# *Laboratory of Biofunctional Chemistry*

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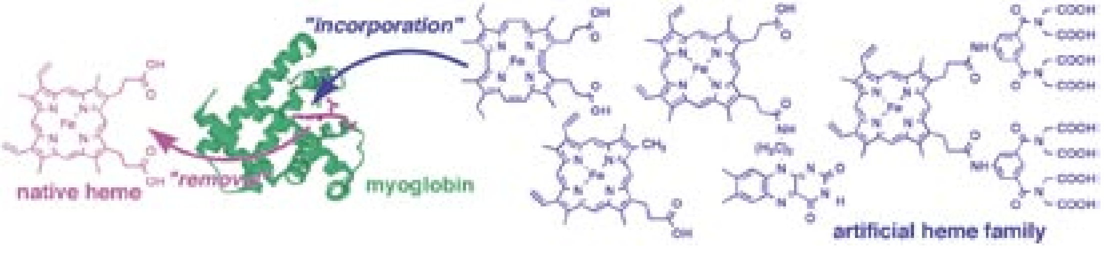
## *Applied Bioinorganic Chemistry*

Metalloproteins are attractive biomaterials, because metal ions generally possess a variety of physicochemical properties and catalytic behaviors. Furthermore, a protein matrix provides an effective metal coordination space that regulates metal ion reactivity. Over the last four decades, many kinds of metalloprotein structures have been determined, and their characteristics give us important and detailed insights into protein functions. Therefore, one of our next research targets is engineering a metalloprotein to create tailor-made biomaterials. To achieve this, we have focused on hemoproteins, typical metalloproteins with a heme (an iron porphyrin complex) prosthetic group. The functions of hemoproteins are varied: electron transfer, $O_2$ storage/transport, $O_2$ and CO sensing, NO transport, oxidation catalysis *etc*., although these proteins have the common prosthetic group, heme. This strongly suggests that the modification of the heme active site will serve as a new strategy to produce a unique functionalized metalloprotein. Thus, our group's efforts have been devoted to the preparation of designed metalloporphyrins and to the construction of unique proteins reconstituted with an artificially created heme. Our goal is to create a new interdisciplinary field, "Applied Bioinorganic Chemistry", based on organic chemistry, coordination chemistry and biochemistry, and then to construct various biomaterials such as biocatalysts, sensors or medical materials.

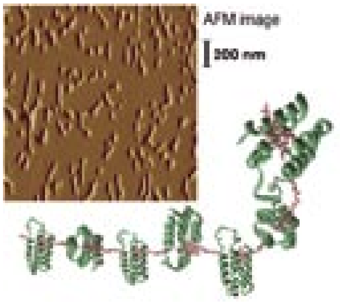
## *Hemoprotein Engineering*

The heme *b* is bound in the heme pocket via non-covalent interactions, making the heme in the protein matrix exchangeable. To enhance and/or modify hemoprotein functions, we have prepared various functionalized artificial heme cofactors and replaced the native heme with them as shown in the following scheme. The obtained reconstituted myoglobins showed unique characteristics: extremely high $O_2$ affinity, remarkable peroxidase activity, reductive $O_2$ activation, and interprotein electron transfer properties. Recently, our research targets have been extended to other hemoproteins such as horseradish peroxidase, cytochrome P450, heme oxygenase, and cytochrome $b_{562}$. Hemoprotein reconstitution with a porphyrin analog is also a powerful tool toward understanding the molecular mechanism of the heme proteins.



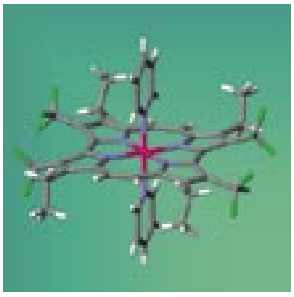
## *Supramolecular Hemoprotein Assembly*

Modifying the architecture of a supramolecular polymer is a promising tactic to mimic a biological system and construct nanomaterials. We found that heme–heme pocket interaction with an affinity of $10^{10}$ –$10^{15}$ $M^{-1}$ serves as a new way to prepare an attractive supramolecular hemoprotein polymer as follows. At the first stage, we introduced an iron porphyrin derivative onto the thiol of the cytochrome $b_{562}$ mutant (H63C). The removal of the native heme from the protein induces the attached external heme to bind into the heme pocket intermolecularly. The subsequent heme–heme pocket interaction produces a unique submicrosized "hemoprotein fiber" containing more than 200 proteins. The present strategy can easily be extended to prepare many kinds of hemoprotein assemblies with various geometries.



## *New Porphyrinoid Chemistry*

Tetrapyrrolic metalloporphyrin derivatives have been widely studied as biomimetic models for hemoproteins. They are also well known as attractive catalysts in organic and coordination chemistry. We have focused on unique porphyrin isomers, particularly those such as porphycene with reduced symmetry, because the LUMO of the 18π-macrocyclic porphycene is dramatically stabilized compared to the corresponding porphyrin. We prepared metalloporphycene having strongly electron-withdrawing trifluoromethyl groups at the pyrrole β-positions to obtain extremely electron-deficient tetrapyrrole macrocycles. The unusual characteristics of porphycene and metalloporphycene species provide a stable low-valent metal complex, a metal-hydride (M–H) complex, an interelement complex containing an M–M bond and a 20π-nonaromatic porphycene.



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