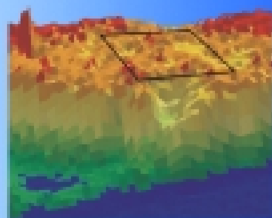
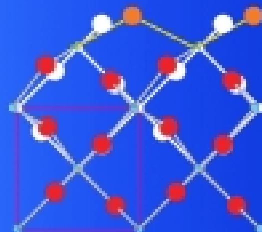
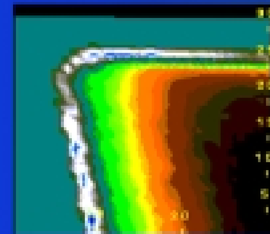
Workshop 2: Structural Biology for the New Millennium

Wayne Anderson and Amy Rosenzweig, organizers

Tuesday, October 9, 2001
8:30 am–Noon & 1:30–5:00 pm

Synchrotron radiation is central to recent efforts in structural biology, including large-scale structural genomics projects, time-resolved crystallography, and high-resolution structure determination. Speakers drawn from academic, industrial, and beamline settings will describe new developments in these areas, focusing on both technical and biologically relevant advances.

8:30–9:15 am — **Structural Genomics of *Haeomophilus influenzae*: Results and Approaches**
Andy Howard, *Illinois Institute of Technology*

9:15–10:00 am — **High-throughput Crystallography and Structure-based Drug Design**
Steven Muchmore, *Abbott Laboratories*

10:00–10:30 am — **Refreshments**

10:30–11:15 am — **Practical Aspects of High-throughput Structural Biology**
Tom Peat, *Structural GenomiX*

11:15 am–12:00 pm — **Diffuse Scattering: Methods for Molecular Structure and Dynamics**
Michael Wall, *Los Alamos National Laboratory*

12:00–1:30 pm — **Lunch**

1:30–2:15 pm — **Molecular Movies and Enzyme Reaction Coordinates**
Andrew Mesecar, *University of Illinois, Chicago*

2:15–3:00 pm — **Molecular Movie of the Photocycle of a Eubacterial Blue Light Receptor from Nanoseconds to Seconds**
Zhong Ren, *Renzresearch*

3:00–3:30 pm — **Refreshments**

3:30–4:15 pm — **High-resolution Structural Studies of the Signal Recognition Particle GTPase**
Doug Freymann, *Northwestern University Medical School*

Structural Genomics of *Haeomophilus influenzae*: Results and Approaches

Andy Howard, *Illinois Institute of Technology, Chicago, IL 60439 USA*

Haemophilus influenzae was the first bacterium for which a complete gene sequence became available, and as such represents a logical target for study by structural genomics. A group from the University of Maryland Biotechnology Institute (UMBI), in conjunction with researchers at the Institute for Genomic Research and Illinois Institute of Technology, have undertaken a broad-spectrum structural genomics effort using this organism. This mesophilic organism represents a typical, i.e. non-extreme, organism, and as such determination of structures from it may help answer the fundamental question: what is the minimal complement of proteins necessary for the functioning of a free-living organism. In the three years of the *Haemophilus* study, 65 proteins have been chosen as targets; all were initially of unknown function, were water-soluble, and had homologues in at least one other sequenced organism. UMBI, TIGR, and IIT researchers have used both x-ray diffraction and NMR spectroscopy in determining structures. Twenty-one structures are now complete, most of them obtained within the last year. Some familiar folds and some novel folds have been found; deducing function from structure has proven possible in a number of instances, particularly where the fold has turned out to be familiar.

High-throughput Crystallography and Structure-based Drug Design

Steven Muchmore, Clarissa Jakob, Ronald Jones, Jeff Olson, Eugene Maslana, Tom Nemcek, and Jeffrey Pan,
Abbott Laboratories, Chicago, IL 60064 USA

High-throughput crystallography has been developed and implemented in order to optimize crystallography-driven structure-based drug design. While much of the driving force behind the current practice of high throughput crystallography is due to large structural genomics efforts, optimizing structure-based drug design efforts also leads to many of the same considerations. Instead of a start to finish approach for automating the crystallography laboratory, our emphasis was placed on automating those aspects of the process which would immediately yield increased throughput. The first instrument constructed was a robot which mounts (and dismounts) a series of crystals on the data collection system, preserving the crystal at liquid nitrogen temperatures at all times. This robot then aligns the crystal in the x-ray beam automatically to permit unassisted data collection 24 hours/seven days a week. An automated data processing scheme was also implemented to process the large volume of crystallographic data being generated. Other robots have designed to facilitate soaking of compound mixtures into crystals to support the CystaLEAD™ process. This has resulted in increased throughput in data collection, and has placed additional demands on protein crystallization efforts. To address this strain, it was necessary to develop a robot to automatically scan and record crystallization experiments was developed, in addition to a crystallization robot designed to be able to meet the demands for high throughput crystallization on a laboratory scale. This robot can be utilized not only for screening, but also for custom crystallization experiments. Appropriate robotic control systems and laboratory database software have been created to support the vastly increased throughput.

