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Abstract Book

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Crystalline Sponge Method: From Origins to the Latest Advances

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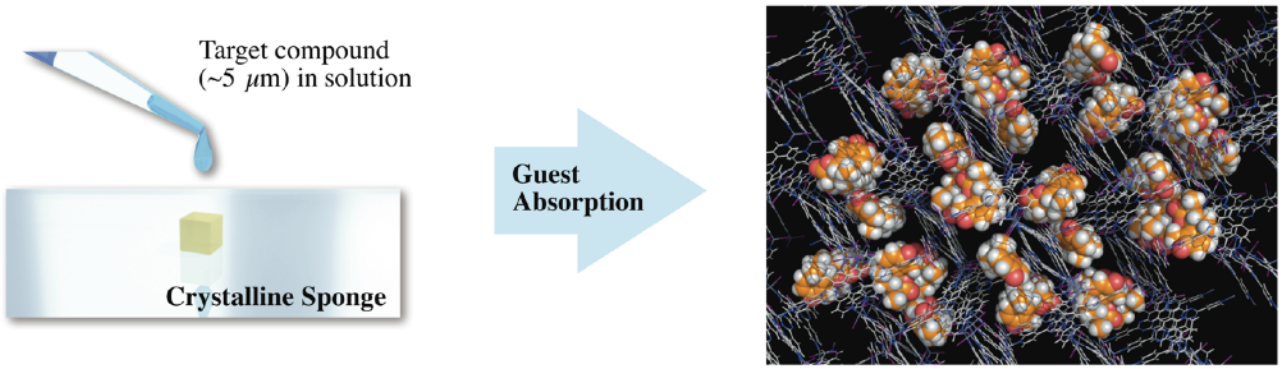
X-ray single crystal diffraction (SCD) analysis has the intrinsic limitation that the target molecules must be obtained as single crystals. Here, we report a new protocol for SCD analysis that does not require the crystallization of the sample.[1-5] In our method, tiny crystals of porous complexes are soaked in the solution of a target, where the complexes can absorb and orient the target molecules in the pores. The crystallographic analysis clearly determines the absorbed guest structures along with the host frameworks. As the SCD analysis is carried out with only one tiny crystal, the required sample amount is of the nano-to-microgram order. With chiral guests, the space group of the crystal turned into chiral, enabling the determination of absolute configuration of the guests by anomalous scattering effect from the host heavy atoms (Zn and I). In this talk, following a general discusson,[6-11] the applications of the method for natural product chemistry, synthetic chemistry, and pharmaceutical research will be discussed. The absolute configurations of elatenyne, first isolated in 1986, has still not been unequivocally confirmed because of its almost achiral meso-formed core structure that results in nearly zero $[\alpha]_D$ specific rotation. This faint chirality, defined only by the slight difference in the two alkyl side-chains, was precisely discriminated by the crystalline sponge and its absolute structure was reliably determined. [12] The total amount required for the experiments was only ~100 μg and the majority of this (95 μg) could be recovered after the experiments.



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Absolute Structure, Absolute Configuration and Racemic Twinning: What the Flack Parameter Can and Cannot Tell Us

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X-ray diffraction is the simplest and most elegant method to determine the absolute configuration of chiral compounds. To this end, an absolute structure parameter, usually the Flack-*x*[1], is calculated. The Flack-*x* possesses values of 0 (correct absolute structure), 1 (inverted structure), or between 0 and 1 (inversion twinning, *i.e.* presence of the opposite configuration). This presentation will explain why a Flack-*x* of zero is not always sufficient to determine the absolute configuration of a molecule and also why sometimes a value of, say, 0.1 does not necessarily indicate the presence of the other enantiomer.

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Precise absolute structure determination for light-atom crystal structures

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A non-centrosymmetric crystal structure cannot be superimposed on its inverted image, and determination of absolute structure amounts to assigning a particular non-centrosymmetric crystal structure to one of two possible structures which are related by inversion. The determination of the absolute structure of an enantiopure molecular crystal can be used to establish the absolute configuration of the molecules that comprise it.[1,2] In Flack's method of absolute structure determination the sample is considered to be a twin composed of a reference domain, which has the absolute structure of the current refinement model, and a second domain in which the absolute structure is inverted. The model thus contains both possible absolute structures, and the absolute structure of the sample is found by determining the relative proportion of the inverted domain present. This proportion is called the Flack parameter.[3] Flack and Bernardinelli[4] have shown that even if a compound is known to be enantiopure, the value of the standard uncertainty of the Flack parameter should be less than 0.1 before any conclusions regarding absolute structure can be made. This precision is rarely obtained in conventional crystallographic least squares refinement for crystal structures containing no atom heavier than oxygen. In this talk, the use of Bijvoet differences and quotients which enable better - and more realistic - precision to be obtained will be described.[5,6,7]

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Metal-Macrocycle Framework (MMF): A Porous Crystal with Multiple Binding Pockets and Active Palladium Sites

