

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

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### Abstract

**Background:** The W.M. Keck Foundation Center for Molecular Structure (CMoS), a core facility of CSUPERB, is the first comprehensive X-ray crystallographic and computational facility located at a predominantly undergraduate institution. CMoS is also the west coast "core node" of the "Science Teaching and Research Brings Undergraduate Research Strengths Through Technology Cyber Diffraction Consortium" (StaBURSTT-CDC). As a "collaboratory" and a "virtual laboratory", CMoS 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, CMoS 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 national workshops are among a variety of courses offered by the Center for Workshops in the Chemical Sciences, a consortium of 12 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 should 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 CMoS' 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, CMoS has enhanced the research and educational infrastructure of the CSU, and students have benefited from the collaborative aspects of molecular science. CMoS 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 multiview detector
- Scintillation detector
- Sealed tube Mo source, normal focus
- SMART CCD detector
- Low temperature LN2 devices
- Brookhaven National Laboratory
- Stanford Synchrotron Radiation Laboratory



#### Biophysical Instrumentation

- Circular Dichroism Spectropolarimeter
- Spectrofluorometer
- Light scattering

#### Crystallization Screening

- Random screening of new proteins
- Optimization of existing conditions
- Cryoprotection/mounting
- Shipping to synchrotrons

### StaBURSTT CyberDiffraction Consortium

#### Science Teaching and Research Bring Undergraduate Research Strengths Through Technology

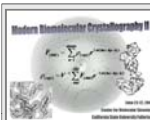
The StaBURSTT-CDC (<http://www.staburstt.org>) grew out of five pre-existing regional instrumentation consortia, the first being CMoS. StaBURSTT's goals are directed towards systematically and significantly changing the research and educational cultures at Predominately Undergraduate Institutions, PUIs, 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. StaBURSTT members are Predominately Undergraduate Institutions, PUIs, Community Colleges, CCs, 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.

### StaBURSTT-CDC



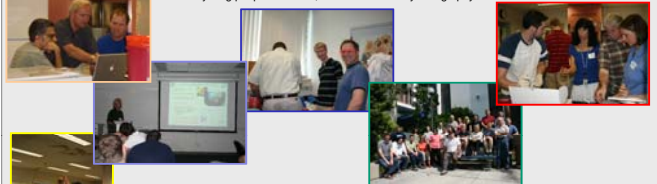
Competitive research and advanced education both require access to a wide range of sophisticated instrumentation. Remote access instruments located at "collaboratories" like those of the StaBURSTT-CDC provide an essential suite of leading-edge capabilities needed for a particular kind of research or 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 StaBURSTT 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 had 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. MBOC 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; 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 gain an understanding of crystallography terminology commonly used in publications, and they learn what is required to successfully undertake, complete and publish a structure determination. Experiments developed for the undergraduate curriculum are described and disseminated. **Our most recent workshop took place June 22-27, 2008 at CMoS (<http://chemistry.csu.edu/CWCS/>).**

**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.

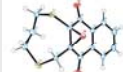
**Elements of Structure-Based Drug Design** is a one-day workshop for CSU faculty that highlights the tools and difficulties related to informatics-driven drug discovery. This workshop discusses the fundamentals of rational drug design based upon structure determination and analysis, and assessment of 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

#### We've never seen anything like this before! - Occidental College

This was one of the weird products that could not be explained. 2-bromo-3-methyl-1,4-naphthoquinone was reacted with propylenedithiol and triethylamine. It was known that the base was strong enough to deprotonate the hydrogen on the methyl group for Michael addition, so in a sense it was expected that the eight-membered ring might form. However, the epoxide was unexpected. The oxygen doesn't come from methanol, but from oxygen in air, so it was odd that the epoxide formed even in such mild conditions. Mystery solved by crystallography. (T. Lam, senior thesis in the laboratory of Tetsuo Otsuki)



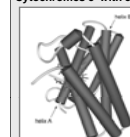
#### Chemistry 480T/168 - CSU Fullerton, Harvey Mudd College, Pomona College

- Amino alcohols are important ligands for asymmetric catalysis of many important reactions. The amino alcohol L-N-cyclohexyl-diphenylalinald was synthesized and crystallized from 7:1 ether:ethanol by slow evaporation. The structure revealed a hydrogen bond from the alcohol hydrogen to the nitrogen of the amine. (A. Hickman, J. Moritt)
- The title molecule (1S, 2R)-2-(N-mesitylsulfonylamino)-1-phenyl-1-chloropropane, C<sub>18</sub>H<sub>19</sub>ClNO<sub>2</sub>S, forms a triclinal crystal system, with two molecules per unit cell. The (+)-enantiomer conformation of the alkyl chloride intermediate C<sub>10</sub>H<sub>13</sub>ClNO<sub>2</sub>S for the synthesis of the Hulme auxiliary was confirmed. This alkyl chloride intermediate has a mesitylene group that is almost orthogonal to the phenyl ring. (J. Hines, K. Lo, G. Ruytermark)
- In the process of creating new ligands for ruthenium-based metathesis catalysts, we have synthesized 4,5-dichlorophthalic acid dimethyl ester from 4,5-dichloro phthalic acid. Though the molecule theoretically possesses two planes of symmetry, the crystal structure we have obtained reveals that one ester is rotated out of the plane of the aromatic ring. This molecule has also been used as a precursor for the synthesis of potential treatments for Alzheimer's disease. (D. Hickster)
- Half-cage isodrin was the first molecule in which the Nuclear Overhauser Effect was observed. The derivative half cage isodrin propionate was synthesized as previously described and crystallized in a 10:1 mixture of hot ethanol to dichloromethane. The structure of half cage isodrin propionate is reported. (S. Sobelman)



