6th International Conference on BIOPHYSICS & SYNCHROTRON RADIATION

August 4-8, 1998
Argonne, Illinois USA

PROGRAM / ABSTRACTS

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, make any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

Program / Abstracts

Contents

International Scientific Advisory Committee

Joan Bordas, Spain
Carl-Ivar Brändèn, Sweden
Peter Colman, Australia
Steve P. Cramer, USA
Steve E. Ealick, USA
Roger Fourme, France
Britt Hedman, USA
John R. Helliwell, UK
Wayne A. Hendrickson, USA
Keith Hodgson, USA
Kenneth C. Holmes, Germany
Hugh E. Huxley, USA
Louise Johnson, UK
Janos Kirz, USA
Dong-cai Liang, PRC
Keith Moffat, USA
David E. Moncton, USA
Glaucius Oliva, Brazil
Michael G. Rossmann, USA
Noriyoshi Sakabe, Japan
Günter Schmahl, Germany
Janet Smith, USA
David I. Stuart, UK
William Thomlinson, USA
Jill Trewhella, USA
Tomitake Tsukihara, Japan

Local Organization and Program Committee

Keith Moffat (Chair), *The University of Chicago*

Cele Abad-Zapatero, *Abbott Laboratories*
Grant Bunker, *Illinois Institute of Technology*
Dean Chapman, *Illinois Institute of Technology*
Andy Howard, *Illinois Institute of Technology*
Tom Irving, *Illinois Institute of Technology*
Alfonso Mondragon, *Northwestern University*
Gerd Rosenbaum, *Argonne Nat'l Lab*
Wilfried Schildkamp, *The University of Chicago*
Susan Barr Strasser, *Argonne Nat'l Lab*
Joy Talsma, *The University of Chicago*
Keith Watenpaugh, *Pharmacia & Upjohn Co.*
Edwin Westbrook, *Argonne Nat'l Lab*

Sponsors

The generous donations of the following sponsors are gratefully appreciated.

Corporate Sponsors

Abbott Laboratories

Eli Lilly and Company

Glaxo Wellcome Inc.

Merck & Co., Inc.

Pharmacia & Upjohn Co.

Schering Plough Research Institute

Searle

Zeneca Pharmaceuticals

Non-corporate Sponsors

Associate Laboratory Director for the APS, Argonne National Laboratory

Associate Laboratory Director for Energy, Environmental Science and Technology, Argonne National Laboratory

Department of Energy, Office of Biological and Environmental Research

International Union of Crystallography

National Institutes of Health

National Science Foundation

The University of Chicago Biological Sciences Division

The University of Chicago Center for Advanced Radiation Sources (CARS)

Universities participating in the "Collaboration of Illinois Universities at the APS, Argonne National Laboratory" grant from the Illinois Board of Higher Education–Higher Education Cooperation Act:

Illinois Institute of Technology
Northern Illinois University
Northwestern University
Southern Illinois University at Carbondale
The University of Chicago
University of Illinois at Urbana-Champaign

Schedule-at-a-Glance

	Monday, August 3	Tuesday, August 4	Wednesday, August 5
MORNING		**Session I** Macromolecular Crystallography	**Session IV** Impact of Synchrotron Radiation on Biology and Biophysics: Past, Present, and Future
AFTERNOON	**Pre-conference Activities** Registration begins and continues throughout the conference.	**Session II** Optics and Special Techniques **Tours of the APS and of Biology/Biophysics Collaborative Access Teams (CATs)**	**Session V** Apparatus and Techniques **Poster Session**
EVENING	**Reception**	**Session III** Hot Topics	**Session VI** "Meet the Experts"
	Thursday, August 6	**Friday, August 7**	**Saturday, August 8**
MORNING	**Session VII** Scattering from Non-Crystalline Systems	**Session X** X-ray, VUV, and IR Spectroscopies	**Session XII** Macromolecular Complexes: The Frontier with Cell Biology
AFTERNOON	**Session VIII** Microscopies and Medical Research	**Session XI** Macromolecular Complexes: The Frontier with Cell Biology **Poster Session**	**Conference Ends**
EVENING	**Session IX** "Meet the Experts"	**Conference Banquet** At The Field Museum, Chicago	

Program

Monday, August 3, 1998

Pre-conference Activities

3:00 - 8:00 p.m. **Registration**
Argonne Guest House Lobby

1:00 - 5:00 p.m. **Vendor Set-Up**
Bldg. 402, Gallery

5:00 p.m. **Reception**
Argonne Guest House Dining Room

Tuesday, August 4, 1998

8:00 a.m. - 5:00 p.m. **Vendor Exhibits**
Bldg. 402, Gallery

8:00 a.m. - 5:00 p.m. **Registration**
Bldg. 401, Atrium
(Continues throughout the conference)