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In the chemistry of porous crystals including zeolites and metal-organic frameworks, molecular recognition is a key basis to enhance their porous functions such as separation, transportation, and catalysis. We have previously developed a molecule-based porous crystal, metal-macrocycle framework (MMF) (Figure 1), through co-crystallization of four stereoisomers of helical Pd^{II}_3-macrocycles.[1] As its one-dimensional pore with a 1.4 × 1.9 nm^2 dimension is equipped with five enantiomerically paired binding pockets, we have utilized the multiple binding pockets on the pore surface to site-selectively arrange various molecules including catalysts and bio-related compounds.[2,3] Furthermore, the multiple binding pockets also enabled to simultaneously arrange not only single but also multiple guest molecules within the pore.[4] Recently, we also found that Pd^{II} sites exposed on the pore surface worked as a catalytic center for olefin migration reactions under photo-irradiation. In this presentation, we will discuss the design of a preferential photo-reaction in MMF with the aid of both molecular binding pockets and catalytic Pd^{II} sites on the pore surface.[5]

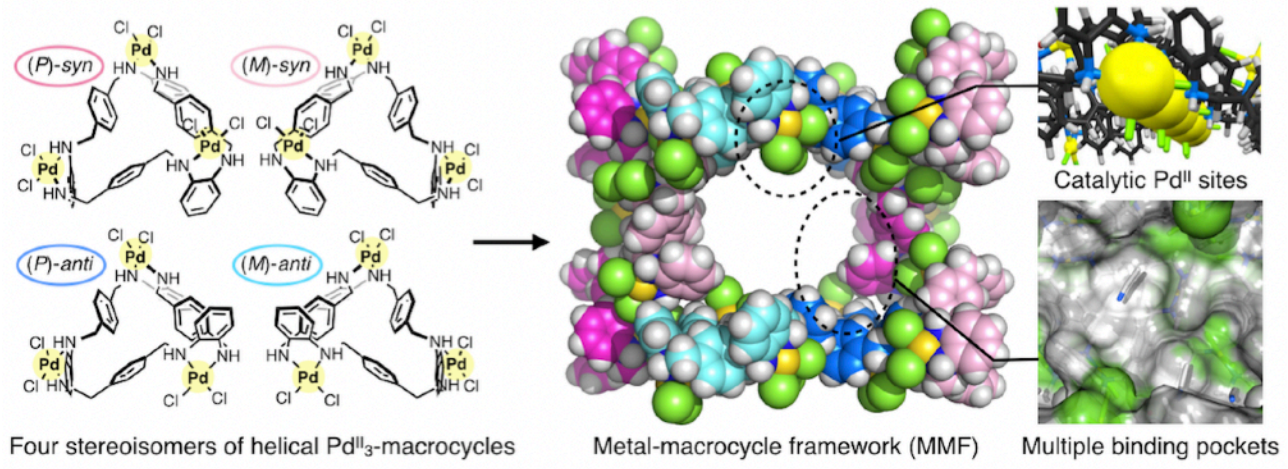

Figure 1 Formation and structure of metal-macrocycle framework (MMF)

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A New Work-flow for the Structure Analysis of Natural Products

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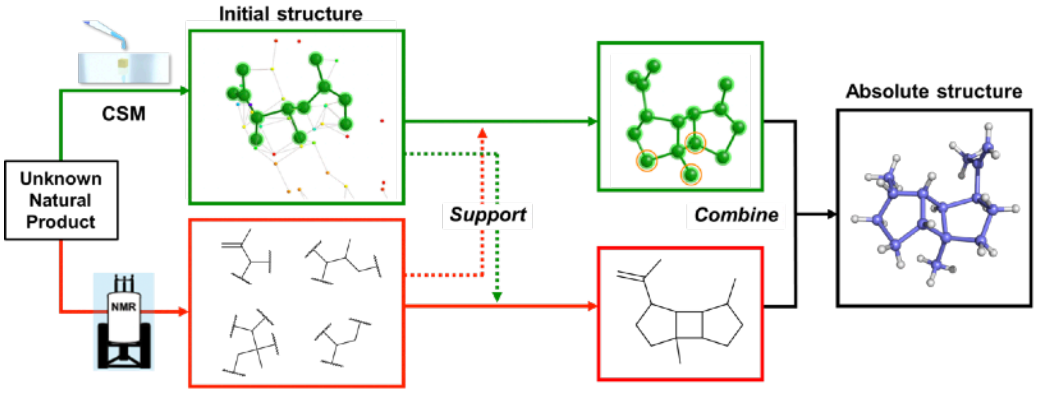
Structure determination of natural products is a challenging work because of their rarity that prevents researchers from utilizing to analytical experiments especially to the X-ray diffraction (XRD) analysis.

