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Forum

Preface: Forum on Biomolecular Design in Inorganic Chemistry

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In his 1902 Nobel Lecture, Emil Fischer foresaw the day when synthetic enzymes would complement their natural counterparts as agents of chemical transformation.[1] In the intervening century, the goal of engineering custom enzymes for therapeutic and industrial applications has been transformed from a fantastical vision into an approachable reality. Fueled by tremendous strides in all aspects of biotechnology, the fundamental tenets of natural protein and nucleic acid structure−function relationships are being elucidated at an ever-accelerating pace. Because most nucleic acids and half of all proteins require metal ions for their biological functions, the role of inorganic chemists is indispensable in tackling the daunting challenge of understanding how metal ions bind to protein ligands, influence their folding pathways, and facilitate catalysis.

Biological macromolecules that require metal ions are, at their very essence, often highly sophisticated Werner complexes assembled from aqueous solution. Aside from their vital roles in contributing to the breadth of chemical reactivity available in enzymatic catalysis, the metal ions bound to these proteins and nucleic acids are also critical components to the assembly, folding, stability, and electrochemistry of the resulting complexes. While the complexity of these natural systems is formidable, the underlying principles of coordination chemistry so familiar to inorganic chemists are the foundation for their unique spectroscopic and functional properties. Thus, the deeper understanding of biological metal complexes in aqueous solution that inorganic chemists can provide is clearly necessary in order to fulfill Fischer's vision.

In this context, *Inorganic Chemistry* is proud to present a Forum on Biomolecular Design in Inorganic Chemistry. This series of contributions highlights recent developments in the study of metal−protein and metal−nucleic acid complexes from an inorganic chemistry perspective. The goal of these researchers is to elucidate the fundamental governors of metalloprotein and metal−nucleic acid structure and function so as to access the unique properties of biological ligands. These designed systems provide a conceptual amalgamation between the inorganic synthetic analogue and biochemical approaches to bioinorganic chemistry. The utility of these biomolecule-based coordination compounds is not only in the unraveling of the fundamental concepts of metallobiochemistry but also in the constructive expansion of biochemical catalysis beyond its current scope.

In the classic 1996 thematic issue of *Chemical Reviews*, Holm, Kennepohl and Solomon detailed the hierarchy of metalloprotein function, i.e., structural, metal-ion storage, electron transfer, ligand binding/transport, and catalysis.[2] In addition, these authors describe the unique properties of protein ligands including allostery, multicenter organization, surface recognition sites, hydrophobic burial, substrate binding/activation, and rack/entatic states. The Articles in this Forum describe systems that use the unique properties of protein ligands to access each of the metalloprotein functions described.

The series of Forum Articles begins with a tutorial overview contributed by Lu detailing the approaches to, and the challenges of, inorganic biomolecular design. Aimed at graduate students and researchers new to the field, the Article describes the process from selecting a design approach through evaluating its success. One of the initial decisions when entering the field is the choice of whether to use a rational design or a combinatorial screening/selection approach. Once this initial decision is made, questions as to whether to use a native or de novo designed scaffold, whether to use empirical or computational design, and whether to use chemical synthesis or biological expression to generate the ligand follow and lead to the diversity of inorganic model systems in this series of Forum Articles. In terms of the first

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(1) *Nobel Lectures, Chemistry 1901−1921*; Elsevier Publishing Co.: Amsterdam, The Netherlands, 1966.

(2) Holm, R. H.; Kennepohl, P.; Solomon, E. I. *Chem. Rev.* **1996**, *96*, 2239−2314.

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decision point, rational metalloprotein design provides for rigorous tests of our understanding of metalloprotein structure−function relationships at all levels, but, in practice, its use must be weighed in light of the power of the combinatorial approach in both lead discovery and optimization. In the case of nonmetalloproteins, the advent of improved molecular mechanics energy functions and minimization algorithms has accelerated the pace of success in computational protein design to be competitive with combinatorial methods. In the case of metalloproteins, one can envision that the future implementation of mixed quantum mechanical−molecular mechanical (QM/MM) computational design will yield comparable success and provide access to the design of inorganic and organometallic catalysts within protein ligands.

In the second paper, Gibney and co-workers provide a review of thiolate-rich metalloprotein design and determine the thermodynamic contribution of $M^{II}(S{\cdot}Cys)_4$ sites to protein assembly and folding. Using traditional coordination chemistry methods, these researchers evaluate the formation constant of a simple, unstructured tetrathiolate peptide derived from a natural ferredoxin protein for Fe(II), Co(II), and Zn(II). The data show the selectivity expected based on the Irving−Williams series and demonstrate that structural $Zn^{II}(S{\cdot}Cys)_4$ sites such as those found in zinc finger transcription factors can contribute up to −21.6 kcal/mol toward metalloprotein assembly and stability. This thermodynamic contribution is an order of magnitude larger than most protein−protein interactions, and its determination provides the requisite data for improving computational metalloprotein design. Indeed, the formation constant of this Zn(II)−peptide is greater than the tightest natural Zn(II)−protein association constant reported, which the authors argue is due to the minimization of the penalty for protein folding.

The strength of metal−thiolate bonds can also be used to drive coiled-coil protein assembly and folding reactions, as discussed by Pecoraro and co-workers. This contribution addresses the complex interplay between the fold encoded in the protein ligand itself and the coordination preferences of the bound metal ion within the larger context of coiled-coil protein design. Using empirical de novo metalloprotein design, these researchers had previously demonstrated a metal-induced protein folding and assembly event via the construction of supercoiled assemblies of helical peptides, or coiled-coil proteins, from unstructured monocysteine peptides upon metal-ion binding. The original design concept of these authors was to make parallel three-stranded coiled-coil scaffolds; however, detailed studies to assess the selectivity of parallel vs antiparallel peptide aggregates, in the presence or absence of metals, had not been reported. In this contribution, it is shown that from either homomeric or heteromeric mixtures of peptides one attains solely homomeric parallel three-stranded coiled coils assembling to give trigonal metal binding sites. In this case, the metal−sulfur bond strengths are not sufficient to alter the originally realized parallel design, even though acceptable trigonal metal binding structures should be possible from heterotrimeric antiparallel structures. Thus, these scaffolds illustrate the highly selective self-assembly of homomeric coiled coils by Cd(II), a hallmark of biological molecular recognition.

