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Final Defense

DE NOVO HEME PROTEIN DESIGN

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Understanding how protein folds, how they function and how to design them is a basic and important scientific endeavor. Imagine the diversity of millions of species on our earth, the complexity of a cell, the intelligence of human beings and the beauty of a flower, the fact that proteins, the fundamentally building blocks of life, are composed of only 20 amino acids with different sequences and lengths is most intriguing. Although the recent success of the genome project has led us to a new level to understand life in a systematic way, we still can not predict protein structure from its sequence, nor can we predict how a protein motions in response to its environmental change.

One fundamental approach to understanding protein folding and function is to design them from scratch, since the most rigorous way to test our understanding of protein is to put these principles together and see if we can design a well-folded and functional protein. Heme is arguably the most versatile prosthetic group in proteins, which serves the function of oxygen transfer, electron transfer, and oxygen activation, et al. How the protein scaffold fine-tunes the heme prosthetic group to achieve such diversity of functions is still not well understood, and designing minimal heme peptide complex can often provide substantial insight into these issues.

Our first step is to construct a library of 15-mer peptides with His ligand in the middle, such that when a peptides folds into ideal α helix, the His ligand coordinates to heme and its hydrophobic residues interact favorably with the hydrophobic heme plane.

while hydrophilic residues such as Glu or Lys point away from the heme, providing solubility. We observed that heme is able to induce sizable α helix formation (30-40%) for these peptides, and in subsequent 2D NMR study, we found that the α helix is well defined in the center, while the structure is dynamic at the terminus. In subsequent experiments, we also found that unnatural solvent such as trifluoroethanol or surface salt bridges can stabilize the helix.

To design a more "native-like" system, we choose to add Cys residues to our 15-mers, thus making parallel tweezers or cycle, depending on the number of disulfide bridges formed. The cyclic peptide Co^{III} porphyrin complex is not only nearly 90% helical and displays well-defined NMR structure, its also unfolds cooperatively.

We have learned that we can design a native-like protein from first principles, thus demonstrating validity of these principles and our design themes. We have also discovered that the helix is tilted, thus only three residues interact with the heme. By making those residues hydrophobic and the rest hydrophilic, we were able to design a more stable protein.

We have also developed an interesting hypothesis for Olfactory Receptor (OR) mechanism. Inorganic chemists know as a rule of thumb that if a volatile molecule is a good metal ligand, it probably smells strongly. We are so sensitive to thiols and amines that the most natural way to explain this is that OR is a metalloprotein. ORs belong to the superfamily of G-Protein Coupled Receptors (GPCRs), which are believed to be 7-helix trans-membrane proteins. We have found a consensus sequence "HXXCE" in the 4-5 loop of ORs, which not only binds strongly to Cu^{2+} and Zn^{2+} , but also turns α helical after metal binding. Since the 4-5 loop is as hydrophobic as the fourth helix of OR,

charge neutralization of metal ion increases its hydrophobicity, thus it might turn into transmembrane helix and replace the fourth helix. Endogenous ligand binding to the metal site might disturb the charge balance and triggers helix motion, which results in a cell signaling cascade, and eventually the sense of smell.