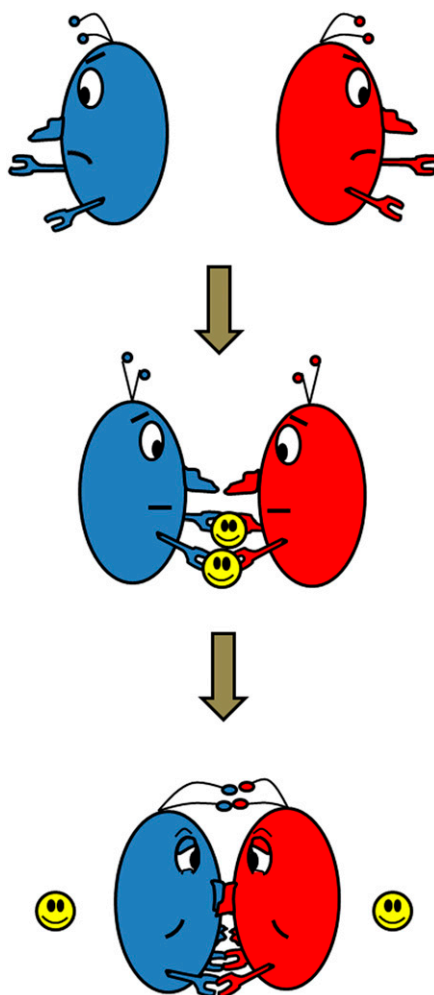# Metal ions as matchmakers for proteins

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Matchmakers have been shown to be valuable for single men and women in both ancient and modern societies. Matchmakers bring together two people who otherwise have little chance of being associated with each other, nurture the closeness between the two parties by enhancing their commonality and smoothing out their differences, and then quietly disappear after the marriage. In this issue, Salgado et al. (1) describe such a matchmaker for single-domain proteins, with significant implications for protein structural evolution and design (Fig. 1).

Just as families are important units for the functioning of society, multiple-domain proteins are vital for biological functions (2). A well-known example is hemoglobin in red blood cells, which transports O_2 from our lungs to other parts of the body. Interfacial interactions between the four domains or subunits of hemoglobin are critical for regulation of the binding and release of O_2; a single-residue mutation at the interface can result in diseases such as sickle-cell anemia. It is no wonder that a large number of proteins are multiple-domain proteins. Even single-domain proteins often do not function alone, and their interactions with other proteins play an important role in many biological functions, ranging from electron transfer in photosynthesis and respiration to ligand binding and activation in biocatalysis.

Despite the widespread recognition of the importance of interfacial interactions, there are few clues as to how proteins evolved to form such strong and tightly regulated interfaces. The chance that a single-domain protein will fold into a structure that can match well with that of another protein simply by random collisions is very small. For a long time, scientists have been searching for ways to induce such selective interactions. Several groups have reported de novo design of protein interfaces through docking of two protein structures, making mutations to minimize steric conflicts and to maximize non-covalent interactions, such as hydrogen bonding, hydrophobic, and electrostatic interactions (3–7). Despite these successes, few studies have addressed the issue of how two proteins with few interfacial interactions are able to find each other by overcoming the entropic barriers in early events of protein structural evolution. In this issue, Salgado et al. (1) propose and demonstrate that metal ions could possibly fulfill such a

Fig. 1. Metal ions (yellow) as matchmakers for single-domain proteins (blue and red).

role by being a matchmaker. In a strategy called metal templated interface redesign (MeTIR), these investigators first designed histidines into the interface of cytochrome cb_{562}, a monomeric protein even at millimolar concentration, to allow Zn^{2+}–histidine coordination to bring monomeric cytochrome cb_{562} together. On obtaining crystal structures of such metal-containing multiple domain proteins, they went on to use a variant of the Rosetta-Design algorithms to optimize side chain rotamer conformations. With as few as six mutations in cytochrome cb_{562}, the proteins formed dimers at a micromolar concentration in the absence of Zn^{2+} or other divalent metal ions.