In this presentation, we introduce our results on the absolute configuration determination of oily natural products using the crystalline sponge method (CSM). We elucidated the complete structure of an unknown natural product using the novel work-flow for structure analysis, NMR-coupled CSM.[1] Combining initial information of XRD study with that of NMR study gave much faster and more reliable method than conventional tandem studies. We also show other results of a structure determination of major components in crude extracts from natural resource by NMR-coupled CSM.[2]

NMR-coupled CSM is expected to accelerate a structural determination of natural products and we believe that obtained novel scaffolds must be useful for the natural product-derived drug discovery research.



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Crystalline Sponge Method Efficiently Reveals Stereo-Configurations of Beer's Bitter Acids and Their Oxides

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The bitter acids and their oxides derived from hops (*Humulus lupulus* L.) affect quality of beer. Information on molecular characteristics of these compounds is particularly important to develop science-based brewing technology. However, their structural analyses, especially stereo-configuration analyses, are very arduous because they have various scaffolds and many continuous quaternary carbon atoms. Here, we showed that the crystalline sponge (CS) method could easily and efficiently reveal the relative and absolute configurations of bitter acids and their oxides. The absolute configurations of (−)-humulone and (−)-*trans*- and (+)-*cis*-isohumulones, the representative bitter acids, were confirmed without the need to prepare single crystals of their derivatives containing heavy atoms or reference chiral centers (Figure 1). The absolute configurations of (+)-tricyclooxyisohumulones, a series of bitter acid oxides, were also determined and 6 novel compounds were identified. Furthermore, the relative configurations of *rac*-scorpiohumulinols and *rac*-dicyclohumulionols, another series of bitter acid oxides, were confirmed by the CS method. As a result, the CS method is highly practical to analyze stereo-configurations of various bitter compounds in beer.

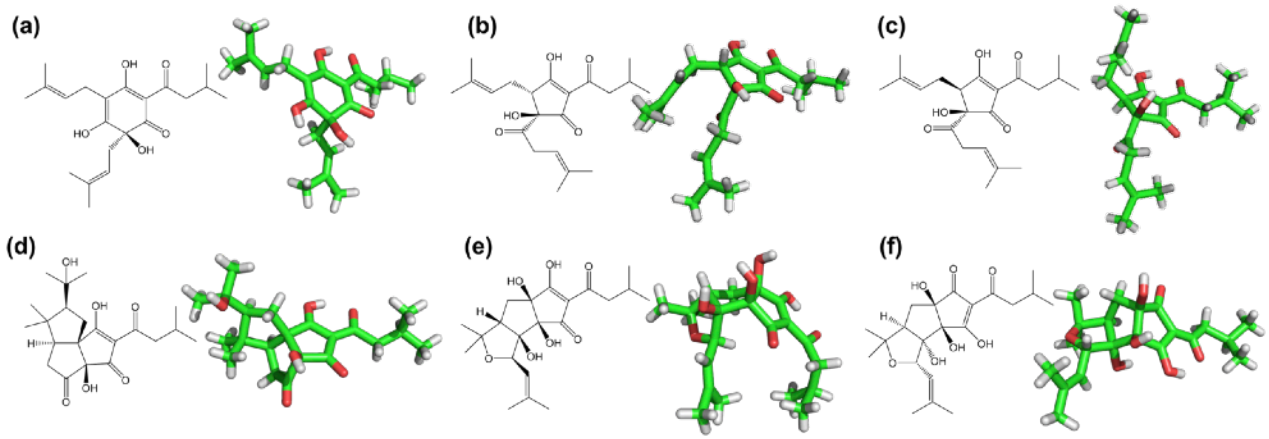

Figure 1 The stereo-configurations determined by the CS method; (a) (−)-humulone; (b) (−)-*trans*-isohumulone; (c) (+)-*cis*-isohumulone; (d) (+)-tricyclooxyisohumulone A; (e) *rac*-scorpiohumulinol A; and (f) *rac*-scorpiohumulinol B

Advancing terpene biochemistry by the crystalline sponge method

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Sesquiterpene scaffolds are the core backbones of many medicinally and industrially important natural products. A plethora of sesquiterpene synthases, widely present in bacteria, fungi, and plants, catalyze the formation of these intricate structures often with multiple stereocenters starting from linear farnesyl diphosphate (FPP) substrates. Recent advances in next-generation sequencing and metabolomics technologies have greatly facilitated gene discovery for sesquiterpene synthases. However, a major bottleneck limits biochemical characterization of recombinant sesquiterpene synthases: the absolute structural elucidation of the derived sesquiterpene prod-ucts. Here, we report the identification and biochemical characterization of LphTPS-A, an sesquiterpene synthase from the red macroalga Laurencia pacifica. Using the combination of transcriptomics, sesquiterpene synthase expression in yeast, and microgram-scale NMR-coupled crystalline sponge X-ray diffraction (XRD) analysis, we resolved the absolute stereo-chemistry of prespatane, the major sesquiterpene product of LphTPS-A, and thereby functionally define LphTPS-A as the first bourbonane-producing sesquiterpene synthase and the first biochemically characterized sesquiterpene synthase from red algae. Our study showcases a workflow integrating multi-omics approaches, synthetic biology, and the crystalline sponge method, which is generally applicable for uncovering new terpene chemistry and biochemistry from source-limited living organisms.

