論文 / 著書情報
Article / Book Information

Title	Use of the confined spaces of apo-ferritin and virus capsids as nanoreactors for catalytic reactions
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Citation	Current Opinion in Chemical Biology, Volume 25, Page 88-97
Pub. date	2015, 1
DOI	https://doi.org/10.1016/j.cbpa.2014.12.026
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Use of the Confined Spaces of Apo-Ferritin and Virus Capsids as Nanoreactors for Catalytic Reactions

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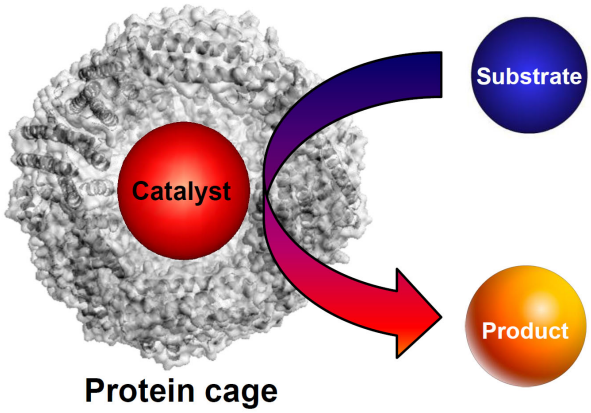
Highlights

- Ferritin cage and virus capsids as nanoreactor
- Catalytic reactions inside apo-Ferritin cage
- Enzymatic reactions in virus capsid
- Protein cages vs. protein needles/tubes

Graphical abstract



Abstract

Self-assembled protein cages providing nanosized internal spaces which are capable of encapsulating metal ions/complexes, enzymes/proteins have great potential for use as catalytic nanoreactors in efforts to mimic confined cellular environments for synthetic applications. Despite many uses in biomineralization, drug delivery, bio-imaging etc., applications in catalysis are relatively rare. Due to their restricted size, protein cages are excellent candidates for use as vessels to exert control over reaction kinetics and product selectivity. Virus capsids with larger internal spaces can encapsulate multiple enzymes and can mimic natural enzymatic reactions. The apo-ferritin cage is known to accommodate various metal ions/complexes and suitable for organic transformation reactions in an aqueous medium. This review highlights the importance, prospects and recent significant research on catalytic reactions using the apo-ferritin cage and virus capsids.

Introduction

Naturally occurring protein assemblies with nanosized scaffolds are of great interest due to their unique structural features, robustness, and uniform size. Such protein assemblies have wide-ranging applications in chemistry, biology and material science [1-5]. The highly symmetric self-assembled protein architectures provided by ferritin, hsp (heat shock protein), dps (DNA-binding protein from starved cells) and virus capsids provide interior nanospaces ranging in size from 6 to 500 nm [6]. Such protein scaffolds are mostly studied for their applications in biomineralization and preparation/storage of inorganic nanoparticles [1,2]. However, current interest is growing with regard to utilizing such protein scaffolds as catalytic nanoreactors similar to cellular microcompartments where several enzymes work together in catalyzing metabolic reactions [7,8]. Inside the cell, catalytically-active species are protected in confined spaces and separated from the surrounding environment. Investigating catalytic reactions in confined spaces is an important effort which will improve our understanding of the complex processes of the cell. Self-assembled protein cages with restricted interior cavities have the capability to accumulate catalytically active metal ions/complexes and enzymes and are thus suitable for development of biomimetic protein compartments. The limited space can influence the reaction kinetics and product selectivity [9,10••]. Furthermore, the small pores of protein cages help substrates and products to freely diffuse in and out of the cavity. Virus capsids with larger interior spaces are suitable for accommodating multiple enzymes simultaneously whereas the other protein cages are suitable for small molecular catalysts [11,12]. In addition to naturally occurring protein cages, several artificial protein assemblies are also being developed for wider applications of protein scaffolds [13-15]. Other molecular cages such as polymerosomes, liposomes etc. are also known to encapsulate enzymes but the monodisperse nature of the self-

assembled protein cages represent an important distinction [16,17]. Protein scaffolds thus provide a powerful platform for development of biomimetic compartments for important synthetic applications. The area is relatively new and offers rich opportunities to study new catalytic reactions using various protein cages.

This review focuses on highlighting recent significant developments in the design and construction of protein assemblies as catalytic nanoreactors. We limit our scope to the apo-ferritin nanocage and virus capsids which are the two most promising natural self-assembled protein architectures for catalytic reactions (Figure 1). We also compare the advantages of protein cages over protein needles/tubes.

Ferritin

Ferritin is a natural intracellular iron storage protein consisting of 24 subunits which self-assemble to form a hollow cage structure with an inner diameter of 8 nm and an outer diameter of 12 nm [18]. The cage is known to store thousands of iron ions as compact minerals. The high stability of ferritin over a wide pH range of 2-11 and temperatures up to 80°C has encouraged the study of this cage in a number of useful and intriguing applications. The cage can accommodate a number of other metal ions/complexes or small organic molecules [19-21]. The pH-dependent assembly/disassembly properties are advantageous with respect to encapsulation of larger metal complexes, organic molecules or nanoparticles [22,23]. Interestingly, the interior cage is connected to eight 3-fold channels and six 4-fold channels. The 3-fold channels used to transfer ions into and out of the protein. These properties make ferritin an important platform for preparing nanomaterials, and for use in drug delivery and catalysis [22-27]. For example, the ferritin cage can encapsulate Gd-HPDO3A (gadolinium-[10-(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid]) chelates via a reassembly/disassembly mechanism

and exhibits 20 times higher relaxivity compared to the free Gd-HPDO3A chelate in water [23]. The well known anticancer drug cisplatin was incorporated into the ferritin cage. Uptake of this encapsulated cisplatin species was found to be increased by 4-fold over free cisplatin [28,29]. Similarly the anticancer drug doxorubicin and a zinc-phthalocyanine based photosensitizer have been incorporated into the apo-ferritin cage with high loading capacities [22,27]. These examples of drug-loaded ferritin also exhibit increased uptake relative to the free drugs alone.

