MS04.14.02  

**DE NOVO DESIGN OF \( \alpha \)-HELICAL PROTEINS: BASIC RESEARCH TO MEDICAL APPLICATION.**


The two-stranded \( \alpha \)-helical coiled-coil is not only an ideal model protein system to determine quantitatively the relative contributions that all the noncovalent interactions have in controlling protein conformation, folding and stability but is nature's way of creating a rod-like molecule for structural and regulatory roles. This universal dimerization motif has been found in a diverse group of over 200 proteins (muscle proteins, DNA binding proteins, viral proteins, enzymes and receptor proteins). We have shown that the minimum polypeptide chain length of coiled-coils is 3-heptads when maximally stabilized by hydrophobic and intra- and interchain electrostatic interactions. By controlling electrostatic interactions we have been able to design peptides which will not form homodimers (exist as random coils) but can form extremely stable heterodimers (K<sub>d</sub> of ~ 1 nM). Our knowledge base has allowed us to use this heterodimerization system for a series of medical applications:

1. as synthetic vaccine delivery vehicles;
2. as a two-stage targeting and drug delivery system;
3. as a dimerization motif for antibody reductants and receptor domains;
4. as a cloning/expression/detection and purification system for peptides and proteins;
5. as a dimerization domain for biosensor applications.

In addition, we have utilized a small interchain disulfide bridged and intrachain lactam bridged homodimeric coiled-coil as a template for displaying combinatorial peptide libraries in a helical conformation. Lastly, the most difficult de novo design challenge is the design of nonhomodimerizing inhibitor molecules of coiled-coil function (both homo- and heterodimers). We have been able to control parallel and antiparallel association of polypeptide chains into either two-stranded or 4-stranded coiled-coils. These designs lay the ground-work for the next generation of pH, salt and metal-ion induced folding of proteins with unique biological activities.

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MS04.14.03  

**THE DESIGN OF PROTEINS WITH NOVEL STRUCTURES AND ACTIVITIES.**

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The research the I will discuss falls into three broad areas: helix-helix packing, the stabilization and design of beta sheet structure and the design and characterization of novel metal-binding sites in proteins.

Our work on helix-helix packing seeks to systematically vary the inter-helical residues in the four helix bundle protein, Rop. We aim to determine how structure and stability change as we repack the hydrophobic core of the protein. Of particular interest is to achieve an understanding of the "non-native" behavior that results from certain repacked cores. Similarly, our work on inter-helical loop connections seeks to determine how the structure and properties of a protein change as the length and topology of inter-helical loop connections is varied.

Our work on beta sheets seeks to understand both the intrinsic and pair-wise contributions of the amino acids to beta sheet stability.

Finally, we are involved in the design and characterization of novel metal binding sites in proteins. The focus of our studies is the design of tetrahedrally coordinated Zn(II) sites. To date we have introduced several such sites onto small protein frameworks.

Our current research involves a detailed characterization of these sites to achieve a better understanding of what constitutes a successful design and the modification of existing designs such that they have the potential to be catalytically active.

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MS04.14.04  

**TOWARDS AN UNDERSTANDING OF \( \beta \)-SHEET STRUCTURE.**

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The design, synthesis and characterization of several beta-sheet based peptidomimetics will be outlined, focusing on the goal of understanding the folding and stability of beta-sheet structure. Our current understanding of both intramolecular and intermolecular beta-sheet formation will be discussed. The ability to promote folding in small peptides, which typically adopt an ensemble of conformations in aqueous solution, is important for understanding the early intermediates in protein folding and has practical applications in medicinal chemistry and materials science which will be outlined.

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MS04.14.05  

**TWO STRATEGIES FOR THERMOSTABILIZATION IN 3-ISOPROPYLMALATE DEHYDROGENASE.**

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Several factors have been proposed to account for the thermostability of proteins. For understanding the availabilities to thermal environments, it is necessary to reveal protein structures with intermediate properties between thermolabile and thermostable proteins. We have determined a crystal structure of 3-isopropylmalate dehydrogenase from the moderate facultative thermophile Bacillus coagulans (BcPMDH) at 3.0 Å resolution with an R-factor of 0.185. A comparison of the structure with that of the enzyme from the extreme thermophile Thermus thermophilus (TtPMDH) shows remarkable differences in the four loop regions though their overall tertiary structures are similar. These loops in BcPMDH have structural redundancies in length of amino acid sequence, flexibility of residues and architecture of the tertiary structure, which seem to be unnecessary to retain the active site. Such redundancies are also found in the enzyme of the mesophile Bacillus subtilis, but those parts are more stabilized in BcPMDH by hydrogen bonds and ion pairs. On the other hand, TtPMDH is different in structural economy so that redundant parts are reduced to adapt at higher temperature. This contrast suggests the two different strategies depending on temperature. One is "reinforcement of structure", which has high enthalpic effects and results in minor changes of thermostabilization. The other is "reduction of structural redundancy", which is required when the former strategy is not sufficient to stabilize the structure at higher temperature.

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MS04.14.06  

**CRYSTAL STRUCTURE OF AN ARTIFICIAL 4-HELIX BUNDLE PROTEIN.**

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A 108 residue artificial protein has been designed, expressed, characterized, crystallized and its three dimensional structure has been solved to 2.9 Å resolution. The protein was designed by connecting four identical \( \alpha \) helices together with short glycine loops. The component helices are modeled after amphipathic helices which interact with each other through flat hydrophobic faces. This 4-helix bundle is the first crystal structure to reveal an ordered, entirely de novo designed protein motif. This motif offers a template for the design of functionality into a 4-helix bundle protein.