Metals Make Proteins Stick

Birte Höcker[1,*]
[1]Max Planck Institute for Developmental Biology, Spemannstr. 35, 72076 Tübingen, Germany
*Correspondence: birte.hoecker@tuebingen.mpg.de
DOI 10.1016/j.chembiol.2010.02.002

Salgado et al. (2009b) report the rational design of protein interfaces based on metal-mediated association. Initially coordinated via zinc, the adapted interface self-associates, even in the absence of metal. The approach is hypothesized to mimic the early evolution of protein assemblies.

Protein-protein interactions are important for numerous biological processes and central to cellular organization. The binding surfaces often consist of many contributing noncovalent interactions. During the course of evolution, these interactions have to accumulate through natural selection to enable stable or functional complex formation. In that context, the existence of some kind of molecular "glue" that makes proteins stick and thereby provides an initial interface that can be optimized would present a great advantage. Salgado et al. (2009b) now suggest that metals could have played this role and mediated initial interactions. In many present day proteins, metals play key roles for structural integrity and functionality. They may have also contributed to the evolution of protein interfaces as a first interaction mediator that later would have been rendered dispensable. Similarly, the energetically favorable metal coordination could provide an advantage to the challenging task of protein interface design from scratch.

The authors took cytochrome c-b562, an engineered four-helix bundle heme protein that remains monomeric at millimolar protein concentrations, and turned it into a self-associating multimer. Despite its lack of association in solution, c-b562 was observed to form pairs in the lattice of a protein crystal with crystal-packing contacts between the antiparallel aligned helices 3. In order to establish two metal coordination sites at the interface of the helices, di-histidine motives at positions i and i+4 were created at either end of helix 3. This variant was found to assemble into a D2-symmetrical tetramer in the presence of Zn^{2+}. The crystal structure of the zinc-mediated complex revealed two interlaced V-shaped dimers with four zinc ions at the interfaces (Salgado et al., 2007). Differently than anticipated, the zinc ions were coordinated by three of the histidines and an aspartate residue present in the wild-type protein. Two of the three interfaces within the tetramer buried rather large surface areas comprised of polar side chains that were forced together by the zinc coordination. This demonstrates the ability of metal coordination to assemble protein building blocks with noninteracting surfaces into superstructures. It further illustrates that only few mutations are necessary to turn a monomeric protein into a multimeric complex, a process that thus could have happened during the course of evolution. The same protein was also found to form a C2-symmetrical dimer and a C3-symmetrical trimer in the presence of copper and nickel, respectively (Salgado et al., 2009a). Hence, the arrangement of the c-b562 superstructure is dictated by the inner-sphere metal coordination geometry, which is tetrahedral for Zn^{2+}, but square-planar for Cu^{2+} and octahedral for Ni^{2+}. The same metal-coordinating mutations can therefore lead to various different superstructures.

If advantageous, such a "forced" complex might evolve toward further stabilization by optimization of the interfaces. Following this hypothetical evolutionary path, the new interfaces of the zinc-mediated complex were improved by computational design using RosettaDesign algorithms (Ambroggio and Kuhlman, 2006). Six mutations were introduced into each of the two larger interfaces generating hydrophobic core and knob-into-hole interactions. Sedimentation velocity experiments confirmed that the interfacial mutations improve self-association in the presence as well as in the absence of zinc. At higher concentrations of equal amounts of protein and zinc, dimers as well as tetramers became more populated, while in the absence of zinc solely increasing amounts of dimers were observed, indicating that only one of the designed interfaces has become independently self-associating. Crystal structures of the two interface-design variants in the presence of zinc confirmed that the overall structure remains the same despite the large number of amino acid changes.

Next the authors wanted to test the strength of the self-associating interactions in a competing environment. The newly introduced mutations were based on the interfaces imposed through tetrahedral zinc coordination. If the engineered interactions were strong enough, they could override the non-tetrahedral copper coordination. Such an effect is called templating. The crystal structure of the c-b562 interface redesign was determined in the presence of copper. It showed a criss-crossed alignment that significantly differs from the copper-coordinated arrangement observed without the interface mutations (Salgado et al., 2009a). The two Cu^{2+} environments still had pyramidal geometries but the coordination was unsaturated, which shows that the noncovalent interactions introduced based on the zinc geometry have only a small templating effect.