The apo-ferritin age as a catalytic nanoreactor

a) **Nanomaterial-based catalyst**:

Due to its uniform size, the ferritin cage is suitable for preparing homogenous nanoparticles (NPs). Since the cage can accommodate other metal ions such as Pd^{2+}, the accumulated metal ions inside the protein cage can be reduced to prepare NPs for catalytic reactions (Figure 2a,2b) [19]. Ueno and co-workers have developed a novel strategy for construction of size-selective hydrogenation catalysts using the apo-ferritin cage [9]. Pd nanoclusters were synthesized inside the apo-ferritin cage by addition of Pd^{2+} ions followed by reduction with $NaBH_4$. The hydrogenation reactions were evaluated in aqueous medium. The catalytic activity was tested using a number of olefins with various functionalities including acid, amide, isopropyl, and *t*-butyl groups. Since the inner surface of the 3-fold channel is negatively charged, the rate of hydrogenation of anionic acrylic acid is lower than the rates of hydrogenation of other substrates. This result indicates that the negatively charged substrate does not efficiently penetrate the 3-fold channel. Molecular modeling studies suggest that small substrates with positive charges will penetrate more efficiently. The entire apo-ferritin cage structure is retained after the reaction. To improve the catalytic activity of (**Pd-NP)·apo-ferritin**, bimetallic Au/Pd core-shell and alloy NPs were prepared inside the Ferritin cage [30•]. The alloy

NPs were prepared by co-reduction of the mixture of Au^{3+} and Pd^{2+} ions with apo-ferritin whereas the core–shell NPs were prepared using a sequential-reduction method. The Au core/Pd NPs have 2.5 to 4.2 fold higher catalytic activities than (**Pd-NP)·apo-ferritin** and alloy Au/Pd NPs in the ferritin cage. The Pd core/Au NPs did not show any catalytic activities whereas the alloy Au/Pd NPs were found to have lower activity than (**Pd-NP)·apo-ferritin**.

Arends and co-workers have recently developed monodisperse Pd nanoclusters inside the core of the hyperthermophilic ferritin from *Pyrococcus furiosus* and investigated catalytic oxidation of primary and secondary alcohols [31•]. The uniform Pd NPs (~5nm) were prepared by H_2-driven reduction of Pd^{2+} encapsulated apo-ferritin. The Pd NPs selectively catalyzed the aerobic oxidation of primary alcohols to aldehydes without over oxidation. The catalyst was recycled at least twice without any significant loss of activity.

Similarly, the metal nanoparticles prepared in the ferritin cage can mimic the enzymatic reactions such as catalase and peroxidase activities to control oxidative stress in living cells (Figure 2) [32-36]. Nie and co-workers have successfully synthesized highly stable Pt nanoparticles (1-2 nm) using the apo-ferritin scaffold. The Pt NPs have catalase and peroxidase activities which are dependent on pH and temperature [33]. Similarly the bimetallic Fe-Pt nanomaterials were prepared in ferritin scaffolds and exhibit enhanced peroxidase activity compared to monometallic NPs [34]. Yan, Liangs and co-workers synthesized magnetite (Fe_3O_4) inside the heavy-chain ferritin cage [35]. The iron oxide core inside magnetoferritin was found to catalyze the peroxide substrate TMB (tetramethylbenzidine) in the presence of H_2O_2 to form a distinct blue color. This property of magnetoferritin was used for targeting and visualizing tumor tissue.

CeO_2 NPs (which are known as nanoceria) were prepared inside the apo-ferritin cage using assembly/disassembly mechanism [36••]. The ROS ($O_2^{\bullet-}$) scavenging activity of the 4.5 nm nanoceria encapsulated apo-ferritin was found to be significantly higher than natural superoxide dismutase (SOD) or the mixture of CeO_2 and free apo-ferritin. The apo-ferritin coating was found to increase the biocompatibility of the nanoceria and induced ferritin receptor-mediated cellular internalization. The low cytotoxicity and high ROS scavenging activity make nanoceria-encapsulated apo-ferritin an excellent artificial redox enzyme candidate for reduction of oxidative stress in living cells.

b) **Metal-based catalyst**:

