# 論文 / 著書情報
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# 論 文 要 旨

THESIS SUMMARY

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| 系・コース：<br>Department of, Graduate major in | 生命理工学 | 系<br>コース | 申請学位（専攻分野）：<br>Academic Degree Requested | 博士<br>Doctor of | （ 理学 ） |
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）


In nature, some proteins self-assemble into shell-like structures which are highly stable systems for encapsulation and surface modifications to optimize functions. Protein cages can be artificially designed to encapsulate enzymes and have multiple applications. However, the current functionalization of protein cages often requires external enzymes, co-factors, or metals, which may result in lower-than-expected activity and can introduce undesirable side effects, toxicity, reduced stability, and low biocompatibility. Residue clusters are groups of amino acids with high concentrations of specific properties (e.g., charge, hydrophobicity) that often have functional or structural importance in proteins. Due to their diverse roles in proteins, residue clusters are engineered to create functional sites in artificial proteins. By designing and engineering such residue clusters, it is possible to directly introduce or enhance functional sites within proteins, enabling precise modulation of their activity and specificity.

In this doctoral thesis, I utilize ferritin, a well-studied protein cage, to do structural and functional design with construction of residue clusters. On the two-fold interface, previously research designed different residue clusters with various positions and immobilize metal ions. This approach allows for creating desired structures and functionalities through residue clusters without using any exogenous components. The cage structure can facilitate the reaction in a confinement environment. My research focuses on structural design, analysis, and activity characterization, to establish and understand the relationship between structure and function. By residue substitution, I seek to overcome the challenges of using external molecules, ultimately creating a more efficient and predictable protein cage system for various applications.

In Chapter 2, I focused on designing a ferritin cage with peroxidase-like activity through histidine substitution. I used the two-fold symmetric interface of ferritin as a scaffold to design various histidine clusters. I precisely designed the histidine clusters with different numbers of histidine at different positions. By employing X-ray structural analysis, I explored the structure of Histidine clusters. Through characterization of peroxidase activity, and molecular dynamics simulations, I elucidated the influence of these histidine clusters on peroxidase activity. The molecular dynamics simulations revealed that the ferritin cage enhances catalytic activity by increasing the frequency of substrate binding to the active site. This finding consisted of the fact that protein cages are effective systems for designing and optimizing enzymatic activity. The insights gained from this study not only provide a deep understanding of the role of histidine clusters in producing peroxidase-like activity but also demonstrate the potential of using protein cages as versatile platforms for enzyme design.

Protein crystals are solid assemblies consisting of protein molecules in a regular arrangement. Through the engineering of amino acid residues, exogenous compounds such as dye molecules and metal complexes can be immobilized to develop hybrid solid materials. Besides small molecules, the immobilization of protein into in-cell crystals is a remarkable method of synthesizing solid-state materials. In Chapter 3, based on the results from Chapter 2, I utilized the mutants developed in the previous chapter and fused ferritin with Crystalline Inclusion Protein A (CipA). This fusion protein enabled ferritin to form protein crystals within cells spontaneously. The resulting ferritin-fused crystals exhibited peroxidase-like activity, with enhanced catalytic activity observed in the mutation. This study presents a straightforward approach to developing functional solid materials, showcasing the potential of using protein crystals for catalytic applications and further expanding the versatility of protein cages in material science.

In Chapter 4, I investigated whether isolated ferritin crystals exhibit any unique structural features. Through X-ray structural analysis, I discovered a unique water network structure made up of five fused pentagonal water rings, which was called semi-clathrate. This semi-clathrate structure is typically observed in antifreeze proteins, where it supports the inhibition of ice crystal growth. I further explored the semi-clathrate structure in ferritin under varying temperatures and designed several mutants to study its formation mechanism. I found that both temperature and alanine residues play important roles in the formation of the semi-clathrate structure. The formation of semi-clathrate is influenced by temperature, and this process is reversible.

In Chapter 5, since ferritin remained stable even after substituting multiple amino acids with histidine at the two-fold symmetric interface, I continued to use the two-fold symmetric interface as a scaffold construct semi-clathrate. Based on the findings in the previous chapter and other studies, poly Alanine can promote the formation of semi-clathrate. Therefore, several mutants were designed with varying numbers of alanine substitutions. As the number of alanine residues increased, the semi-clathrate structure gradually formed. This chapter primarily uses X-ray structural analysis to elucidate the formation process of the semi-clathrate structure and explore the formation mechanism. These findings suggest that ferritin can be further engineered to produce more semi-clathrate structures, potentially serving as antifreeze materials.