Bromination Improves Dramatically Structural Analyses of Aroma Compounds

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The crystalline sponge (CS) method is a method of single crystal X-ray diffraction (SC-XRD) analysis without crystallization of target compound. The guest molecules are ordered periodically in the CS by intermolecular interactions and the structure is determined by SC-XRD analysis. However, the structure determination of some aroma compounds by CS method was difficult due to formation of weak interactions between the guest compound and the CS. Here we report structural analyses of aroma compounds by utilizing halogen interactions [Figure 1]. The structure analyses of several compounds by the CS method resulted in no observation of their structures. After bromination of them, the structural analyses of the obtained bromides were examined by the CS method. The structures were clearly observed by formation of halogen interactions between the bromine atoms and the CS framework, enabling determination of their original structures.

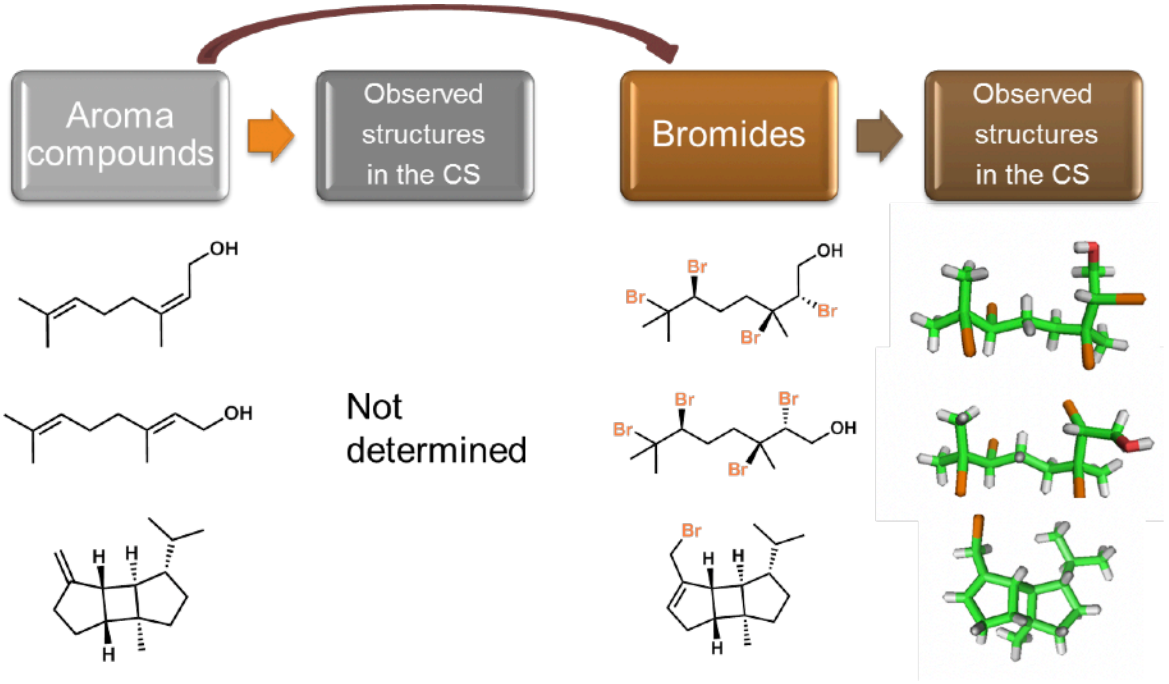

Figure 1 **Observed structures in the CS of aroma compounds and their bromides**

Development of a crystalline sponge tag method for structural analysis of amino acids

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Amino acids are added to a wide range of foods, supplements, pharmaceuticals, cosmetics and many other products. To provide safe, high-quality amino acid products, a rapid and highly sensitive structural determination technique is required for detecting trace amounts of byproducts, often other amino acids or amino acid metabolites from fermentation processes.

The crystalline sponge method reported by Fujita *et al.* is a groundbreaking technique for performing X-ray crystal structure analysis, on even trace quantities, without crystallization[1]. However, it is difficult to analyze amino acids and related compounds with the crystalline sponge method because of their wide diversities in such properties as size, charge and hydrophobicity. Therefore, we developed a crystalline sponge tag method (CS-Tag Method) applicable to amino acids and related compounds, based on amino group derivatization with a specific tag. The potency of the CS-Tag Method was evaluated with eleven proteinogenic amino acids. Tagged amino acids were successfully incorporated into the crystalline sponge. Specific binding of the tag moiety to the crystalline sponge was then observed.

These results indicated that using a CS-Tag for an amino group would enable application of crystalline sponge technology to amino acids and related compounds. In this symposium, the authors will describe the tag and its optimization.

