Protein Structures

DOI: 10.1002/anie.200801169

Metals Coordinate Protein–Protein Interactions**

*Kagan Kerman and Heinz-Bernhard Kraatz**

bioinorganic chemistry · de novo design · metal coordination · protein structures · protein–protein interactions

For decades, scientists in diverse fields have been intrigued by the ability of an amino acid sequence to dictate the formation of a unique protein structure. As our knowledge of the structure of proteins has increased, major advances in the field have enabled the design and engineering of specific protein structures. In proteins, the combination of noncovalent forces is encoded in the sequence of amino acids, which constitutes the primary structure. As these noncovalent forces (hydrogen bonding, electrostatic and van der Waals interactions) have been understood well in physicochemical terms, it has been possible to design and synthesize non-natural polymers/oligomers with compact, well-defined three-dimensional structures by selecting appropriate amino acid sequences.

This idea led to the birth of the de novo design of artificial "proteins" from scratch.[1] These de novo molecules, or foldamers,[2] can include abiotic building blocks, such as aromatic rings with redox or photoactive groups that give these foldamers specific functions. Developments in the design and engineering of the bioinspired foldamers include the investigation of the sequence specificity of various secondary structures, the stabilization of the secondary structures in water, and the creation of tertiary structures, such as helical bundles, barrel-like assemblies, and new stable helical foldamers.[2,3] Abiotic-foldamer research has focused mainly on the design of the monomer geometry to modulate the structure of the homooligomers. In particular, the objective of the research has shifted to the introduction and manipulation of foldamers with specific chemical functions (recognition, binding, and selection). Emil T. Kaiser, a pioneer in this field, formulated the ground rules for de novo protein design.[1c] DeGrado and co-workers[1f] recently discussed various aspects of foldamer design and summarized the use of foldamers as versatile frameworks for the design and evolution of function, and Schueler-Furman et al.[1e] reviewed recent progress in the modeling of protein structures and interactions.

[*] Dr. K. Kerman, Prof. H.-B. Kraatz
Department of Chemistry
The University of Western Ontario
1151 Richmond Street, London, N6A 5B7, Ontario (Canada)
Fax: (+1) 519-661-3022
E-mail: hkraatz@uwo.ca

[**] This research was supported by the NSERC and the Ontario Ministry of Research and Innovation.

Various research groups have used coordination chemistry for the assembly of well-defined supramolecular peptide assemblies.[4] The interplay between the ligand on the peptide foldamer and the metal ion determines the coordination environment around the metal ion and drives the supramolecular peptide assembly (Figure 1). This approach has been used to study peptide–peptide interactions and, more recently, peptide–protein interactions. A recent success is the de novo design of a metalloprotein from smaller peptide precursors by Ogawa and co-workers.[4b–i] Metal coordination was exploited to change the oligomerization of an α-helical coiled coil and thus enable the incorporation of electron-transfer functionality into the assembly from the bottom up. The final de novo metalloprotein exhibits gated electron transfer and was exploited as a probe for the configurational dynamics of peptide–protein complexes.[4d]

Figure 1. Metal–ligand interactions have been exploited for the construction of de novo metalloproteins that can often mimic some of the properties of naturally occurring metalloproteins. Geometric constraints imposed by the metal–ligand interactions as well as the interaction of substituents on the ligating peptide determine the tertiary structure of the supramolecular construct.