The success of producing metal NP-loaded apo-ferritin with various catalytic activities has motivated researchers to incorporate synthetic metal complexes into the ferritin cage in efforts to develop biocatalysts. The metal complexes can freely move inside the protein cage through the 3-fold channel and gain access to suitable amino acid residues for immobilization on the interior surface of the cage. Thus the metal complexes are stabilized and become water soluble. Ueno and co-workers have focused on understanding the nature of metal coordination inside the apo-ferritin cage and evaluated the catalytic activities of metal complexes inside the cage [37,38]. The organometallic $[Pd(allyl)Cl]_2$ complex was incorporated into the apo-ferritin cage by adding into the apo-ferritin solution in 50 mM Tris/HCl (pH 8.0) with stirring followed by dialysis and gel purification. The crystal structure of the composite **Pd(allyl)·apo-rHLFr** shows that there are two Pd binding domains in each subunit of apo-ferritin which are centered at Cys48 in the accumulation center and at Cys126 in the 3-fold channel. In each center, the Pd atoms maintain the dinuclear structure with thiol-bridging ligation of a cysteine residue. The catalytic activity of **Pd(allyl)·apo-ferritin** was subjected to a Suzuki-Miyaura cross-coupling

reaction in 0.15 M NaCl and in the presence of NaOH at 50 °C. The Pd catalyst has a very high turnover frequency (TOF) of ~3500 h^{-1} for the C-C coupling reaction of 4-iodoaniline with phenylboronic acid (Figure 2). Various apo-ferritin mutants were used to change the coordination structure of Pd and to study the effects of these changes with respect to their catalytic activities. In the apo-H49A-ferritin mutant, the dinuclear structure of Pd(allyl) units is retained and the composite has a similar TOF (~3400 h^{-1}). The apo-H114A-ferritin mutant has a trinuclear Pd cluster in the 3-fold channel which blocks the penetration of substrates inside the cage. As a result, there is a 4-fold decrease in activity (TOF ~900h^{-1}) relative to native ferritin. The Pd binding sites were abolished in apo-C48A/H49A-ferritin and apo-C126A-ferritin mutants. As a result, the catalytic activities of these mutants are 1.8- and 4-fold lower than native ferritin, respectively. The catalytic activities of apo-E45C/C48A-ferritin (4200 h^{-1}), apo-E45C/R52H-ferritin (~4300 h^{-1}) and apo-E45C/H49A/R52H-ferritin (~4200 h^{-1}) are higher than that of native ferritin. Size exclusion chromatography of the reaction solutions indicates that the entire ferritin cage structure is retained during the reaction.

Similar to $[Pd(allyl)]_2$ complex, the organometallic $[Rh(nbd)Cl]_2$ complex was incorporated into the apo-ferritin cage for polymerization of phenyl acetylene aiming that the restricted space of apo-ferritin could control the molecular weight distribution of polymer formed (Figure 2) [10]. The **Rh(nbd)·apo-ferritin** was prepared using the same method used for preparation of **Pd(allyl)·apo-ferritin**. The crystal structure shows three different binding sites, one of which is located at 3-fold channel. The other two sites are located at the accumulation center. In the 3-fold channel, His114 coordinates to one Rh atom and the other two Rh atoms bind to His49 or Cys48 in the accumulation center. The polymerization of phenylacetylene (3000 equiv) was carried out in an aqueous solution of 0.15 M NaCl in the presence of NaOH at RT.

The progress of the reaction was visualized by observing color changes from colorless to yellow, indicating the formation of polyphenylacetylene. The same reaction using only the $[Rh(nbd)Cl]_2$ complex results in the formation of water-insoluble polymers which appear as a precipitate. The number-average molecular weights (M_n) of polymers produced using **Rh(nbd)·apo-ferritin** were estimated to be 13.1(±1.5) × 10^3 [M_w/M_n = 2.6(±0.3)] whereas the number-average molecular weight of the free $[Rh(nbd)Cl]_2$ complex was estimated to be 63.7(±4) × 10^3 [M_w/M_n = 21.4(±0.4)]. This indicates the influence of the apo-ferritin cage on production of polymers with a narrow molecular weight distribution. Approximately eight polymer chains were formed inside the cage. Theoretical investigations indicate that all of the Rh atoms are not involved in catalysis. Various phenyl acetylene derivatives bearing carboxylic acid, phosphonic acid, or amino groups were tested under similar experimental conditions and only the monomer with the amino group was found to undergo polymerization. This indicates that anionic substances do not efficiently pass through the 3-fold channel.

Virus capsids

Virus capsids are empty protein cages of viruses. Like ferritin, virus capsids have been the subject of much interest for potential use as natural nanocompartments for storage and as vessels for chemical reactions [1,2,8,39]. Unlike the ferritin cage, the virus capsids provide larger interior spaces and have a variety of shapes and sizes. The main function of the virus capsids is to store genomic materials such as DNA/RNA which can be replaced with artificial cargo such as proteins, enzymes etc. The highly symmetrical protein architectures of virus capsids are formed by self-assembly of several hundred protein subunits which can be manipulated both genetically and chemically. Like the ferritin cage, they have assembly/disassembly characteristics within particular pH ranges which are suitable for triggering encapsulation and

release of cargoes. Thus, virus capsids have great potential for use as natural nanoreactors for chemical reactions [40-42]. For example, the icosahedral cowpea chlorotic mottle virus (CCMV) viruses which have a 28-nm outer diameter and an 18-nm interior diameter were used to prepare highly monodisperse Prussian blue nanoparticles with an average diameter of 18±1.7 nm [43]. Similarly, P22 capsids are functionalized with Gd(III) chelates for studying magnetic resonance (MR) relaxivities [44].