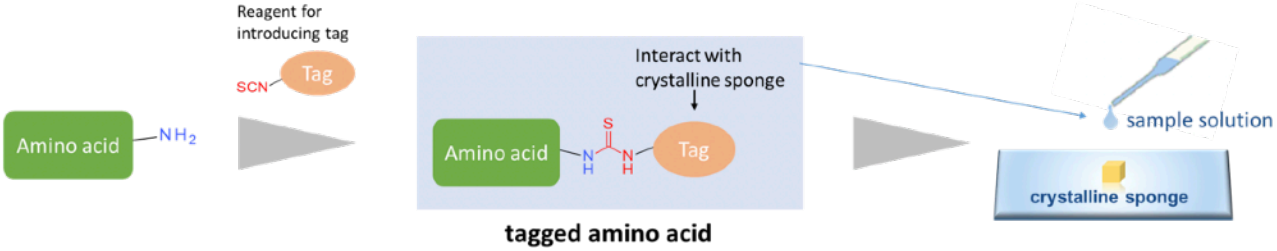

Figure 1. Crystalline Sponge Tag method

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Determination of substitution position of heteroatom-containing compounds by the crystalline sponge method

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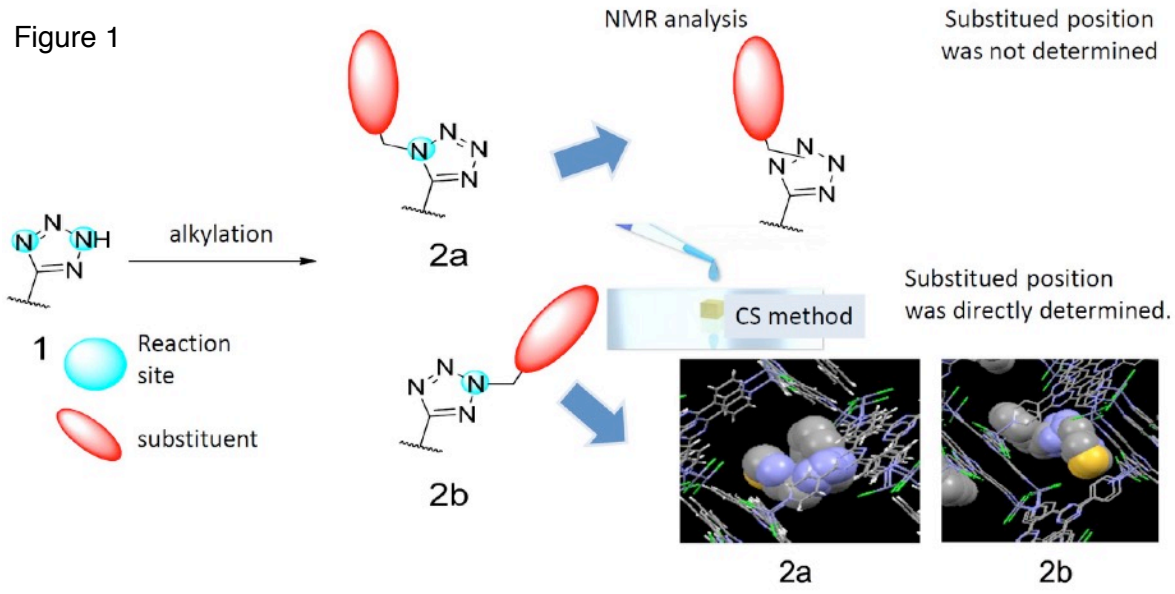
Heteroatom-containing compounds are widely found in pharmaceuticals and pesticides because the heteroatom greatly contributes to their activity and physical properties. Furthermore, introduction of substituents to the heteroatom enables development of new pharmacophores. Therefore, accurate determination of the position of substituents in their introduction to the heteroatoms is quitely important for design and synthesis of new drug candidate. However, determination of the position of substituents introduced to the heteroatoms by conventional analytical methods (such as NMR) is often difficult.

Herein, we applied the crystalline sponge (CS) method[1] to determination of the position of the substituents in their introduction to tetrazoles which have two reaction sites. N-substituted tetrazoles 2a, 2b were synthesized by the alkylation of tetrazole **1**. But determination of the substitution position of both compounds by 2D-NMR analysis was difficult. On the other hand, the CS method enabled to determine the position of the substituent directly (Figure 1).

Figure 1



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Introduction to "Structure Analysis by Crystalline Sponge Method" Service of Nanotechnology Platform Program in IMS

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The crystallization-free crystallographic analysis called "Crystalline sponge method (CSM)" was originated from the Fujita group at the University of Tokyo in 2013[1]. CSM has been developed in academic and industrial fields over the past five years. In order to make more people accessible to CSM easily, it is now being transferred to Institute for Molecular Science (IMS), the leading institute of Molecular & Material Synthesis Platform (M&MS), MEXT Nanotechnology Platform Program. On October 1st, 2018, "Structure Analysis by Crystalline Sponge Method" is starting as one of the supporting program of M&MS in IMS.

