

The W.M. Keck Foundation Center for Molecular Structure: A Core Facility of CSUPERB and Core Node of the StARBURSTT-CyberDiffraction Consortium

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Abstract

Background: The W.M. Keck Foundation Center for Molecular Structure (CMoIS), a core facility of CSUPERB, is the first comprehensive X-ray crystallographic and computational facility located at a predominantly undergraduate institution. CMoIS is also the west coast "core node" of the "Science Teaching and Research Brings Undergraduate Research Strengths Through Technology Cyber Diffraction Consortium" (StARBURSTT-CDC). As a "collaboratory" and a "virtual laboratory", CMoIS provides faculty and student investigators throughout the 23-campus California State University, regional colleges and universities, and the United States with local and remote access to instrumentation, software and databases for research and training involving both small and macromolecular structure. Furthermore, CMoIS offers experiments and tutorials for the undergraduate curriculum, as well as annual professional development workshops for undergraduate faculty, covering small molecule crystallography, macromolecular crystallography, molecular modeling and simulation, and structure-guided drug design. These 12 national workshops are among a variety of courses offered by the Center for Workshops in the Chemical Sciences, a consortium of 24 universities funded by the National Science Foundation.

Methods: Practically all information about the molecular structure of matter at atomic resolution is the result of crystallographic analysis. Diffraction methods have contributed to our fundamental understanding of chemical bonds, chemical reactions and biochemical pathways, the composition and properties of minerals and ceramics, and to the design of material properties, pharmaceuticals, engineered crystals and engineered enzymes. Many contexts exist in which crystallography can be introduced in undergraduate research and education, and formal courses in crystallography should be available to senior undergraduates and graduate students. Professional development beyond the degree is also a necessary aspect of crystallography training, particularly in areas where crystallography is increasingly being outsourced abroad. Maintaining the vitality of crystallography is important to university departments advancing science. Education and training today will contribute to the production of a successful workforce that will assist the nation to prosper in a world of global economic competition.

Results: This poster describes CMoIS' research, training and remote access capabilities, as well as its contributions to curriculum development, including workshops, short courses and undergraduate laboratory experiments. Recent system-wide research projects are also highlighted.

Conclusions: Through its networks and partnerships, CMoIS has enhanced the research and educational infrastructure of the CSU, and students have benefited from the collaborative aspects of molecular science. CMoIS has given the scientific community "a whole new way to think about our science (crystallography)". - Carole Brook, Editor of *Acta Crystallogr. B*.

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Core Facilities

Crystallographic Instrumentation

18kW rotating anode, Cu source, fine focus
HI-STAR multiwire detector
Scintillation detector
Low Temperature LN2 device
Sealed tube Mo source, normal focus
SMART CCD detector
Brookhaven National Laboratory *Fedex crystallography
Stanford Synchrotron Radiation Laboratory *Fedex crystallography



Biophysical Instrumentation

Circular Dichroism Spectropolarimeter
Spectrofluorometer
Static and dynamic light scattering
Crystallization Screening



StARBURSTT CyberDiffraction Consortium

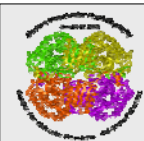
Science Teaching and Research Bring Undergraduate Research Strengths Through Technology
The StARBURSTT-CDC (<http://www.starburstt.org>) grew out of five pre-existing regional instrumentation consortia, the first being CMoIS. StARBURSTT's goals are directed towards systematically and significantly changing the research and educational cultures at Predominately Undergraduate Institutions, PUIs, with the value added benefits flowing from this change to our "customers" (the nation's major research universities, R&D and production organizations) through the currency of our students. The project has a series of interlinked and naturally synergistic components that will facilitate a major and simultaneous increase in the depth of undergraduate research and educational experiences. StARBURSTT members are Predominantly Undergraduate Institutions, PUIs, Community Colleges, CSs, Historically Black Colleges and Universities, HBCUs, Hispanic Serving Institutions, HSIs, and Tribal Colleges, TCS. We also collaborate closely with a range of affiliate members such as PhD granting Universities, Government Labs, Non-Profit Organizations, and Companies.

StARBURSTT-CDC



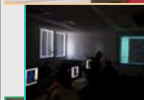
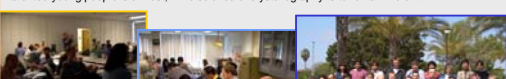
Competitive research and advanced education both require access to a wide range of sophisticated instrumentation. Remote access instruments located at "collaboratories" like those of the StARBURSTT-CDC provide an essential suite of leading-edge capabilities needed for a particular kind of research or education, giving faculty and students the ability to design and execute experiments remotely and automatically over the Internet. This distributes resources more effectively across the scientific community and manages costs by optimizing travel, equipment use and information value. If researchers and instructors can perform an experiment "in their own backyard", remotely and automatically, while at their desk or the lab bench with students, the value added benefit is that more students are exposed to "real" science, leading-edge instrumentation, and good mentors. The leaders of StARBURSTT have successfully demonstrated implementation of remote access to diffraction instrumentation and crystallography education tools, and we are taking the necessary steps to make single crystal diffraction a core tool in undergraduate research.