The ability to design specific supramolecular structures with proteins allowed the development of experimental and bioinformatic approaches for probing protein–protein interactions,[5] which play a critical role in many cellular functions. Although advanced computational techniques have been developed to predict these interactions,[6] there is a lack of well-defined model systems. A significant number of well-defined systems are necessary to enable an adequate comparative evaluation of theoretical predictions. Furthermore, high-throughput experimental methods are necessary to generate a suitably large number of biophysically well characterized protein complexes. Once again, coordination chemistry is making a significant contribution to this area. Tezcan and co-workers[7] recently described a potentially versatile approach to the generation of a large number of

well-defined protein complexes by exploiting metal–ligand coordination, in which some amino acid side chains function as metal-ligating sites to drive the assembly of the protein building blocks. Four copies of the well-characterized small protein cytochrome b_{562} (Cyt b_{562}) were coordinated around Zn^{2+} ions through imidazole side chains of His and a carboxylate side chain of Asp (Figure 2). A tetrahedral

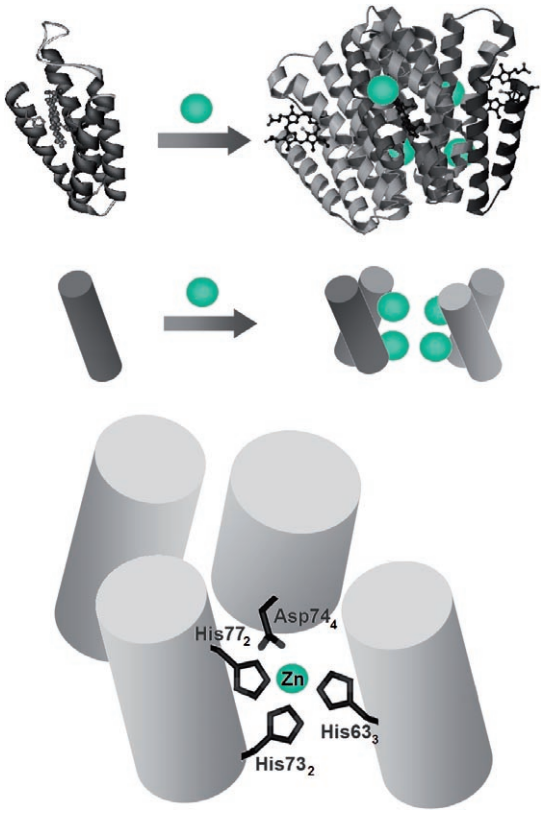

Figure 2. Four Cyt b_{562} building blocks are brought together by the coordination of Zn^{II} ions to the imidazole and carboxylate side chains of histidine and aspartic acid, respectively. The strength, directionality, and selectivity of metal–protein interactions provide the basis for controlling protein–protein interactions. Thus, specificity and affinity in the construction of a protein complex do not require extensive binding surfaces.

coordination environment around each metal center resulted in one of the first examples of protein–protein interactions caused by metal coordination. The benefits of an approach based on coordination chemistry are clear. Whereas protein–protein interactions are generally guided by the superposition of the noncovalent forces that are spread over large surfaces, making the control and design of such models a challenge, metal–ligand interactions are strong, highly directional, and, in most cases, under thermodynamic control. De novo protein design provides an attractive approach to the critical testing of the structural and functional features of metalloproteins and will thereby undoubtedly contribute significantly to our understanding of the function of many metal-based enzymes and proteins.[1,4]

The choice of Cyt b_{562} as a test case is an interesting one. The protein is a hemoprotein expressed in the periplasm of the gram-negative bacterium *E. coli* and has been studied extensively, which makes it an ideal test case for the generation of protein assemblies by metal coordination. Cyt b_{562} comprises four antiparallel α helices that wrap into a left-handed bundle. The heme group is inserted into the helix bundle and held in place by Met7 and His102, which occupy axial coordination sites of the heme iron. The ease of overexpression of this protein in *E. coli* and the availability of structures for both the apo- and oxidized holoproteins have made it the subject of intense investigation in several laboratories. The structure of Cyt b_{562} was determined initially at a resolution of 2.5 Å in 1979,[8] and later refined to a resolution of 1.4 A.[9] The solution structure of oxidized Cyt b_{562} was reported by Arnesano et al.[10] The protein has well-defined and fast folding kinetics on a sub-millisecond time scale, whereas other heme proteins, such as Cyt c, require 10 s or more for complete refolding.[10] Fast kinetics is observed only for the holoprotein, and heme dissociation significantly hampers the refolding dynamics. In fact, a covalent linkage between the heme unit and the polypeptide chain in a variant of Cyt b_{562} causes a substantial increase in the stability of the protein and faster folding kinetics.[11]