The use of metal ions as matchmakers for single-domain proteins makes sense. About 40% of the natural amino acids are capable of binding metal ions; therefore, the chance for metal-coordinating amino acids to be exposed on the surface is quite high. During the early events of evolution, metal ions were abundant on earth and their presence and concentrations were not as highly regulated as they are now in cells, making it possible for a variety of metal ions to interact with surface-exposed amino acids. Because the metal–ligand bond is much stronger than all non-covalent interactions, such coordination may be strong enough to overcome steric conflicts, electrostatic repulsions, and other noncovalent interactions that prevent two monomeric proteins from associating with each other. Once the two proteins are brought together by metal ions, random mutations in the interface of the two proteins may produce a stronger interface to result in multiple-domain proteins without the help of metal ions. Another support for MeTIR playing a key role in protein structural evolution is the presence of a number of metal-binding sites between domains of proteins, such as the dinuclear copper center in hemocyanin and Cu_Z center in nitrous oxide reductase (8). Perhaps these metal ions were matchmakers in action, and they have been retained because they can perform additional functions, such as O_2 binding or nitrous oxide reduction.

In addition to providing insight into how single-domain proteins are evolved to form multiple domain proteins, this work expands our understanding of the role of metal ions in biological systems. Previous studies have shown that metal ions can help peptides to fold into rigid and pre-defined structures within a single domain of protein, such as DNA-binding zinc finger proteins (9). In this issue, Salgado et al. (1) indicate that metal ions can play structural roles between domains of proteins. It has been shown that 47% of structurally determined proteins require metals, with 41% containing metals at their catalytic center (10). The work by Salgado et al. (1) suggests that metal ions can play important roles as matchmakers

for single-domain proteins, even though metal ions are not present in the final product. Therefore, metal ions manifest their importance in far more than the ~50% of proteins discovered so far.

This study not only provides fresh perspectives into the past on topics such as protein structural evolution and the roles of metal ions, but opens avenues for the future in terms of protein design. Protein–protein interactions are at the heart of many diverse biological functions, such as regulation of O_2 binding in hemoglobin and signal transduction in neurobiology. Understanding these interactions and being able to control the protein–protein interactions rationally and predictably will advance many areas of biological and biomedical sciences. One example is the impact of the yeast two-hybrid system (11) in advancing our understanding of biological systems and in discovering molecules involved in protein–protein interactions. Because of complicated noncovalent interactions, it has been very difficult to control these interactions well. Using the power of metal–amino acid coordination, MeTIR provides a general method for engineering protein interfacial interactions.

This study by Salgado et al. (1) will also have a significant impact in metalloprotein design and engineering. Accounting for almost half of all proteins, metalloproteins catalyze some of the most difficult and important reactions in nature, such as photosynthesis, carbon fixation, and water oxidation. Designing metalloproteins is a touchstone whereby our knowledge can be tested and metalloenzymes with un-

precedented properties for biotechnological and pharmaceutical applications can be obtained (12, 13). Most current focus is on designing metal-binding sites within a single domain of proteins.

MeTIR provides a general method for engineering protein interfacial interactions

Because there are many metal-binding sites between domains of proteins, designing those metal-binding sites is just as important but has not been explored extensively. The study by Salgado et al. (1) indicates that the redesigned interface that binds Zn^{2+} provides sufficient driving force so that when Cu^{2+} binds to the same site, the site imposes an unsaturated coordination environment around Cu^{2+}, making it possible to design copper enzymes.

Although this work is exciting, the designed interface and metalloprotein are not ready for application, and thus have room for improvement. For example, the strongest dissociation constant (K_d) between the two monomeric proteins achieved in this study is 40 μM; to be useful for practical applications, a K_d in the nanomolar range or tighter is preferred. Furthermore, unlike blue or type 1 copper proteins, where the protein provides a rigid network of noncovalent

interactions to enforce a geometry not preferred by Cu^{2+} (14–16) while also fine-tuning its potential without any perturbation to the primary coordination sphere (17), the designed interface is sufficiently flexible to allow Cu^{2+} to conform to the square planar geometry it prefers instead of the tetrahedral geometry the study designed. To improve the design, other interactions in the interface, including those in the second shell as demonstrated within single-domain proteins (13, 17, 18), may be considered and improved computing algorithms may be employed. The protein may also be subjected to directed evolution (19, 20).

The next time we see multiple-domain proteins with an intimate interface between them, the work by Salgado et al. (1) may remind us of the possible involvement of metal ions as a matchmaker in helping these single-domain proteins associate into a functional unit. Although it may be debatable whether MeTIR played an important role in the evolution of protein structure and function, it is clear that MeTIR will provide a unique approach for protein designers to engineer protein–protein interfaces for multiple-domain proteins, including many metalloproteins.

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