Virus capsids as catalytic nanoreactor

Virus capsids are mostly studied for biomineralization and controlled synthesis of inorganic nanomaterials due to their monodisperse nature. Recently, there is growing interest with regard to using the larger space of virus capsids to incorporate enzymes/proteins in efforts to mimic the confined cellular environment. We are highlighting the recent significant research on virus capsids as catalytic nanoreactors.

Cornelissen and coworkers developed a virus-based single-enzyme nanoreactor where the enzymatic activity of horseradish peroxidase (HRP) was studied at the single-molecule level inside the CCMV capsid [45]. The CCMV disassembles at pH 7.5 into dimer capsid proteins and reassembles at pH 5.0. HRP was added to the disassembled state and the concentration was adjusted in such a way that the capsids can either encapsulate a single enzyme molecule or fail to encapsulate a single enzyme molecule after reassembly. The enzymatic activity of the HRP-encapsulated CCMV capsid was examined using nonfluorescent dihydrorhodamine 6G as a substrate which is oxidized in presence of HRP to yield the highly fluorescent rhodamine 6G. The observation of bright fluorescence after a 10-min incubation period confirmed the activity of HRP inside the CCMV capsid. The confocal microscopic images also identified diffusion of substrate into the cavity as well as conversion and diffusion of product out of the cavity. The

control reaction using the mixture of empty capsid and free HRP showed only localized fluorescence with much less intensity. No fluorescence was observed for free HRP. This could be due to denaturation of the enzyme. Furthermore, a new method was developed to incorporate multiple enzymes. Cornelissen and coworkers used a coiled-coil protein as a non-covalent anchor to connect the target proteins/enzymes with a capsid protein dimer to form a target-capsid protein complex prior to assembly [46]. For encapsulation, the target-capsid protein complex was mixed with wild-type capsid proteins isolated from the CCMV virus at pH 5.0 for self-assembly to form the capsid. Enhanced green fluorescent protein (EGFP) was used as a target model protein and the observation was made that a maximum of 15 EGFP molecules can be incorporated per capsid. This number can be changed by varying the ratio of the EGFP-capsid protein complex with wild-type capsid protein. The effect of confinement using the enzyme *pseudozyma antartica (*formerly *Candida) lipase B* (PalB) was also investigated [47•]. It was observed that the overall reaction rate of PalB increases when it is encapsulated inside the capsid. The activity remained the same when additional enzyme molecules were incorporated into the capsid. This indicates that the activity per enzyme molecule decreases. This decrease is due to the increased local concentration (~1 mM) of enzymes inside the capsid.

Virus-like particles (VLPs) have been developed in a manner similar to development of the virus capsids. The VLPs are formed by self assembly of viral coat proteins (CPs) and have hollow structures similar to those of native viruses without any genomic materials. The homogeneous, robust, and uniform protein architectures of VLPs are as useful as virus capsids for encapsulating artificial cargoes. Douglas and coworkers have developed such VLPs derived from the bacteriophage P22 which is capable of encapsulating the enzyme alcohol dehydrogenase D, AdhD (from *Pyrococcus furiosus*) [48••]. The enzyme catalyzes the ketone to

alcohol reduction reaction using NADH. The unique VLP system can undergo structural changes upon heating and yields a larger internal volume along with changes in capsid porosity which is important for accessing the substrate by encapsulated enzymes for the enzymatic reaction. The encapsulation was accomplished by heterologous co-expression of fused AdhD-scaffold protein (AdhD-SP) with P22 coat protein (CP) in *E. coli*. Approximately ~250 enzyme molecules were incorporated into the protein cage. This is the highest number of enzymes loaded into VLPs to date. The enzyme-encapsulated P22VLP followed Michaelis-Menten kinetics for the enzymatic reaction. The encapsulated AdhD did not exhibit the substrate inhibition which is typically observed in the free AdhD enzyme. The overall catalytic efficiency at 50 ^{o}C was nearly the same as that of the free AdhD enzyme. This indicates that the enzymatic activity is retained after encapsulation.

A new hybrid nanoreactor was developed by coupling AdhD with an organometallic $[Cp^{*}Rh(phen)Cl]^{+}$ catalyst within the confined space of P22VLP for promotion of cascade reactions (Figure 3) [49•]. The Rh catalyst is known to convert NAD^{+} to NADH in the presence of formate. The NADH formed in this reaction can be utilized by AdhD to reduce a ketone (3-hydroxybutan-2-one) to alcohol (2, 3-butanediol). To prepare the hybrid catalyst, the phenanthroline ligand of $[Cp^{*}Rh(phen)Cl]^{+}$ was functionalized with iodoacetamide for direct conjugation with a cysteine residue. The scaffold proteins and coat proteins were engineered to introduce cysteine residues (S141C and K118C). The AdhD-P22VLP was prepared by co-expression with SC and CP followed by labeling with functionalized $[Cp^{*}Rh(phen)Cl]^{+}$ to prepare the hybrid catalyst AdhD-P22VLP-Rh. The catalytic reduction of NAD^{+} by Rh in the hybrid catalyst, AdhD-P22VLP-Rh was found to be lower than that of free $[Cp^{*}Rh(phen)Cl]^{+}$. A possible explanation can be found in the partial decomposition of the metal complex in the

protein environment based on ICP-MS data. The activity of AdhD in the absence of ketone was found to be similar to that of previous reports. This excludes the possibility of any interference due to Rh attachment [48]. The overall cascade reactions using the hybrid AdhD-P22VLP-Rh were performed in the presence of substrate ketone (for AdhD), NAD^+ (for Rh) and formate. The reaction was monitored by measuring NADH absorption at 340 nm and the final product was detected by NMR spectroscopy. The results indicate that the production and consumption of NADH is dependent upon the formate and substrate concentration which can be adjusted to tune the overall reaction.