Practical Aspects of High-throughput Structural Biology

Tom Peat, *Structural GenomiX, San Diego, CA 92121 USA*

A short introduction to the SGX process will be followed by a more detailed discussion of the SGX-CAT beamline design. This will cover some of the design parameters and some of the capabilities of this new beamline. This will be followed by some discussion on the software platform we use and the quality control measures we have put into place. This includes our processed diffraction data capture system and general aspects of our validation/deposition system. This system allows not only for automation but also scalability for the larger number of structures being solved each year.

Diffuse Scattering: Methods for Molecular Structure and Dynamics

Michael Wall, *Los Alamos National Laboratory, Los Alamos, NM 87545 USA*

Methods for measuring diffuse features in x-ray diffraction from protein crystals will be reviewed, including use of diffuse scattering to characterize protein dynamics. The potential for integrating diffuse scattering into structure determination will be discussed. It will be argued that use of all of the diffraction data, and not just the Bragg peaks, would improve experimental models.

Molecular Movies and Enzyme Reaction Coordinates

Andrew Mesecar, *University of Illinois at Chicago, Chicago, IL 60612 USA*

One of the remaining and great unsolved questions in chemistry and biology from the twentieth century is the nature of enzyme catalysis—How do enzymes accelerate the rates of chemical reactions over 10^{17} times faster than their corresponding uncatalyzed reactions, and how do they achieve such high degrees of substrate specificity? The central hypothesis of our laboratory is that enzymes achieve these remarkable properties, not by some unconventional enzy"magical" property, but by combining general physical-chemical and structural properties with dynamic processes. The focus of this presentation is on the role of protein conformational changes in positioning the substrates into proper alignment for catalysis to take place, and in discriminating between potential substrates. Although an enormous body of data on enzyme structure and catalysis exists, there are relatively few examples of studies that directly and experimentally quantitate the physical-chemical and dynamic factors that contribute to the catalytic power and specificity of enzymes. Therefore, we are currently investigating in detail, the kinetic, chemical and structural mechanisms of two model enzyme systems—isocitrate dehydrogenase and trihydroxytoluene dioxygenase, in order to quantitate the contribution of the individual physical-chemical, structural and dynamic properties of these enzymes to their specificity and catalytic power. We are attempting to construct atomic level "Molecular Movies" of these enzymes as they carry out their catalytic reaction cycles. Because of the complex nature of enzymatic catalysis, a variety of techniques from the fields of chemistry, biology and physics, such as steady-state and time-resolved x-ray crystallography, small-angle x-ray scattering, enzyme kinetics, isotope effects, synthetic organic chemistry, bioinformatics, computational chemistry, and molecular biology, are being utilized. We are using a multi-faceted, multi-disciplinary and integrated approach to the elucidation of the nature of enzymatic catalysis, and deem that such an approach is not only necessary, but is also becoming feasible with the advent of emerging synchrotron capabilities in this New Millennium.

Molecular Movie of the Photocycle of a Eubacterial Blue-light Receptor from Nanoseconds to Seconds

Zhong Ren, *Renzresearch*

Laue crystallography recently offers new opportunity for monitoring the structural changes in a biochemical reaction at ambient temperature, which may be an attractive alternative in addition to the chemical and cryogenic trapping techniques in studying reaction pathways. The photocycle of the bacterial blue-light photoreceptor, photoactive yellow protein, was stimulated by illumination of single crystals by a seven nanosecond laser pulse. The molecular events were recorded at high resolution by time-resolved x-ray Laue diffraction as they evolved in real time, from one nanosecond to seconds after the laser pulse. The complex structural changes during the photocycle at ambient temperature are displayed in a movie of difference electron density maps relative to the dark state. The step critical to entry into the photocycle is identified as flipping of the carbonyl group of the 4-hydroxycinnamic acid chromophore into an adjacent, hydrophobic environment rather than the concomitant isomerization about the double bond of the chromophore tail. The structural perturbation generated at the chromophore propagates throughout the entire protein as a light-induced "protein quake" with its "epicenter" at the carbonyl moiety of the chromophore. Combining the

molecular movie with a series of hypotheses on the kinetic mechanism model of the photocycle, a computational approach is proposed to identify the correct kinetic model and to deconvolute the intermediate structures involved in the photocycle.

High-resolution Structural Studies of the Signal Recognition Particle GTPase

Doug Freymann, *Northwestern University Medical School, Chicago, IL 60611 USA*

The prokaryotic SRP GTPase, Ffh, mediates interaction between the signal recognition particle (SRP) and its membrane receptor during co-translational protein targeting. Ffh comprises two functional domains—the 'M' domain that mediates signal sequence recognition and the 'NG' GTPase domain. Our goal is to use high-resolution x-ray crystallography to understand the structural logic of the GTPase as it undergoes interaction with signal sequence, GTP, and receptor. The recent structure of the GMPPNP-complex of NG reveals an unexpected binding mode for the nucleotide analog that suggests entry of GTP into its 'active' conformation is regulated, perhaps by interaction with receptor. The crystal structure of the apo NG domain refined at 1.1 Å resolution reveals significant anisotropic disorder of the N domain. Analysis of packing interactions at the NG interface indicates a region of low packing density that facilitates a functionally important interdomain motion. These structures provide insight into how the SRP GTPases are activated by a mechanism distinct from other GTPases, and support the notion that an 'empty' conformation is functional during the SRP GTPase cycle.