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Functionalization of protein crystals with metal ions, complexes and nanoparticles

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Self-assembled proteins have specific functions in biology. With inspiration provided by natural protein systems, several artificial protein assemblies have been constructed via site-specific mutations or metal coordination, which have important applications in catalysis, material and bio-supramolecular chemistry. Similar to natural protein assemblies, protein crystals have been recognized as protein assemblies formed of densely-packed monomeric proteins. Protein crystals can be functionalized with metal ions, metal complexes or nanoparticles via soaking, co-crystallization, creating new metal binding sites by site-specific mutations. The field of protein crystal engineering with metal coordination is relatively new and has gained considerable attention for developing solid biomaterials as well as structural investigations of enzymatic reactions, growth of nanoparticles and catalysis. This review highlights recent and significant research on functionalization of protein crystals with metal coordination and future prospects.

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Introduction

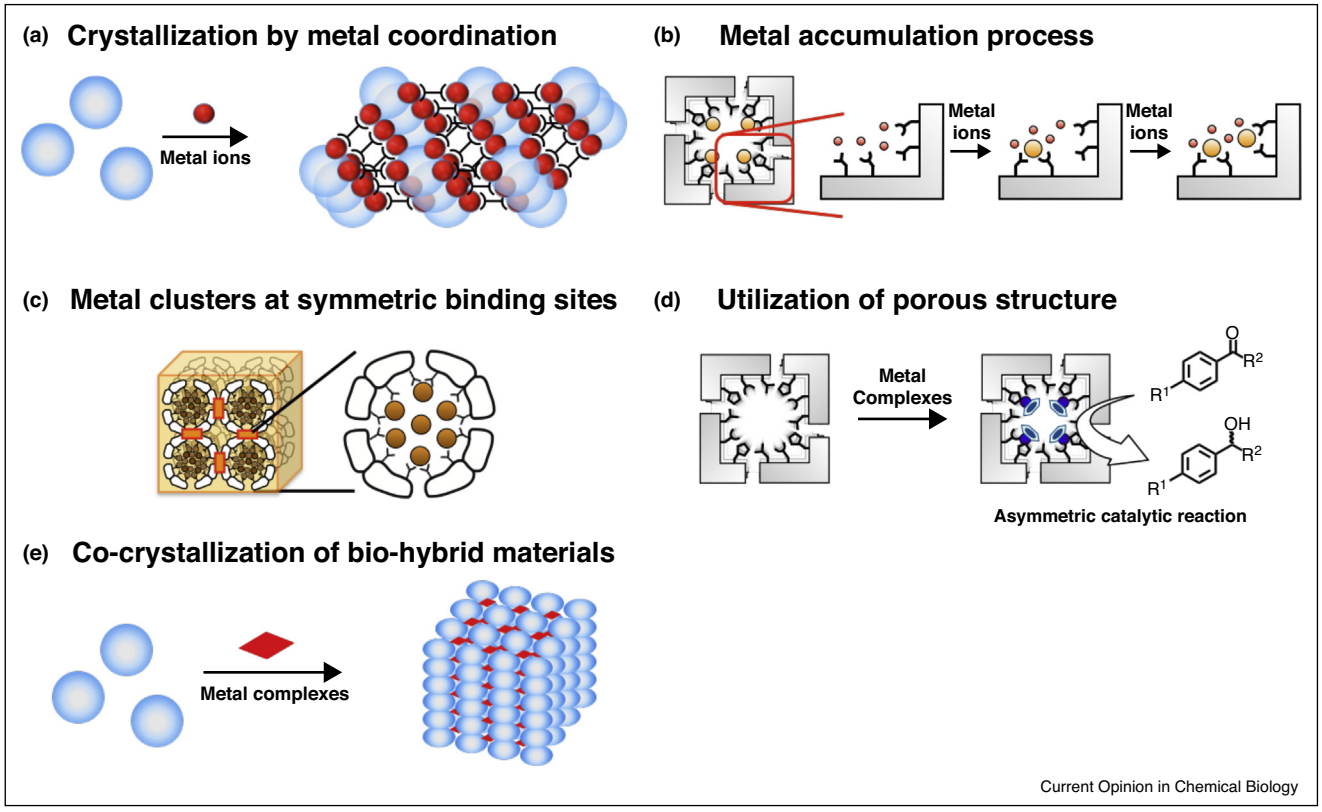
Proteins have specific functions such as metal transport, catalysis of enzymatic reactions, and electron transfer which harness the power of coordinated metal ions and metal complexes in biological systems. Using genetic and chemical methodology, several artificial metalloproteins have been developed where metal coordination is employed to provide unnatural activity of metal ions and complexes in protein scaffolds in efforts to understand the range of natural functions of metalloproteins [1–5]. Some proteins exist as assembled structures, such as protein cages and tubes, to immobilize multiple metal ions and complexes. Self-assembled proteins are of particular interest in efforts to take advantage of both the properties of supramolecular structures and the functions of immobilized metal compounds. The interior spaces of the protein assemblies such as ferritin and virus particles have been utilized as templates to accumulate metal ions/complexes for synthesis of metal nanoparticles and catalytic reactions in the cages [4,6].