For academic users, we offer CSM for the structure determination of your compounds as a collaborating work without charging. Not just structure analyses of unknown compounds by CSM but also new strategic proposals regarding CSM are welcome. For industrial users in private companies, the service will be available on April 1st, 2019.

Detail information will be accessible on website (http://nanoims.ims.ac.jp/ims/). If you want to know more, please feel free to contact us (kiyo-adachi@ims.ac.jp).

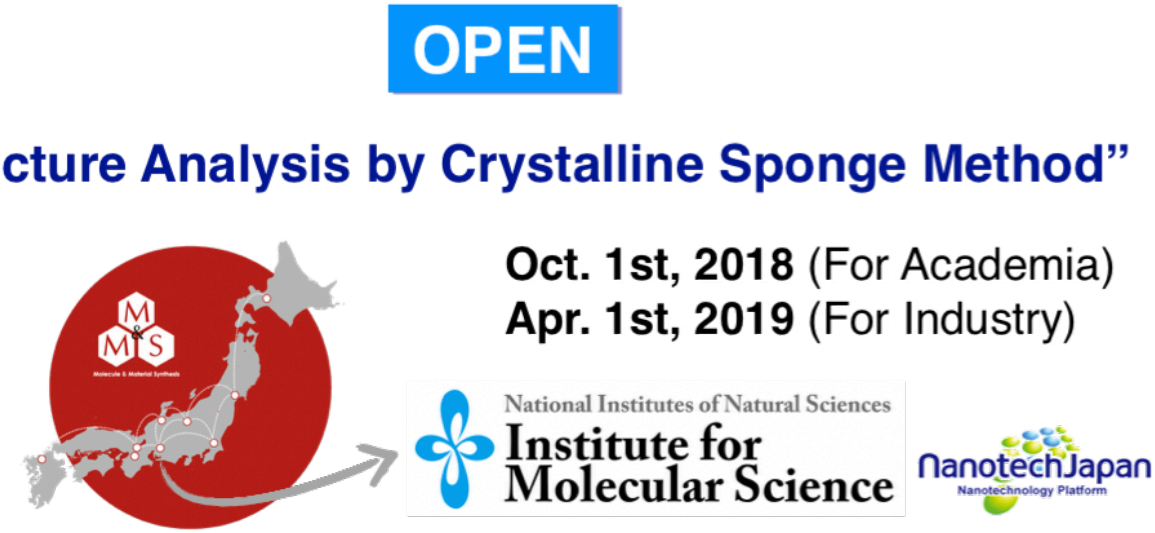

Figure 1 "Structure Analysis by Crystalline Sponge Method" starts in IMS on Oct. 1st, 2018.

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Functions designed in Crystalline Protein Assembly

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Protein assemblies have recently become known as potential molecular scaffolds for applications in bioinorganic chemistry. Efforts to design protein assemblies for construction of protein-based hybrid materials with metal ions, metal complexes, nanomaterials and proteins now represent a growing field with a common aim of providing novel functions and mimicking natural functions. However, the important roles of protein assemblies in coordination and biosupramolecular chemistry have not been systematically investigated and characterized. Currently, we are studying the accumulation of metal ions in protein crystals and protein cages, which are known to provide unique reaction environments, and have shown that these reaction scaffolds are suitable for controlling the reactivity of metal compounds. In particular, by using the protein assemblies, it becomes possible not only to apply to the cascade reaction by accumulation of different metal complexes, to observe the accumulation process of metal ions, but also to control the interaction of metal ion-protein in living cells. I would like to introduce functionalizations of various metal compounds by crystalline protein assembly.

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Molecular Borromean Rings based on Half-Sandwich Metal Fragments

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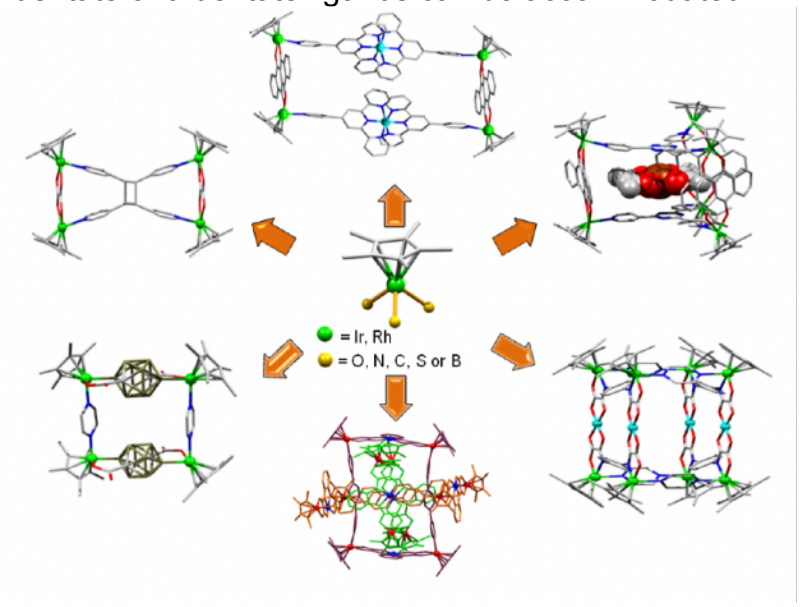
The construction of new inorganic and organometallic macrocycles and cages with interesting structural features and technologically useful functions have been topics of intense study with considerable potential.[1] One of the chief motivating factors to growth in this field is the development of new, functional and tunable donor building blocks that can bridge transition metals. Ideal building blocks should be easily accessible, exhibit high affinities toward transition metals, and possess facial coordination sites can undergo exchange reactions with various ligands. Half-sandwich transition metal complexes (Cp*M, Cp* = ^{5}-C_5Me_5) are useful model compounds in which one hemisphere of the coordination shell is blocked by the voluminous Cp* rings. In the protected space below the Cp* ligands, various bidentate or tridentate ligands can be accommodated.



