain PA, Curry S, Tsuchida E (2004) *J Am Chem Soc* 126:14304–14305
28. Mahammed A, Gross Z (2005) *J Am Chem Soc* 127:2883–2887
29. Marchetti M, Mangano G, Paganelli S, Botteghi C (2000) *Tetrahedron Lett* 41:3717–3720
30. Reetz MT, Jiao N (2006) *Angew Chem Int Ed* 45:2416–2419
31. Hunter TM, McNae IW, Liang XY, Bella J, Parsons S, Walkinshaw MD, Sadler PJ (2005) *Proc Natl Acad Sci USA* 102:2288–2292
32. Aime S, Frullano L, Crich SG (2002) *Angew Chem Int Ed* 41:1017–1019
33. Ishii D, Kinbara K, Ishida Y, Ishii N, Okochi M, Yohda M, Aida T (2003) *Nature* 423:628–632

34. Iwahori K, Yoshizawa K, Muraoka M, Yamashita I (2005) *Inorg Chem* 44:6393–6400
35. Kramer RM, Li C, Carter DC, Stone MO, Naik RR (2004) *J Am Chem Soc* 126:13282–13286
36. Meldrum FC, Heywood BR, Mann S (1992) *Science* 257:522–523
37. Wong KKW, Mann S (1996) *Adv Mater* 8:928
38. Blum AS, Soto CM, Wilson CD, Cole JD, Kim M, Gnade B, Chatterji A, Ochoa WF, Lin TW, Johnson JE, Ratna BR (2004) *Nano Lett* 4:867–870
39. Douglas T, Strable E, Willits D, Aitouchen A, Libera M, Young M (2002) *Adv Mater* 14:415–418
40. Klem MT, Willits D, Solis DJ, Belcher AM, Young M, Douglas T (2005) *Adv Funct Mater* 15:1489–1494
41. Mao CB, Solis DJ, Reiss BD, Kottmann ST, Sweeney RY, Hayhurst A, Georgiou G, Iverson B, Belcher AM (2004) *Science* 303:213–217
42. Casini A, Mastrobuoni G, Temperini C, Gabbiani C, Francese S, Moneti G, Supuran CT, Scozzafava A, Messori L (2007) *Chem Commun* 156–158
43. Razavet M, Artero V, Cavazza C, Oudart Y, Lebrun C, Fontecilla-Camps JC, Fontecave M (2007) *Chem Commun* 2007:2805–2807
44. Hayashi T, Hiseada Y (2002) *Acc Chem Res* 35:35–43
45. Ozaki SI, Roach MP, Matsui T, Watanabe Y (2001) *Acc Chem Res* 34:818–825
46. Watanabe Y, Nakajima H, Ueno T (2007) *Acc Chem Res* 40:554–562
47. Douglas T, Young M (1998) *Nature* 393:152–155
48. Douglas T, Dickson DPE, Betteridge S, Charnock J, Garner CD, Mann S (1995) *Science* 269:54–57
49. Liu XF, Theil EC (2005) *Acc Chem Res* 38:167–175
50. Yang XK, Chasteen ND (1996) *Biophys J* 71:1587–1595
51. Mackle P, Charnock JM, Garner CD, Meldrum FC, Mann S (1993) *J Am Chem Soc* 115:8471–8472
52. Mann S, Meldrum FC (1991) *Adv Mater* 3:316–318
53. Tsukamoto R, Iwahori K, Muraoka M, Yamashita I (2005) *Bull Chem Soc Jpn* 78:2075–2081
54. Ueno T, Suzuki M, Goto T, Matsumoto T, Nagayama K, Watanabe Y (2004) *Angew Chem Int Ed* 43:2527–2530
55. Yamashita I, Hayashi J, Hara M (2004) *Chem Lett* 33:1158–1159
56. Dominguez-Vera JM, Colacio E (2003) *Inorg Chem* 42:6983–6985
57. Yang Z, Wang XY, Diao HJ, Zhang JF, Li HY, Sun HZ, Guo ZJ (2007) *Chem Commun* 3453–3455
58. Comellas-Aragones M, Engelkamp H, Claessen VI, Sommerdijk N, Rowan AE, Christianen PCM, Maan JC, Verduin BJM, Cornelissen J, Nolte RJM (2007) *Nat Nanotechnol* 2:635–639
59. Dujardin E, Peet C, Stubbs G, Culver JN, Mann S (2003) *Nano Lett* 3:413–417
60. Lee SW, Mao CB, Flynn CE, Belcher AM (2002) *Science* 296:892–895
61. McMillan RA, Paavola CD, Howard J, Chan SL, Zaluzec NJ, Trent JD (2002) *Nat Mater* 1:247–252
62. Nam KT, Kim DW, Yoo PJ, Chiang CY, Meethong N, Hammond PT, Chiang YM, Belcher AM (2006) *Science* 312:885–888
63. Shenton W, Douglas T, Young M, Stubbs G, Mann S (1999) *Adv Mater* 11:253–256
64. Varpness Z, Peters JW, Young M, Douglas T (2005) *Nano Lett* 5:2306–2309
65. Zeth K, Offermann S, Essen LO, Oesterhelt D (2004) *Proc Natl Acad Sci USA* 101:13780–13785
66. Davies RR, Distefano MD (1997) *J Am Chem Soc* 119:11643–11652
67. Kruithof CA, Casado MA, Guillena G, Egmond MR, van der Kerk-van Hoof A, Heck AJR, Gebbink R, van Koten G (2005) *Chem Eur J* 11:6869–6877
68. Panella L, Broos J, Jin JF, Fraaije MW, Janssen DB, Jeronimus-Stratingh M, Feringa BL, Minnaard AJ, de Vries JG (2005) *Chem Commun* 2005:5656–5658
69. Ueno T, Koshiyama T, Abe S, Yokoi N, Ohashi M, Nakajima H, Watanabe Y (2007) *J Organomet Chem* 692:142–147