Tezcan and co-workers used four copies of Cyt b_{562} as building blocks to construct a 16-helix bundle by coordination of the building blocks to four Zn^{II} ions (Figure 2).[7] The resulting metal–protein complex crystallized, and detailed structural analysis (PDB ID: 2QLA) was carried out at a resolution of 2.9 Å. From the point of view of coordination chemistry, this structure is one of the most complex biological ligand systems to be characterized structurally. The individual protein building blocks are arranged in a parallel fashion in a V shape, whereby two Cyt b_{562} building blocks are related by a two-fold symmetry axis with an interprotein angle of ≈37°. The two V substructures are wedged into one another in an antiparallel fashion. Interprotein Zn coordination to ligating sites on the protein is responsible for both the formation of the V substructures and their interlacing. Each Zn ion is located in an identical distorted-tetrahedral $Zn(His)_3(Asp)$ coordination environment that involves amino acid side chains from three protein building blocks. Thus, this supramolecular structure is assembled with little or no thermodynamic bias from specific protein–protein contacts.

The results of Tezcan and co-workers certainly demonstrate that protein building blocks with noninteracting surfaces can be assembled into superstructures through metal coordination. The choice of metal ions to drive complex formation and the choice of the ligating site in the protein are critical for this approach to be successful and truly live up to the versatility that it promises for the generation of a large number of protein complexes with well-defined architectures. It is hoped that this approach in combination with detailed bioinformatics studies will prove a powerful tool for the investigation of protein–protein interactions in a series of related and well-characterized protein complexes.

Published online: July 24, 2008

[1] a) D. H. Busch, *Chem. Rev.* **1993**, *93*, 847 – 860; b) W. F. De Grado, Z. R. Wasserman, J. D. Lear, *Science* **1989**, *243*, 622 – 628; c) E. T. Kaiser, *Acc. Chem. Res.* **1989**, *22*, 47 – 54; d) T. Sasaki, E. T. Kaiser, *J. Am. Chem. Soc.* **1989**, *111*, 380 – 381; e) O. Schueler-Furman, C. Wang, P. Bradley, K. Misura, D. Baker, *Science* **2005**, *310*, 638 – 642; f) C. M. Goodman, S. Choi, S. Shandler, W. F. DeGrado, *Nat. Chem. Biol.* **2007**, *3*, 252 – 262.

[2] a) M. Costas, M. P. Mehn, M. P. Jensen, L. Que, Jr., *Chem. Rev.* **2004**, *104*, 939 – 986; b) J.-U. Rohde, M. R. Bulowski, L. Que, Jr., *Curr. Opin. Chem. Biol.* **2003**, *7*, 674 – 682; c) K. S. Akerfeldt, J. D. Lear, Z. R. Wasserman, L. A. Chung, W. F. DeGrado, *Acc. Chem. Res.* **1993**, *26*, 191 – 197; d) K. W. Hahn, W. A. Klis, J. M. Stewart, *Science* **1990**, *248*, 1544 – 1547; e) A. Grove, M. Mutter, J. E. Rivier, M. Montal, *J. Am. Chem. Soc.* **1993**, *115*, 5919 – 5924; f) M. R. Ghadiri, J. R. Granja, L. K. Buehler, *Nature* **1994**, *369*, 301 – 304.