### Biomolecular Crystallography

#### Cytochromes c' with engineered properties - Katherine Kantardjief, CSU Fullerton

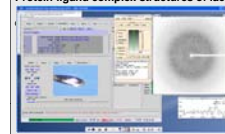


Detailed energetic and structural knowledge of biomolecular association is fundamental to understanding processes such as electron transfer, catalysis and gene regulation. Moreover, *de novo* design and redesign of metalloproteins, by revealing key aspects of folding and electrochemical function, can afford novel molecules with applications as biosensors and therapeutics. In these regards bacterial cytochromes c' (CPs) are intrinsically interesting and distinctly suited to provide new information. We aim to elucidate the sequence-dependent features of CPs that control folding dynamics, molecular assembly and reduction potential, using the high-resolution crystal structures of CP from *Rb. sphaeroides* 2.4.1 (RSCP) and *R. palustris* (RPPC) as model systems. Cytochromes c' (CPs) are periplasmic heme proteins found in metabolically diverse prokaryotes, including photosynthesizers, sulfur oxidizers and methylotrophs. These proteins represent the largest and most widespread class (IIa) of bacterial c-type cytochromes. Although CPs are alleged to function as electron carriers, increasing evidence supports an additional biochemical role as a nitric oxide carrier that protects bacterial cells against nitrosative stress. Several pathogens have genes encoding CP, suggesting that CP may protect pathogenic bacterial cells against attack by NO produced by the mammalian immune system.

CPs have highly conserved physicochemical and structural properties over a wide range of bacterial species, but extensive sequence homology is generally restricted to the carboxyl terminal region incorporating the heme binding sequence C-X-Y-C-H, similar to that of low-spin cytochromes c. CPs occur primarily as 28kD homodimers, with each polypeptide chain folded into an elongated right-handed four  $\alpha$ -helix bundle incorporating the covalently bound heme. In CPs, the hemes are axially coordinated by a single histidine ligand, resulting in normally five coordinate iron centers, paramagnetic in both the Fe<sup>3+</sup> (oxidized) and Fe<sup>2+</sup> (reduced) forms at neutral pH, and pH-dependent reduction potentials spanning -10 to +150mV. Monomers typically bind small ligands axially to the heme in both the oxidized and reduced forms, although recent crystallographic and spectroscopic studies indicate that exogenous ligands such as NO and CO exhibit a novel proximal coordination geometry, with the ligand residing at the previously occupied axial histidine binding site. Binding of exogenous ligands has been shown to bring about conformational changes leading to dissociation of dimers to monomers.

Our crystal structure of RSCP provides new insights into the structural determinants of dimer topology and heme redox activity. The disposition of charged groups plays a role in establishing an equilibrium mixture of monomer and dimer in solution, and heme iron reduction potential is controlled by the hydrophobic profile and hydrophobic packing of residues in the heme pocket. The specific aims for this project are to 1) complete crystal structure determinations of the reduced forms of native RSCP and RPPC; 2) use advanced protein design and docking algorithms at CSUF to investigate the chemical features that control RSCP dimer assembly (the RSCP and RPPC interface formed by helices A and B are being redesigned *in silico* to form fully monomeric CP forms, dimers of greater binding affinity than wild-type RSCP, and dimers with designed topology); 3) physically characterize native and recombinant proteins by electron magnetic resonance, circular dichroism spectrometry, light scattering, fluorescence spectroscopy, gel filtration and X-ray crystallography; and 4) assess the effects of mutants designed to modulate the reduction potential by standard spectroscopic methods and direct electrocatalysis using film-modified electrodes. Crystals of reduced cytochrome c' and co-crystals bound to various small ligands (such as NO and CO) are currently being analyzed.

#### Protein-ligand complex structures of lactate dehydrogenase - CSU Fullerton, CSU Channel Islands and SSRL



At CSUF and CSUIC, the majority of the upper division biochemistry laboratory is a 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 are conducting comprehensive structure determination and analysis using protein crystallography, a modern method of structural genomics. They have grown crystals of native protein and several protein-ligand complexes, which are flash cooled and shipped to SSRL, where X-ray diffraction data are collected remotely. Research students in Kantardjief's laboratory at CSUF have completed 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. 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.

#### Dialkylphenylphosphates as selective inhibitors of butyrylcholinesterase - CSU Fullerton and CSU Long Beach



Cholinesterase inhibitors have been used not only as chemical weapons, but also as a class of administered to slow the progression of Alzheimer's disease (AD). The severity of AD parallels reduction in levels of acetyl choline as well as activity, whereas activity of butyrylcholinesterase increased. Since both enzymes hydrolyze acetylcholine, the use of cholinesterase inhibitors as treatment of AD is based on the assumption that inhibiting the activity of these enzymes will increase the concentration of acetylcholine in the brain. Current treatment for AD involves use of reversible dual inhibitors to suppress activity of both enzymes. It has been suggested that the next generation of cholinesterase inhibitors to treat AD should include those that are selective for butyrylcholinesterase. Structurally and functionally, both enzymes are serine hydrolases belonging to the esterase family with higher eukaryotes, and sequence identity between human enzymes is 51%. The active site in each enzyme is located at the base of a deep -20Å, hydrophobic gorge. The microenvironment of the butyrylcholinesterase gorge, notably the larger volume (~200Å) and greater hydrophobicity, is exploited structurally to virtually screen Markovitch libraries of dialkylphosphates, phosphonates, phosphinates and phosphoramidates. Virtual screening is done by Kantardjief and students at CMoS, while synthesis is done in the laboratory of Kensaku Nakayama (CSULB) and enzymology is performed in the laboratory of Roger Acey (CSULB). (Law et al. Biochem Biophys Res Commun, 2007, 355: 371-378)

### Support