Workshops



Practically all information about the molecular structure of matter at atomic resolution is the result of crystallographic analysis. Substantial advancements in crystallographic techniques made over the last 25 years allow individuals with quite diverse background and preparation, and sometimes little training, to use crystallography as a tool to address a specific hypothesis-driven structural problem. Ironically, as a result of methodological advances, crystallography as a science as been misunderstood in recent years, sometimes thought of as too easy or irrelevant beyond the solid state.

Crystallography continues to make major contributions to the pharmaceutical industry as part of the process of rational drug design, to the field of synthetic chemistry by facilitating synthetic processes, to materials science in relating structure to function, and to medicine by identifying on the molecular level, structural features that play key roles in disease processes. The need for skilled crystallographers has never been greater, and teaching crystallography in a way that attracts the most talented young people is a must, if the science of crystallography is to remain vibrant.



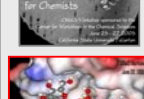
Modern Biomolecular Crystallography is a five-day workshop that familiarizes faculty with the process of macromolecular structure determination by single crystal x-ray diffraction. MBC discusses theory and methods, and it provides participants hands-on experience in protein production and crystallization, structure solution and refinement, interpretation and validation of protein crystal structures, as well as aspects of structural bioinformatics such as targeting, *in silico* modeling and mutagenesis, and structure-guided drug design. Remote access to instrumentation is also demonstrated. Participants will gain an understanding of crystallography terminology commonly used in publications, and they will learn what is required to successfully undertake, complete and publish a structure determination. Experiments developed for the undergraduate curriculum will be described and disseminated.



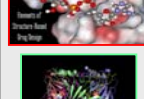
Crystallography for Chemists (Crystallography and others!) is a five-day workshop that familiarizes faculty with modern instrumentation and software commonly utilized for both small and macromolecular crystallography. This workshop discusses theory and methods, and it provides participants hands-on experience in structure solution and refinement, as well as interpretation and critical assessment of crystal structure analysis. Participants will gain an understanding of crystallography terminology commonly used in publications, and they will learn what is required to successfully undertake, complete and publish a structure determination. Experiments developed for the undergraduate curriculum and remote access to instrumentation will also be described.



Elements of Structure-Based Drug Design is a one-day workshop for CSU faculty that highlights the tools and difficulties related to informatics-driven drug discovery. This workshop discusses the fundamentals of rational drug design based upon structure determination and analysis, and assessment of the "druggability" of a compound. Computational approaches, including virtual screening and comparative modeling are also described. Participants work through actual case studies of rational design of enzyme inhibitors.



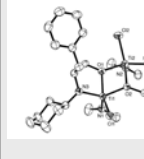
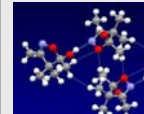
Molecular Modeling - Visualizing Structure-Function Relationships is a half-day workshop for secondary science teachers that describes the fundamentals of structure determination and the importance of structure in explaining function at the atomic level. Participants learn about structure or coordinate files, the information within them, what databases contain small molecule and macromolecule coordinate files, and how to obtain these files. This workshop provides hands-on experience with free software that teachers can use to visualize molecules and communicate chemistry in their classroom.



Chemical Crystallography

Structure-Reactivity Studies on Alkyl-β-Monooximes. Oximes have positive implications in medicine as a treatment for poisoning from neurotoxins and nerve agents, such as organophosphorous compounds. There are potential side effects via enzymatic oxidation of oximes and formation of the reactive intermediates (radical ions), which cause cellular damage leading to cancer, aging, and a variety of diseases. To understand the reactivity of oximes in biochemical processes and improve their potential use in medicine, students in Peter de Lijser's laboratory at CSUF are investigating the structure-reactivity effects of substituted oximes. (de Lijser et al, *J Chem Cryst* 34(2): 103-110, 2004)

Studies on Diastereomeric Titanium Complexes with Amino Acid Derived N- and O-π Donor Ligands.
The organometallic chemistry of the early transition metals is dominated by complexes containing the cyclopentadienyl group and its derivatives. There has been a growing interest in alternative donor ligands such as amides, alkoxides, aryloxides, sulfonamides and amidates, because the dramatically different steric and electronic environments they provide can result in novel reactivity of the resulting complexes. Titanium complexes with amide and alkoxide ligands are known to carry out carbon-carbon bond formations, and chiral titanium complexes with amino alcohol ancillary ligands would be expected to carry out stereoselective reactions. Diastereomeric complexes of the type $Ti(O)(NMe_2)_2(OCH_2CH(CH_2Ph)(N(R)_2))_2$ were synthesized in Adam Johnson's laboratory at Harvey Mudd College, by protonolysis of $TiCl_4(NMe_2)_2$ with the corresponding N-substituted amino alcohols. The complexes bridge through the amino alcohol oxygen atoms and contain terminal chlorides. The dimeric nature of the complexes was established at CMoIS by X-ray crystallography (Ho et al, *Inorg Chim Acta*, 431, 71-76, 2002). HMC and CMoIS have now entered into a formal collaboration engaging HMC undergraduate research students.