Another programmed encapsulation was performed using a tetrameric enzyme, *β*-glucosidase (CelB) inside the P22 capsid which can function as a catalytic nanoreactor for the hydrolysis of beta-linked disaccharides [50]. The encapsulation was performed according to a method similar to that used for AdhD (heterologous protein expression). Approximately 80 monomers were encapsulated per capsid. The *β*-glucosidase activity was examined using 4-nitrophenyl *β*-D-glucopyranoside (PNPG) which, upon cleavage of the glycosidic bond, releases 4-nitrophenol which has a strong absorbance near 400 nm. The overall kinetics of the CelB enzyme remained constant after encapsulation.

To mimic the cellular compartment, multiple enzymes were incorporated into P22VLP to perform coupled cascade reactions (Figure 4) [51••]. Three different enzymes were used; tetrameric *β*-glucosidase (CelB), monomeric ATP-dependent galactokinase (GALK) and dimeric ADP dependent glucokinase (GLUK). CelB hydrolyses lactose to galactose and glucose. GALK utilizes ATP to convert galactose to galactose-1-phosphate and produces ADP which is used by GLUK to convert glucose to glucose-6-phosphate. Thus the sequential reactions can mimic the natural sugar metabolism reactions. First, two enzymes (GLUK and CelB) were incorporated and

their reaction kinetics were characterized. A total of 61 copies were encapsulated per capsid. The arrangement of enzymes within the fusion protein (CelB-GLUK-P22 vs GLUK-CelB-P22) can have important effects in VLP assembly as well as enzymatic activity. Co-encapsulated enzymes were found to have similar activities as in the 1:1 mixture of the individually encapsulated enzymes. This removes the advantage of close proximity of enzymes inside the cavity. When the rate of the first reaction was reduced by changing the buffer, the advantage of close proximity was observed. This indicates the involvement of kinetic balance between two enzymes. Kinetic balance was confirmed by the mathematical model and additional studies. Therefore, the overall enzymatic activity for the individual enzymes co-encapsulated inside the cage is maintained with proper arrangement. The kinetic studies indicate that the intermediate channeling between sequential enzymes is dependent on both the inter-enzyme distance and the balance between the kinetic parameters of the two enzymes. To prepare the three-enzyme encapsulated system, the P22-scaffold protein was first fused with all of the enzymes which were linked together through flexible polyglycine peptides. The spacer peptide was found to contribute to proper folding and formation of the native oligomeric structure. Then, the fused scaffold protein was co-expressed with coat protein for encapsulation (GALK-GLUK-CelB-P22VLP). A total of 15 copies of the tri-functional fusion protein were encapsulated inside the cage. The sequential catalytic activity was measured using only lactose and ATP as substrates in the citrate buffer medium. For the three-enzyme construct (GALK-GLUK-CelB-P22VLP), the reaction rate was 2-fold higher than that of the two-enzyme construct (GLUK-CelB-P22VLP), indicating successful incorporation of the three enzymes which are active in the cascade reactions.

Catalytic antimicrobial nanoparticles were developed by incorporating NADH oxidase (NOX) into the P22-VLP. Homodimeric NOX is known to generate hydrogen peroxide from the

reduction of oxygen using NADH [52●]. The enzyme was encapsulated by heterologous coexpression of NOX-SP fusion protein with CP followed by purification. The enzymatic activity was studied by monitoring the absorbance at 340 nm for oxidation of NADH to NAD^+. The enzymatic activity of P22-NOX was found to increase with increasing temperature. The addition of flavin co-factor (FAD) to the reaction mixture was found to produce a six-fold enhancement of the activity of free NOX relative to P22-NOX. This indicates that access of FAD to the encapsulated NOX is inhibited. However, the overall enzymatic activity of P22-NOX constructs were found to be similar to or slightly less than that of free NOX. This indicates that there is retention of activity upon encapsulation without any significant loss. This method was applied to study bacterial growth inhibition since H_2O_2 induces oxidative damage of *E. coli* bacterial cultures. The increase in optical density at 600 nm indicates inhibition of bacterial growth by P22-NOX as well as free NOX. P22-NOX was found to retain its activity when immobilized onto silica bead particles, indicating the robust nature the P22capsid. This is a favorable characteristic for development of new materials.

