SINGLE CRYSTAL X-RAY DIFFRACTION

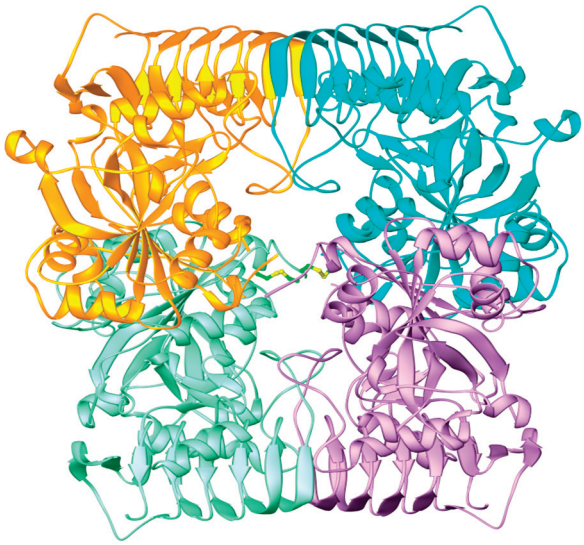
techniques are the major focus of our research to elucidate the structures of biologically important proteins and protein/nucleic acid assemblies. These high-resolution structural "snap shots" reveal a wealth of information regarding the biology, mechanism and chemistry of these biological molecules and assemblies.

Structural Studies of Components of the TFIIIB Complex. RNA pol III transcription initiation requires TFIIIB which consists of three polypeptides, TBP, BRF and a 90 kilodalton polypeptide B″. We are currently attempting to determine the TFIIIB/DNA complex structure at atomic resolution. The structure will detail the interactions between each of the proteins in the complex and elaborate the complex interaction of the complex with DNA, propelling our understanding of the molecular details of Pol III transcription initiation.

SNAPc is a five protein complex required for the initiation of all snRNA genes by both RNA Pol II and Pol III. It is one of the few factors that is involved in both Pol II and Pol III initiation and is therefore a key target for understanding the similarities and differences between these two systems. We have developed a co-expression strategy that allows us to co-express and purify this complex to high levels in an active form. We are in the process of crystallizing and determining the structure of this complex. This will be one of the largest protein complexes involved in transcription to be structurally characterized at atomic resolution. We have also determined the structure of the Oct-1/DNA/SNAP-190 peptide complex, the first structure of a transcriptional activator interacting with a partner in the basal transcriptional machinery.

Structure and mechanism of enzymes. The conversion of glucose 6-phosphate to 1L-myo-inositol 1-phosphate (MIP) by 1L-myo-inositol 1-phosphate synthase (MIP synthase) is the first committed and rate limiting step in the *de novo* biosynthesis of inositol in all eukaryotes. Inhibition of MIP synthase is important in the treatment of both seizure disorder and manic depression. We have determined the structure of MIP synthase both alone and bound to an inhibitor. These structures reveal that an unprecedented refolding event is required for substrate binding involving 20-60 amino acids. The newly folded domain completely encapsulates the inhibitor/substrate indicating that a folding transition is necessary for binding.

More recently we have determined the structures of two of the three enzymes in the starch biosynthetic pathway. Our most recent structure is of ADP-glucose pyrophosphorylase, an allosteric enzyme that regulates the entire pathway. From this structure we are obtaining a detailed, molecular understanding of how this enzyme is regulated by activators and inhibitors. Our eventual goal is to use this information to redesign the enzyme to be more active, potentially increasing the starch content in cash crops.



Potato tuber ADP-glucose pyrophosphorylase.

Structure and mechanism of blood proteins and angiogenesis inhibitors. The blood coagulation pathway is a central target for pharmaceuticals aimed at clot prevention, stroke, heart disease etc. We are working on several structures of blood protein/inhibitor complexes to use rational drug design to produce better drugs to treat these conditions. We have also recently determined the structure of the anti-angiogenesis factor Angiostatin. Angiogenesis inhibitors have attracted recent interest as potential antitumor agents since tumors require angiogenesis for growth and metastasis. Angiostatin is currently in phase two clinical trials. We have also determined the structure of the angiostatin/PAM complex, the first structure of ligand-bound angiostatin.

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Ext. 234

Selected Publications

The Crystal Structure of the Potato ADP-glucose Pyrophosphorylase, Jin, X. Ballicora, M., Preiss, J. and Geiger, J. H., *EMBO J.* **2005**, *in press.*

Geiger, J. H., Jin, X., *The Structure and Mechanism of Myo-inositol-1-phosphate Synthase*, in *Subcellular Biochemistry: Biology of Inositols and Phosphoinositides;* Ed. Majumder, A.; Plenum Press, NY, **2005**; Volume 39, *in press* (invited).

What the Structure of Angiostatin May Tell Us About its Mechanism of Action, Geiger, J. H., Cnudde, S. E., *J. Throm. and Haem.* **2004**, *2*, 23-24.

The Structure of the 1L-myo-inositol 1-phosphate Synthase/NAD+/2-deoxy-D-glucitol 6-(E)-vinylhomophosphonate Complex Demands a Revision of the Enzyme's Mechanism, Jin, X., Foley, K. M. and Geiger, J. H. Jin, X., Foley, K. M. & Geiger, J. H., *J. Biol. Chem.* **2003**, *279*, 13889-13895.

The X-ray Crystallographic Structure of Escherichia coli Branching Enzyme, Abad, M. C., Binderup, K., Rios-Steiner, J., Arni, R. K., Preiss, J. and Geiger, J. H., *J. Biol. Chem.* **2002**, *277*, 42164-42170.

The Crystal Structure and Mechanism of 1-L-myo-inositol-1-phosphate Synthase, Stein, A. J. and Geiger, J. H., *J. Biol. Chem.* **2002**, *277(11)*, 9484-9491.

Activator Recruitment by the General Transcription Machinery: X-ray Structural Analysis of the Oct-1 POU Domain/Human U1 Octamer/SNAP190 Peptide Ternary Complex, Hovde, S., Hinkley, C. S., Strong, K., Brooks, A., Gu, L., Henry, R. W., Geiger, J. H., *Genes and Development* **2002**, *16*, 2772-2777.