DESIGN STRATEGY FOR CREATING CATALYTICALLY ACTIVE METAL BINDING PROTEINS

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Metalloenzymes catalyze a wide variety of reactions in nature by taking advantage of the versatility and reactivity of transition metals. Despite the diversity of reactions catalyzed by natural proteins, there is still a demand for designer enzymes. In many cases, all that is needed is routine re-engineering of the native enzymes to perform efficiently under the demanded application conditions. In other cases, the reaction or reaction condition desired differs so much from natural conditions that mere redesign of natural proteins is not practical. *De novo* enzymes, which are generated entirely from first principles rather than modified from natural proteins, are ideal for these situations. These *de novo* enzymes would allow us to generate enzymes that can survive at much higher temperatures, work in many different solvents and solutions, or perform completely novel functions.

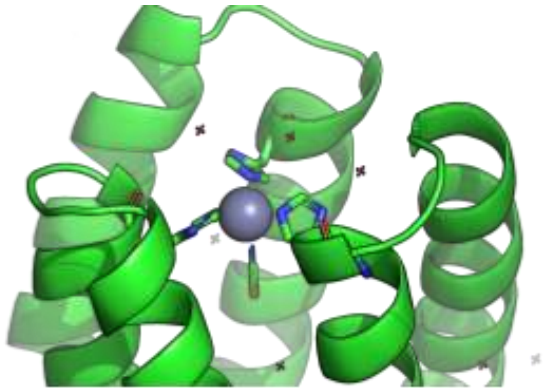

Figure 1 – Metal binding site of designed zinc – binding helical bundle with inverted tetrahedral complex

Currently, most new metalloenzymes are still developed via mutations or evolution of natural proteins. While there are previous examples of designed metalloenzymes in *de novo* scaffolds, these designs are generally limited to incorporation of the metal binding motif into monomeric three and four helix scaffolds. The limitations of this shape greatly limits the functionalities possible for these enzymes. With recent advances in *de novo* design of proteins, it is now possible to generate a wide array of helical bundle oligomers, and create a diverse set active site topologies to enable a variety of novel reactions. Figure 1 shows the crystal structure of a zinc binding protein created using our design methods. Here we present a strategy for design of *de novo* proteins around a desired metal coordination site. This process allows for the generation of myriad topologies custom-designed for the reaction of interest, while still maintaining the stability that designed proteins can afford.