Overall, the experimental outline follows a hypothetical evolutionary pathway (Figure 1). It starts with a non self-associating protein (1) that becomes a metal-mediated multimer (2) through the acquisition of metal-coordinating residues at its surface. The new interface created through metal binding is optimized further (3) until complex formation is independent of it (4). The authors present thoroughly tested examples for each step of the pathway, thereby illustrating the feasibility of this evolutionary process. However, the lack of information about metal regulation

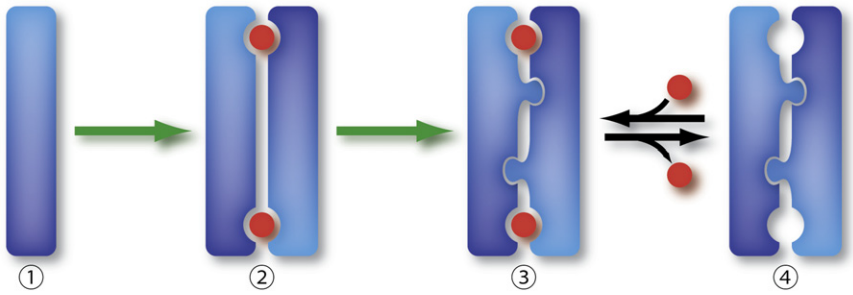

Figure 1. Outline of the Experimental Path
A non self-associating protein (1) becomes a homo-oligomer (2) through the acquisition of metal-coordinating residues at its surface. The new interface is optimized further (3) until complex formation is independent of metal-coordination (4). Metals are shown as red spheres; green arrows depict mutational events.

in early cellular environments renders it purely hypothetical.

Nonetheless, the pathway can be taken as a route to design protein interfaces as suggested, the rational approach being termed Metal Templated Interface Redesign (MeTIR). How feasible and efficient is this approach? The first step of introducing metal-coordinating residues turned out to be very effective in this case, though not entirely predictive: only one of the two designed sites led to zinc binding and in an unforeseen manner. Although a dimer was expected, a tetramer was found. A more precise prediction of metal-coordination and the resulting interfaces is desirable. Use of available computational methods for the placement of side chains with predefined geometries onto a known protein backbone might improve the accuracy of coordination site design at crystallographic interfaces. Precision is extremely important, since metal binding will be the template for the interfacial interactions to be designed. Therefore, structural studies of this intermediate construct are essential. The second part of MeTIR deals with the adaptation of the new interface, which has worked especially well for one of the interfaces in Zn:c-b562. It thus presents another good example for the successful use of the Rosetta software (Kuhlman and Baker, 2000) to optimize protein packing not only within proteins but also between them. The combination of the two steps, metal-mediated association followed by interface optimization, allows incremental improvement from a defined starting point. But one would hope that once computational protein interface design is better understood and more reliable the initial step of metal-coordination is not necessary anymore.

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Nucleic Acid Detection using MNAzymes

Yulia V. Gerasimova[1] and Dmitry M. Kolpashchikov[1,*]
[1]University of Central Florida, 4000 Central Florida Boulevard, Orlando, FL, USA
*Correspondence: dkolpash@mail.ucf.edu
DOI 10.1016/j.chembiol.2010.02.003

Deoxyribozymes are promising biotechnological tools. In a recent *JACS* article, Mokany et al. reported on the design of multi-component deoxyribozyme (MNAzyme) sensors based on 10-23 and 8-17 DNA enzymes. The sensors can detect down to 5 pM of a specific nucleic acid. The versatility of MNAzyme platform allows the design of catalytic cascades for signal amplification. This work is a step forward to PCR-free molecular diagnostics.

The discovery of catalytic DNA molecules, known also as deoxyribozymes, DNA enzymes, or DNAzymes, by Breaker and Joyce (1994) has introduced a new versatile scaffold for the design of a variety of biotechnological tools (reviewed by Schlosser and Li [2009] and by Baum and Silverman [2008]). Sharing the advantages of biocompatibility and simplicity of structural prediction and modification with ribozymes, DNA enzymes are more stable and have lower cost of chemical synthesis than their RNA counterparts. DNAzymes have a great, though not completely explored, potential for sensing a variety of analytes (Liu et al., 2009), mainly due to the possibility of catalytic signal amplification.

Among all possible applications of DNAzyme-based sensors, the detection of specific RNA/DNA sequences is of