Protein cages vs. protein needles/tubes

The foregoing discussion has indicated that the nanospaces provided by protein cages such as apo-ferritin and viruses provide important platforms for several applications, most notably catalytic reactions. Other protein assemblies such as protein needles/tubes are also promising candidates for development as artificial metalloenzymes [53]. The nanocup of the protein needle known as $(gp27\text{-}gp5)_3$ has been modified with Fe(III) protoporphyrin and the resulting modified species has been demonstrated to catalyze H_2O_2-dependent sulfoxidation of thioanisole with activity 6–10 times higher than that of free Fe(III) protoporphyrin complex [54]. Similarly, flavin molecules aligned on the triangular plane of $[(gp5\beta f)_3]_2$ can accelerate an azide-

alkyne [3+2] cycloaddition reaction in the presence of Cu(I) ion through the formation of a mononuclear Cu(I) coordination structure [55]. Various metal complexes such as $Ru(bpy)_3$ and $Re(bpy)(CO)_3Cl$ aligned on the surface of $[(gp5\beta f)_3]_2$ were found to induce photocatalytic reduction of CO_2 to CO through electron transfer from the Ru center to the Re center in the presence of 1-benzyl-1,4-dihydronicotinamide (BNAH) and visible light irradiation [56]. Similarly, an artificial Sc(III) enzyme was developed on the surface of $[(gp5\beta f)_3]_2$. This artificial enzyme promotes an epoxide ring-opening reaction [57]. When considering catalytic reactions, protein cages have several advantages over the protein needles/tubes. Firstly, the restricted interior size of the protein cages can directly influence reaction kinetics and product selectivity. Protein needles/tubes lack this advantage. Secondly, ferritin has a well-defined metal accumulation center which is known to conveniently encapsulate various synthetic metal complexes for a variety of catalytic reactions achieved by simple mixing and stirring. Needles/tubes lack such a metal accumulation center but require additional steps to immobilize a synthetic metal catalyst by functionalization with organic ligands. A third advantage is that in protein cages such as ferritin, the metal encapsulated cage can be crystallized easily for determination of the coordination structure of metal catalysts immobilized on the inner surface. Protein needles/tubes have not yet demonstrated this advantage. A fourth advantage is that the protein cages have specific reassembly/disassembly mechanisms in different pH ranges which provide a means to encapsulate larger molecules inside the cage. The protein needles/tubes do not have such property. Finally, a fifth advantage is that, due to their homogenous uniform and symmetrical structures, protein cages are suitable for preparing monodisperse nanoparticles. The protein needles/tubes do not have such advantages.

Although the protein cages have several advantages, it should be mentioned that the pores connecting the interior cavity to the bulk solution may lead to disadvantages. For example, the small pores in the ferritin cage do not allow larger substrate molecules to penetrate into the interior cage for catalytic reactions.

Summary and future perspectives

It has been demonstrated that the self-assembled protein cages have great potential for use as catalytic reaction vessels. The confined interior cages connected by small channels with uniformity of structure, symmetry and flexibility are the main advantages of utilizing such self-assembled protein cages for catalytic reactions. Initially, the applications of such cages were restricted to investigations of biomineralization. However, recent reports have provided proof of concept for other applications, particularly in catalysis. The ferritin cage and virus capsids are the most suitable protein cages for catalytic applications. Metal catalysts immobilized in a ferritin cage and enzymes in viral capsids have been proven to be active. It has also been proven that the restricted protein cage influences the overall reaction kinetics.

Thus, we believe that catalytic reactions may be effectively investigated using such natural and confined protein containers. Design of catalysts encapsulated within protein compartments could give rise to important synthetic applications and insights into the fundamental questions regarding the complex processes occurring in living cells. However, literature reports on catalysis with detailed kinetic studies are rare. Incorporating multiple catalytic centers into the protein cage could be the best strategy to mimic confined cellular environments and such investigations have been initiated for virus capsids in efforts to provide larger internal spaces [49,51]. The limited space of the ferritin cage is suitable for accommodating small molecular catalysts. However, it is possible to incorporate multiple

synthetic metal catalysts into the ferritin cage for serial reactions. Such efforts are in progress in our laboratory.

Acknowledgement

Parts of this work were supported by the Funding Program for Next Generation World-Leading Researchers (for T.U.), and research fellowship for young scientists for B.M from the Japan Society for the Promotion of Science.

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- • of special interest
- •• of outstanding interest

