

# James H. Geiger

## Structural Biology Using X-ray Crystallography



### ASSOCIATE PROFESSOR

(b. 1962)

- B.S., 1984, New College of USF (Florida)
- Ph.D., 1991, Princeton Univ.
- American Cancer Society  
Postdoctoral Fellow 1994, Yale Univ.;
- Postdoctoral Fellow, 1996, Yale Univ.

517-355-9715 Ext. 234

The major focus of our research is to elucidate the structures of biologically important proteins, enzymes, enzyme/substrate, protein/ligand and protein/nucleic acid complexes. These high-resolution structural “snap shots” reveal a wealth of information regarding the biology, mechanism and chemistry of these biological molecules and assemblies. We then take these insights and verify them by mutagenesis and various assays. We are also involved in applying our structural insights in protein design applications.

***Eukaryotic Transcription:*** 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. 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:*** We have determined the structures of all three of the enzymes in the starch biosynthetic pathway, ADP-glucose pyrophosphorylase, Branching enzyme and glycogen/starch synthase. ADP-glucose pyrophosphorylase is an allosteric enzyme that regulates the entire pathway. From this structure we 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. Our structure of glycogen synthase showed for the first time that several glycogen binding sites exist outside the enzyme's active site. Though the function of these sites is not clear, they are important for the enzyme's activity and understanding their role is a focus of our future work. We have also identified seven glycan binding sites external to the active site of Branching enzyme. Our mutational work has demonstrated that several of these sites are also critical for the enzyme function and we are in the process of determining their role. In general, it appears that these enzymes that act on polymeric substrates often have external binding sites to orient and localize the enzymes to the polymers. In collaboration with the Walker lab, we have also investigated the structure, mechanism and specificity of some of the enzymes involved in Taxol biosynthesis, including phenylalanine aminomutase (PAM) and benzoic acid CoA ligase. The structures of 2 PAMs have been determined, and a variety of benzoic acid CoA ligase substrate structures have been determined for use in rationally extending the substrate specificity of these enzymes.

***A rhodopsin protein mimic:*** In collaboration with the Borhan lab, we have been involved in redesigning small cytosolic proteins to be rhodopsin mimics. We have redesigned cellular retinoic acid binding protein II and cellular retinol binding protein II to bind and form a protonated Schiff base with the retinal chromophore. Further, we have constructed a spectrum of protein pigments that bind the same chromophore retinal, but alter the absorbance of this chromophore over 219 nm. We have also developed new fluorescent proteins that can be used as fluorescent protein fusion tags, extending the range of fluorescent proteins and adding pH sensing to their repertoire.

***Plasmin and pathogen infection:*** The blood coagulation pathway is a central target for pharmaceuticals aimed at clot prevention, stroke, heart disease etc. We have focused our attention on plasminogen, the enzyme responsible for the dissolution of blood clots by cleavage of fibrinogen. Plasminogen is also involved in the infectivity of a number of bacterial pathogens including *streptococcus*, *staphylococcus*, bubonic plague, impetigo and others. These pathogens have cell surface plasminogen receptors and activators, allowing them to co-opt plasminogen's ability to degrade the body's extracellular matrix, allowing infection to spread. We determined the first structure of one of these receptors, Plasminogen-activating group A *streptococcus* M-like protein bound to a plasminogen fragment. Our goal is to use this and further structural information to develop novel antibiotics against these pathogens.<sup>1-7</sup>

## References:

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