Recently, protein crystals (crystalline protein assemblies), have been functionalized by providing sites for metal coordination within solvent channels in efforts to develop new biomaterials [7]. Synthetic porous crystalline materials such as metal-organic frameworks (MOF) and porous organic frameworks are promising solid materials for new applications in adsorption of gaseous molecules, catalytic reactions, molecular recognition, and structural analysis of immobilized molecules [8–10]. Protein crystals are believed to be ideal candidates for creation of functional porous crystalline materials because (1) protein crystals have highly ordered arrangements of protein monomers giving a variety of porous structures, (2) the functional groups of amino acid residues which are periodically aligned on the surface of protein crystals provide sites for accumulation of functional molecules by metal coordination, chemical modification and synthesis of inorganic nanoparticles for applications in heterogeneous catalysis and drug delivery, and (3) X-ray crystal structure analysis can be applied to characterize processes of metal accumulation and cluster formation and to investigate interactions of metal drugs with proteins [11–17]. In addition, metal coordination at the intermolecular contact positions can further promote crystallization of proteins and peptides [18,19]. Crystal engineering of proteins with metal coordination is a new research area which has already led to development of a number of potential applications. This review highlights recent significant research in the area of protein crystal engineering with metal coordination and future perspectives (Figure 1).

Protein crystallization mediated by metal coordination

Metal coordination with functional amino acid residues at protein interfaces is one of the most useful strategies for improving protein crystallization. For example, the light chain (L) apo-ferritin can be crystallized via coordination of Cd^{2+} ions to the outer surfaces of the caged structures [20]. Heavy chain (H) apo-ferritin was crystallized by site-specific mutagenesis to introduce the metal binding sites at the contact inter-surfaces of ferritin molecules [21].

Figure 1



Functionalization of protein crystals with metal coordination. **(a)** Novel protein crystal lattices by using designed metal coordination sites. **(b)** Protein crystals were employed for observation of metal accumulation process on the protein surface using X-ray crystal structure analysis. **(c)** Metal cluster formation at the symmetric interfaces in the crystals. **(d)** Immobilization of metal complexes within porous protein crystals for asymmetric catalysis. **(e)** Co-crystallization of metal complexes and proteins to create bio-hybrid materials.

This technology has been applied to create novel crystal lattice structures. Yeates and coworkers reported that the surface of T4 lysozyme (T4L) and maltose-binding protein (MBP) were modified with histidine (His) or cysteine (Cys) residues to induce symmetric assembly structures by coordination of metal ions, such as Cu^{2+}, Ni^{2+} and Zn^{2+} [18]. The symmetric units were crystallized with new lattice structures of T4L and MBP. The lattice structures of the crystals were regulated according to the positions of the designed metal binding sites.

Tezcan and coworkers have established an intriguing methodology for formation of 1D, 2D and 3D protein assembly structures by design of metal coordination sites at the protein interface [22,23]. Recently, they synthesized a metal organic framework (MOF) with engineered ferritin [24,25••]. The 3-fold channel of the ferritin cage was engineered to bind to Zn^{2+} ions which subsequently connected to another ferritin cage unit through a pyridyl linker, thus forming a unique body-centered cubic crystal lattice which differs from the crystals produced by ferritin and Zn ions. This strategy was expanded to ferritin-MOF crystals using various metal ions (Zn^{2+}, Ni^{2+}, and Co^{2+}) and dihydroxamate linkers. The combination of metal ions and organic linkers was found to influence crystal lattice structures and unit cell dimensions of ferritin-MOF [25••].

Fujita and coworkers have reported preparation of crystalline peptide assemblies by metal coordination with arranged helical structures of peptides [26]. Short peptides including the Gly-Pro-Pro sequences were folded into the crystals by coordination of Ag^{+} ions. The peptide crystalline assemblies can be utilized as porous materials for applications in recognition of chiral molecules.

Observation of accumulation process of metal ions on the protein surface by X-ray structure analysis

X-ray crystal structure analysis is useful for elucidation of accumulation process of metal ions into the protein assemblies, such as the ferritin cage. Theil and coworkers have investigated the process of entry of Fe^{2+} ions into the ferritin cage and the catalytic ferroxidase site by X-ray crystal structure analysis [27,28]. The Fe^{2+} ions pass through the 3-fold channel and move to the ferroxidase