Using similar parallel coiled-coil protein scaffolds, Ogawa and co-workers demonstrate the utility of metalloprotein design in the study of biological electron-transfer function. In a two-stranded coiled-coil scaffold that is folded in the absence of metal ions, they appended ruthenium-based redox-active metal complexes to solvent-exposed histidine residues to generate synthetic electron-transfer proteins. The inorganic photochemistry of these constructs provided a means with which to study a variety of electron transport fundamentals, including the roles of the helix−helix interface, of the electrostatic environment of the inorganic cofactors, and of protein conformational gating on the kinetics of the photo-induced electron-transfer event. With a coiled-coiled protein bearing a pair of cysteine residues one helical turn apart on each helix, the authors present a study of a metal-induced protein folding and assembly event, in which the coordination preferences of the bound metal ion dictate the protein oligomerization state. For example, Zn(II) does not result in oligomerization of peptides, Cd(II) generates a $Cd^{II}(S{\cdot}Cys)_4$ site from a pair of peptides, and Au(I) incorporation yields a hexamer. The most exciting construct involves the spontaneous self-assembly of a multinuclear thiolate−Cu(I) cofactor inside a four-stranded coiled-coil protein that displays room-temperature luminescence similar to that observed in the copper chaperone Cox17. The ability of this protein to act as an electron-transfer reagent is described in a demonstration of the expansion of protein design to include functionally active Cu(I) metalloproteins.

The role of protein dynamics in heme protein function is investigated by Benson and co-workers using peptide-sandwiched mesohemes, or PSMs. As described in their contribution, the PSMs are covalent porphyrin−peptide adducts that, upon histidine coordination to the iron, elicit a unimolecular metal-induced protein folding event. Detailed studies of the metal−ligand bond thermodynamics in two bis(histidine) PSMs demonstrate that the strength of the iron−histidine bonds is at least as important to modulating the heme iron reduction potential as is heme solvent exposure. This Article also puts the current literature in heme functional tuning into context and provides fresh insight into the fundamental differences between six-coordinate heme proteins involved in electron-transfer reaction and those involved in ligand transport and sensing. In the broader context of natural heme proteins, the amount of dynamic strain at the heme binding site may be a critical factor in determining the biological function of the protein. In the PSMs, the dynamic strain produced by restricting the conformational mobility of the peptides upon metal-ion coordination allows exogenous ligands like CO to compete effectively for the endogenous histidine ligands, as observed in natural heme sensor proteins such as CooA. In fact, most de novo designed bis(histidine)-coordinated ferrous heme proteins react with CO, which suggests they may all possess ligand sensor or ligand transport activity. In the case of natural electron-transfer heme proteins with bis(histidine) ligation, such as cytochrome b_5, the lack of dynamic strain

restricts the ability of the heme to bind exogenous ligands. Thus, the dynamical differences between six-coordinate low-spin heme proteins are key to their biological function. This proposal also supports the general concept that, because metal ions are inherently reactive, the role of the protein in a metalloenzyme may not be to accentuate one reactive pathway over all of the others but rather to quash the majority of reactive pathways, leaving only one available. Thus, the challenge for heme protein designers is now not only to control the structure and electrochemical function of their constructs but also to develop methods to map out and modulate their dynamics in order to manage the inherent reactivity of the heme cofactor.

Controlling the function of a metalloprotein active site goes well beyond the primary coordination sphere, and metalloprotein design provides the ability to tailor all aspects of the protein ligand. In their contribution, Franklin and co-workers investigate the role of the protein context on active-site reactivity in a lanthanide-dependent metallonuclease designed as a chimera between a Ca(II)-binding EF-hand motif and a helix−turn−helix (HTH) DNA binding protein. The protein fold, the metal-ion affinity, and the catalytic activity are shown to rely critically on a second-coordination-sphere aspartic acid in the EF-hand that hydrogen bonds to a metal-bound water. Furthermore, the authors show that the global context of the metal-binding domain can dramatically influence reactivity, despite retaining primary and supersecondary local contexts. By expansion of the scaffold into which the EF-hand loop was constructed from a small peptidic HTH motif to the full three-helix bundle homeodomain (of which the HTH was a subset), the same metal site showed new behavior. In this case, designing the lanthanide-binding motif within the context of the full homeodomain results in a loss of catalytic function, but not in metal-ion affinity, relative to its peptidic counterparts. This illustrates that the context of the active site is critical in the design of functional metalloenzymes.

This series of Forum Articles is presented to highlight current research efforts and challenges in metalloprotein and metal−nucleic acid design. The goal in organizing this series of Articles is to illustrate the importance and excitement of this rewarding field of interdisciplinary research for the broader inorganic community. These articles present not only an overview of the various approaches to the field and a description of the major research objectives but also insight into the opportunities for innovation. The challenges inherent to metal−protein and metal−nucleic acid design require the development of new design concepts and methods by creative and skilled inorganic chemists. We hope that the Articles in this *Inorganic Chemistry* Forum on Biomolecular Design in Inorganic Chemistry will encourage others to join us in contributing toward the fulfillment of Emil Fischer's vision in a bioinorganic context.

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