[3] a) M. Mutter, S. Vuilleumier, *Angew. Chem.* **1989**, *101*, 551 – 571; *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 535 – 554; b) M. Mutter, G. G. Tuchscherer, C. Miller, K.-H. Altman, R. I. Carey, D. F. Wyss, A. M. Labhardt, J. E. Rivier, *J. Am. Chem. Soc.* **1992**, *114*, 1463 – 1470; c) R. Mimna, M.-S. Camus, A. Schmid, G. Tuchscherer, H. A. Lashuel, M. Mutter, *Angew. Chem.* **2007**, *119*, 2735 – 2738; *Angew. Chem. Int. Ed.* **2007**, *46*, 2681 – 2684; d) H.-B. Kraatz, *Angew. Chem.* **1994**, *106*, 2143 – 2144; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 2055 – 2056; e) S. K. Dey, H.-B. Kraatz, *Bioconjugate Chem.* **2006**, *17*, 84 – 89; f) S. Chowdhury, G. Schatte, H.-B. Kraatz, *Angew. Chem.* **2006**, *118*, 7036 – 7038; *Angew. Chem. Int. Ed.* **2006**, *45*, 6882 – 6884; g) S. Chowdhury, D. A. R. Sanders, G. Schatte, H.-B. Kraatz, *Angew. Chem.* **2006**, *118*, 765 – 768; *Angew. Chem. Int. Ed.* **2006**, *45*, 751 – 754.

[4] a) M. R. Ghadiri, M. A. Case, *Angew. Chem.* **1993**, *105*, 1663 – 1670; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1594 – 1597; b) M. V. Tsurkan, M. Y. Ogawa, *Inorg. Chem.* **2007**, *46*, 6849 – 6851; c) J. Hong, O. A. Kharenko, M. Y. Ogawa, *Inorg. Chem.* **2006**, *45*, 9974 – 9984; d) O. A. Kharenko, M. Y. Ogawa, *J. Inorg. Biochem.* **2004**, *98*, 1971 – 1974; e) M. V. Tsurkan, M. Y. Ogawa, *Chem. Commun.* **2004**, 2092 – 2093; f) L. Liu, J. Hong, M. Y. Ogawa, *J. Am. Chem. Soc.* **2004**, *126*, 50 – 51; g) A. Fedorova, A. Chaudhari, M. Y. Ogawa, *J. Am. Chem. Soc.* **2003**, *125*, 357 – 362; h) A. Fedorova, M. Y. Ogawa, *Bioconjugate Chem.* **2002**, *13*, 150 – 154; i) M. V. Tsurkan, M. Y. Ogawa, *Biomacromolecules* **2007**, *8*, 3908 – 3913.

[5] a) V. Collura, G. Biossy, *Subcell. Biochem.* **2007**, *43*, 135 – 183; b) M. E. Cusick, N. Klitgord, M. Vidal, D. E. Hill, *Hum. Mol. Genet.* **2005**, *14*, R171 – R181; c) E. Ho, R. Webber, M. R. Wilkins, *J. Proteome Res.* **2008**, *7*, 104 – 112.

[6] a) C. L. Vizcarra, S. L. Mayo, *Curr. Opin. Chem. Biol.* **2005**, *9*, 622 – 626; b) T. Kortemme, D. Baker, *Curr. Opin. Chem. Biol.* **2004**, *8*, 91 – 97; c) R. J. P. van Berlo, L. F. A. Wessels, D. de Ridder, M. J. T. Reinders, *J. Bioinf. Comput. Biol.* **2007**, *5*, 839 – 864.

[7] E. N. Saldago, J. Faraone-Mennella, F. A. Tezcan, *J. Am. Chem. Soc.* **2007**, *129*, 13374 – 13375.

[8] F. S. Mathews, P. H. Bethge, E. W. Czerwinski, *J. Biol. Chem.* **1979**, *254*, 1699 – 1706.

[9] K. Hamada, P. H. Bethge, F. S. Mathews, *J. Mol. Biol.* **1995**, *247*, 947 – 962.

[10] F. Arnesano, L. Banci, I. Bertini, J. Faraone-Mennella, A. Rosato, *Biochemistry* **1999**, *38*, 8657 – 8670.

[11] J. Faraone-Mennella, F. A. Tezcan, H. B. Gray, J. R. Winkler, *Biochemistry* **2006**, *45*, 10504-10511.