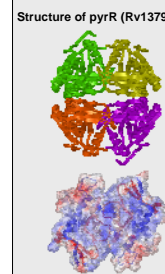


Biomolecular Crystallography

Structure determination and analysis of lactate dehydrogenase from *Gallus* spp. (see poster by L. Grant et al.) at CSU Fullerton, the majority of the upper division biochemistry laboratory is devoted to the study of lactate dehydrogenase (LDH), an essential enzyme in carbohydrate metabolism, from chicken breast muscle. Although amino acid data and atomic coordinate structure information are available for orthologous homologs, the structure of LDH from chicken breast muscle had not yet been determined. Students have conducted a comprehensive structure determination and analysis using protein crystallography, a modern method of structural genomics. They have grown crystals of native protein and several ligand complexes, which are flash cooled and shipped to SSRL, where X-ray diffraction data are collected remotely. Research students in Katherine Kantardjjeff's laboratory at CSUF have completed the model building, refinement and analysis for native and pyruvate complexes of chicken muscle LDH. Results of these studies are providing new insights into the structural features of LDH that govern its kinetic and stability properties. The overall topology is consistent with crystal structures of orthologous homologs, although some secondary structure elements have shifted. Evolutionary alterations in the flexibility of LDH suggest that the active site is an "extended unit" involving most of the enzyme's structure, and substrate binding drives the flexible loop region to fold over the active site. Studies on LDH have been adopted in the biochemistry laboratory course at CSU Channel Islands by Blake Gillespie, who attended MBCI in June 2005.

Preliminary crystallographic studies on two Pyrococcal flavoproteins. The microorganisms in the genus *Pyrococcus* are strict anaerobes isolated from hydrothermal vents and having an optimal growth temperature of ~100 °C. In addition to their ability to survive at extremely high temperatures, these members of the kingdom Archaea have an array of metabolic adaptations which allow them to survive in their subsurface environment. They are only able to grow under extremely reducing conditions and are able to use either elemental sulfur (S⁰) or protons as final electron acceptors, producing hydrogen sulfide or hydrogen gas, respectively. The fact that they are heme-deficient makes them of special interest to a flavin biochemist, since they may be more dependent on flavin chemistry to catalyze their redox reactions. Studies on two Pyrococcal flavoproteins, NOX1 and CoADR, in the laboratory of E.J. Crane at Pomona College, hope to elucidate the role of these enzymes in the metabolism of *Pyrococcus*, and provide insights into how these microbes survive in their harsh environment. Undergraduate research student Cameron Blume has been working at CMoIS to screen for crystals suitable for x-ray diffraction experiments.

Structure of pyrR (Rv1379) from *Mycobacterium tuberculosis*: A persistence gene and protein drug target. The *Mycobacterium tuberculosis* pyrR gene (Rv1379) encodes a protein that regulates expression of pyrimidine nucleotide biosynthesis (pyr) genes in a UMP-dependent manner. Because pyrimidine biosynthesis is an essential step in the progression of TB, the gene product pyrR is an attractive antitubercular drug target. The 1.9 Å native structure of a tetrameric form of Mtb PyrR has been determined by x-ray diffraction (PDB ID: 1W30) in trigonal space group P3₁21 and two molecules in the asymmetric unit. The 3D structure and residual uracil phosphoribosyltransferase activity point to a common phosphoribosyl transferase ancestor for PyrR. However, while PRPP and UMP binding sites have been retained in Mtb PyrR, a distinct dimer interaction among subunits creates a deep, positively charged cleft capable of binding pyr mRNA. *In silico* screening of pyrimidine nucleoside analogs has revealed a number of potential leads compounds that, if bound to Mtb PyrR, could facilitate transcriptional attenuation, particularly cyclopentyl nucleosides. This work illustrates the validity and success of a structural genomic and structure-guided approach to discovering potential new antimycobacterial therapeutics. Undergraduates Carolina Vasquez and Peter Castro (CSUF), and high school intern Nancy Warfel (La Verne HS), contributed to this work. (Kantardjjeff et al, *Acta Crystallogr.* 2005, D(61): p. 355-364.)



Support