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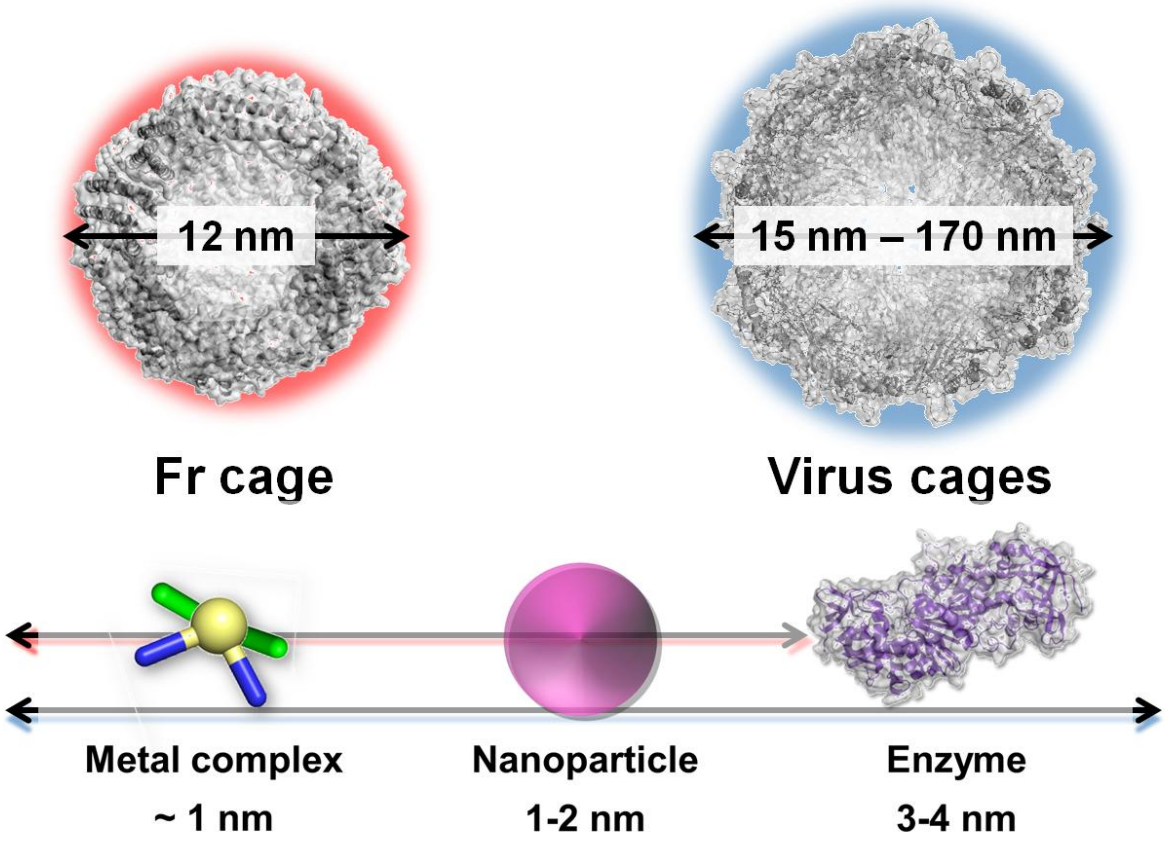

Fig.1 Self-assembled protein cages and the size of encapsulating cargoes.

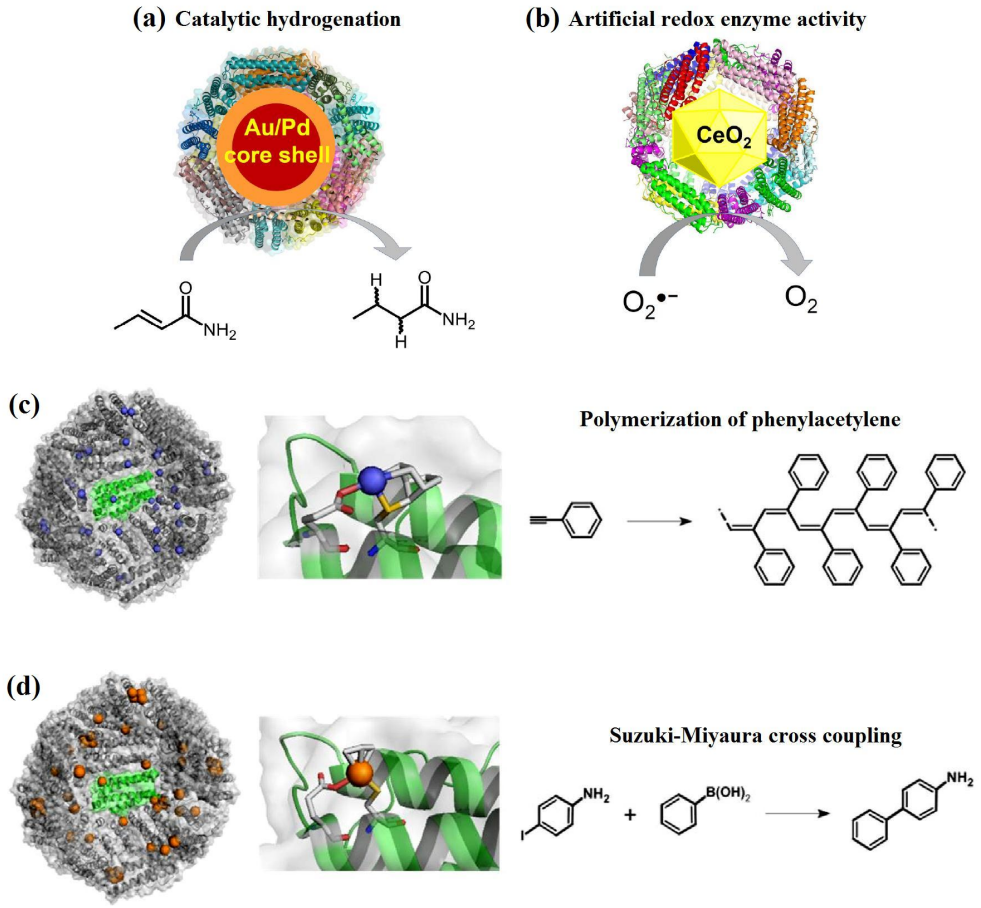

Fig.2 Catalytic reactions by nanomaterials and organometallic complexes in apo-Ferritin. (a) Au/Pd core shell nanoparticles and (b) Nanoceria (CeO_2) particles. (c) Accumulation of Rh(nbd) complex (Rh atom: blue sphere, coordinated amino acid resides: stick indication). (d) Accumulation of Pd(allyl) complex (Pd atom: orange sphere, coordinated amino acid resides: stick indication).