70. Schemberg J, Schneider K, Demmer U, Warkentin E, Muller A, Ermler U (2007) *Angew Chem Int Ed* 46:2408–2413
71. Ferreira KN, Iverson TM, Maghlaoui K, Barber J, Iwata S (2004) *Science* 303:1831–1838
72. Loll B, Kern J, Saenger W, Zouni A, Biesiadka J (2005) *Nature* 438:1040–1044
73. Messerschmidt A, Huber R, Poulos T, Wieghardt K (eds) (2001) *Handbook of metalloproteins*, vol 1. Wiley, Chichester
74. Karlsson A, Parales JV, Parales RE, Gibson DT, Eklund H, Ramaswamy S (2003) *Science* 299:1039–1042
75. Katona G, Carpentier P, Niviere V, Amara P, Adam V, Ohana J, Tsanov N, Bourgeois D (2007) *Science* 316:449–453
76. Kovaleva EG, Lipscomb JD (2007) *Science* 316:453–457
77. Schlichting I, Berendzen J, Chu K, Stock AM, Maves SA, Benson DE, Sweet BM, Ringe D, Petsko GA, Sligar SG (2000) *Science* 287:1615–1622
78. Unno M, Chen H, Kusama S, Shaik S, Ikeda-Saito M (2007) *J Am Chem Soc* 129:13394–13395
79. Koshiyama T, Yokoi N, Ueno T, Kanamaru S, Nagano S, Shiro Y, Arisaka F, Watanabe Y (2008) *Small* 4:50–54
80. Ueno T, Koshiyama T, Tsuruga T, Goto T, Kanamaru S, Arisaka F, Watanabe Y (2006) *Angew Chem Int Ed* 45:4508–4512
81. Uchida M, Flenniken ML, Allen M, Willits DA, Crowley BE, Brumfield S, Willis AF, Jackiw L, Jutila M, Young MJ, Douglas T (2006) *J Am Chem Soc* 128:16626–16633



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