Motivated by interest in supramolecular chemistry with organometallic half-sandwich complexes, we have initiated a new approach for preparing organometallic macrocycles via C-H and B-H activations with Terephthalate and dicarboxylate carborane.[2] We report herein an efficient method for synthesizing molecular macrocycles of half-sandwich iridium and rhodium complexes via C-H and B-H activation directed muticomponent self-assembly under mild condition. [3]

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Synthetic Molecular Wireframes Reinforce Protein Stability through Molecular Chaperone-like Structural Refolding Effects

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Spatial isolation of molecules is often a powerful strategy for regulating their molecular behavior. Biological systems well-employ such mechanisms, however, scientists have yet to rival nature, particularly for macromolecular substrates. Our team has recently demonstrated that only a “wireframe” molecular scaffold is sufficient to improve the structural and enzymatic properties of a protein encapsulated within (Figure 1). The three-dimensionally confined enzyme (cutinase-like enzyme (CLE): a protein for plastic degradation) did not show any structural melting by DSC experiments up to 130 °C (the T_m of native CLE is around 50 °C). Remarkable stability in a 10:90 aqueous–acetonitrile solution for tens of days was observed, even at room temperature. A kinetic assay of the enzymatic reaction revealed that the key to this stability is the isolated space, which aids protein refolding in a manner reminiscent of molecular chaperones (Figure 2). Although the encapsulated enzyme did partially denature in solutions containing high proportions of organic solvent, it refolded back to the original tertial structure when half-aqueous solvent conditions were restored. Isotope-labeled NMR studies also supported such refolding behavior. The key to protein encapsulation is the self-assembly of giant wireframe hollow metal complexes, some of which are the largest known artificially self-assembled objects that still possess a precise atomic composition.[1,2] This protein reinforcing methodology has many potential applications for both industrial and research use of enzymes.

Figure 1.

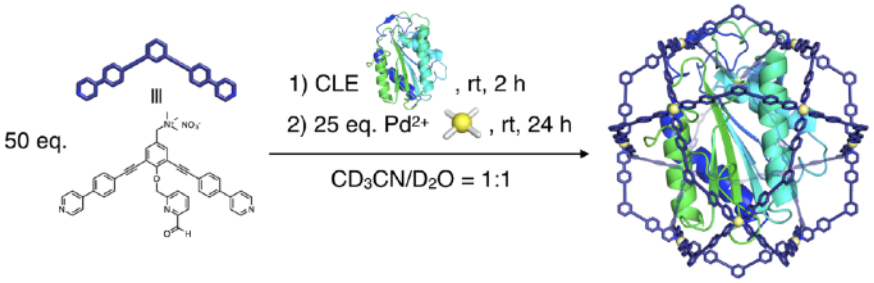
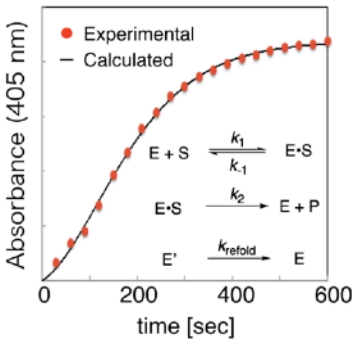


Figure 2.



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Structure Tuning of Metal Nanoparticle by Encapsulating within Anionic Porous Coordination Cages

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To overcome environmental issues associated with fossil fuels, hydrogen was proposed to be an alternative renewable energy source. Dehydrogenation of ammonia borane to generate hydrogen at mild conditions is important but requires a catalyst. However, the metal nanoparticle catalyst suffers from its heterogeneity, broad particle size distribution, and agglomeration. Because of their high porosity, adjustable cavity size, and tunable surface property, porous coordination cages (PCCs) can provide a potential platform to encapsulate and stabilize the nanoparticles. Herein we demonstrate the utility of PCCs as a nanocluster container by using an anionic PCC to encapsulate M NPs (M= Ru, Rh, Pd, Ni and Co), which are uniform in size (2.5 nm) and truncated octahedral structure. The encapsulated nanoparticle shows unusual face-cubic-centered (*fcc*) single crystalline form, and exhibits record-high catalytic activity in dehydrogenation of ammonia borane.