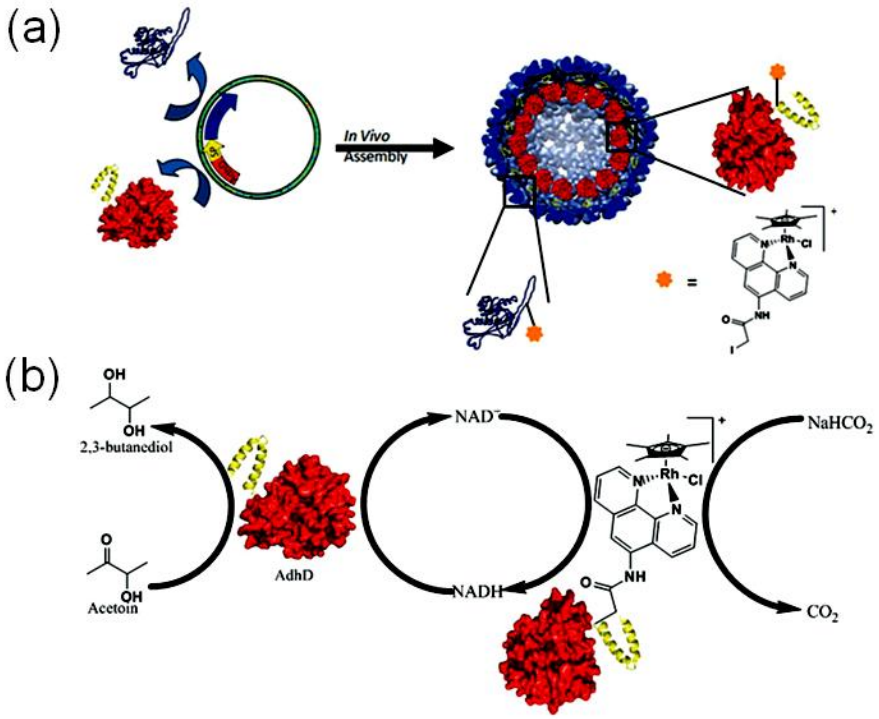

Fig.3 Construction of hybrid catalyst and the catalytic reactions. (a) The schematic representation of *in vivo* recombinant expression and encapsulation of the enzyme AdhD fused with the scaffold protein (SP). The truncated SP is shown in yellow and the assembly of P22 is initiated at C-terminus. The Rh catalyst is attached with both CP and SP in the engineered cysteine residue. (b) Scheme for the AdhD and rhodium catalyzed reactions (adapted with permission from John Wiley and Sons [49•]).

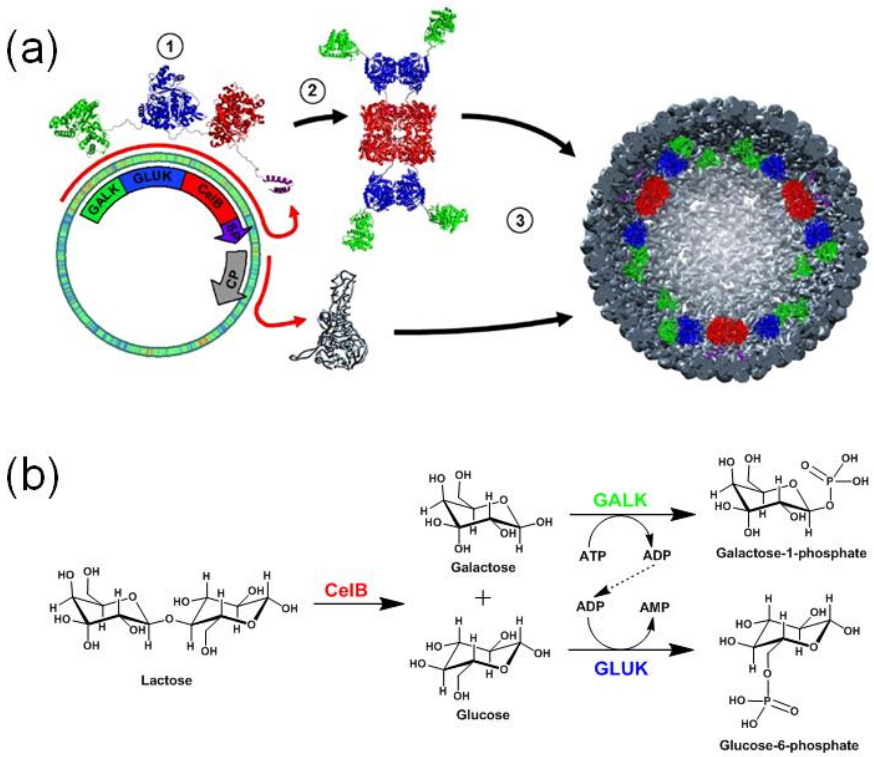

Fig.4 (a) The scheme showing the assembly of P22 in three steps. (1) gene expression of the coat protein (CP) and fusion protein (SP) containing multienzymes linked through flexible spacer peptide, (2) association of the tetrameric CelB and dimeric GLUK, and (3) Co-expression of SP domains and CP subunits for the encapsulation of multienzyme gene product. The color codes are as follows: CP (gray), SP (purple), CelB (red), GLUK (blue), GALK (green). (adapted with permission from ACS [51••]). (b) Coupled enzymatic reactions of CelB, GALK, and GLUK to form the synthetic metabolon.