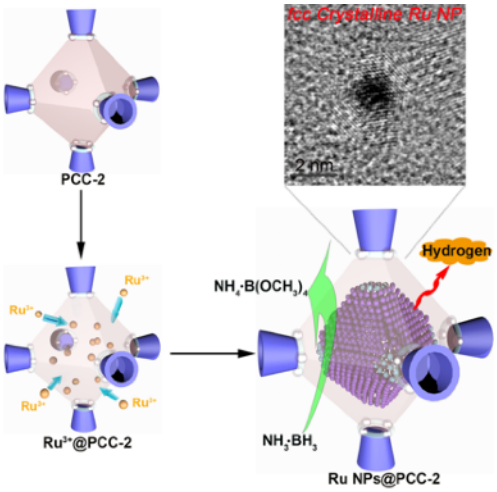

Figure 1 *fcc* Ru nanoparticle encapsulated within porous coordination cage.

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Encapsulation of protein in a hollow protein crystal

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Molluscan hemocyanin is a huge protein complex with a molecular mass of approximately 4 MDa, which is composed of a cylindrical wall and inner domains. We previously determined crystal structure of hemocyanin from Japanese flying squid[1,2], which revealed that the cylindrical hemocyanin stack in the crystal (Fig. 1 left). As a consequence, crystal of hemocynin has a hollow structure with a diameter of approximately 10 nm. Previous report demonstrated that a vacant space of crystal can be used for encapsulating guest molecules, which further enables to determine structure of the guest encapsulated. The hollow of hemocyanin crystal is enough large for encapsulating most proteins, suggesting that hemocyanin crystal may be applicable for structure determination of the biomacromolecular guests such as protein and DNA. In the present study, we encapsulated proteins in the hollow space of the hemocyanin crystal.

For encapsulating proteins in the hemocyanin crystal, three methods were considered to be possible, i.e., (1) soaking the guest into hemocyanin crystal previously formed, (2) crystallizing hemocyanin encapsulating guests, and (3) co-crystallizaion of hemocyanin with the guest. We confirmed that hamocyanin encapsulated guest protein by all these three methods (Fig. 1 right). In this conference, I will discuss the advantage and disadvantage of these methods in protein encapsulation.

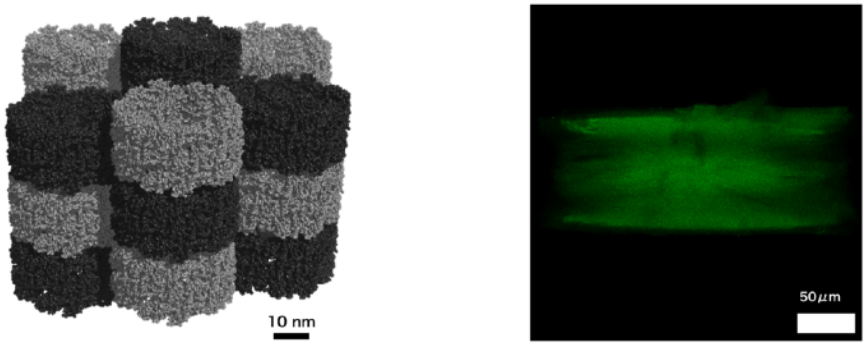

Figure 1 Packing of hemocyanin in the crystal (left) and confocal microscopy image of hemocyanin crystal encapsulating GFP by soaking method (right).

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Application of coordination chemistry approach to structural analysis of carbohydrate chains of biological interest

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Carbohydrate chains that modify proteins and lipids mediate a variety of biological and pathological processes on cell surfaces as well as in intracellular environments. To gain deeper insights into the functional mechanisms of the carbohydrate chains, detailed structural characterization is necessary at the atomic level. However, the crystallographic analysis of carbohydrate chains is hampered by considerable degrees of motional freedom with respect to their glycosidic linkage conformations. In view of this situation, we developed a method for characterizing dynamic conformational ensembles of oligosaccharides in solution by employing NMR spectroscopy in conjunction with molecular dynamics simulation[1]. In this method, a lanthanide chelating tag is chemically attached to the reducing terminus of the target carbohydrate chain in order to observe paramagnetic effects as sources of geometrical information regarding spatial arrangements of individual atoms with respect to the unpaired electron. This technique has enabled us to explore conformational spaces occupied by the carbohydrate chains that control protein fate determination in cells through interactions with a series of lectins.

We have also developed hybrid supramolecules of a self-assembled, spherical complex and carbohydrate chains in collaboration with Drs. Makoto Fujita and Sota Sato[2,3]. These cyborg glycoclusters have unique properties in terms of biomolecular interactions as exemplified by homophilic hyper-assembly through specific carbohydrate–carbohydrate interactions.

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