Session I

9:00 - 9:15 a.m. **Introductory Remarks**
Bldg. 402, Lecture Hall

Keith Moffat, *The University of Chicago and Chair of Local Organization and Program Committee*
David Moncton, *Associate Laboratory Director for the Advanced Photon Source*
Alan Rosenthal, *Abbott Laboratories, Vice President of Pharmaceutical Discovery and Scientific Affairs*

Macromolecular Crystallography (*Morning, full session*)
Bldg. 402, Lecture Hall

9:15 - 9:30 *Advances in Macromolecular Structure Determination*
Wayne Hendrickson, Session Chair (USA)

9:30 - 10:00 *MAD Becoming Sane?*
Janet Smith (USA)

10:00 - 10:30	*Anomalous Dispersion and the Phase Problem in Biocrystallography* Roger Fourme (France)
10:30 - 11:00	**Refreshments** Bldg. 402, Gallery
11:00 - 11:30	*Protein Crystal Structures at Atomic Resolution* Keith Wilson (UK)
11:30 - 12:00 p.m.	*Time-resolved Crystallographic Studies of Isocitrate Dehydrogenase Using Intermediate Trapping, Photolytic Triggering, and Synchrotron Radiation* Barry Stoddard (USA)
12:00 - 12:30	*Crystal Structures of Intermediates Occurring along the Reaction Pathway of Cytochrome P450cam* Ilme Schlichting (Germany)
12:30 - 2:00	**Lunch** Bldg. 402 Lower-level Patio, under the Tent

Session II

Optics and Special Techniques (*Afternoon, first half session*)
Bldg. 402, Lecture Hall

2:00 - 2:10	*Optics and Special Techniques: Advances and Trends* Glaucius Oliva, Session Chair (Brazil)
2:10 - 2:30	*Picosecond Snapshots of Chemical and Biological Reactions Using Pulsed Synchrotron Radiation* Michael Wulff (France)
2:30 - 2:50	*Nanosecond Time-resolved Macromolecular Crystallography: Probing Photo-initiated Protein Relaxation* Vukica Srajer (USA)
2:50 - 3:10	*X-ray Microprobe Applications to Life Science* Wenbing Yun (USA)
3:10 - 3:30	*Protein Micro-crystallography at the ESRF Microfocus Beamline* Christian Riekel (France)
3:30 - 3:50	*Present State of Experimental Phase Determination by Three-beam Diffraction from Macromolecular Crystals* Edgar Weckert (Germany)
4:00 - 5:30	**Tours of the APS and the facilities of the biophysics-oriented Collaborative Access Teams** (*Afternoon, second half session*) Tours begin in the Atrium, Bldg. 401

Session III

Hot Topics (*Evening*)
Bldg. 402, Lecture Hall

This session includes 15-minute presentations by each of eight graduate students or postdoctoral appointees selected by a blue-ribbon panel from abstracts submitted in advance for the poster sessions. Each presenter will receive a $500 award; funds were provided by the International Union of Crystallography.

7:30 - 7:40 Hans Deisenhofer, Panel and Session Chair (USA)

7:40 - 9:40 Speakers and Titles; see p. 12, Hot Topics Awards

Wednesday, August 5, 1998

8:00 a.m. - 5:00 p.m. **Vendor exhibits**
Bldg. 402, Gallery

Session IV

The Impact of Synchrotron Radiation on Biology and Biophysics: Past, Present, and Future (*Morning, full session*)
Bldg. 402, Lecture Hall

The first applications of hard x-rays emitted by synchrotron sources were to problems in biology in 1970-1971 at Hamburg. What motivated those scientists? How would they perceive those earliest efforts now? In the following decades, the disciplines and phrases "biotechnology" and "structural biology" were coined, and these disciplines now have a strong synchrotron component. From an industrial perspective, what is and will be the impact of synchrotron radiation on biotechnology?

Novel uses of synchrotron radiation in biophysics are contemplated or underway, for example in experiments that exploit the coherence properties of the source, seek to generate and use ultra-short x-ray pulses, or subject the sample to unusual conditions. Fourth-generation x-ray sources that are based on linear accelerators rather than on circular rings are being considered. As we look into the crystal ball, what might we expect the next five to 10 years to hold?

History

9:00 - 9:05 a.m. *Impact of Synchrotron Radiation on Biology and Biophysics*
Gerd Rosenbaum, Chair (USA)

9:05 - 9:30 *Synchrotron Radiation and Muscle Contraction*
Kenneth Holmes (Germany).

9:30 - 9:55 *The Evolution of Synchrotron Radiation as a Key Enabling Technology in Structural Biology and Biophysics*
Keith Hodgson (USA)

9:55 - 10:20	*Early Days of Biologically Oriented XAS at SSRL* Dale Sayers (USA)
10:20 - 10:50	**Refreshments** Bldg. 402, Gallery
	Impact on Biotechnology
10:50 - 10:55	*Synchrotron Radiation and Industry* Tony Kossiakoff, Chair (USA)
10:55 - 11:30	*The Impact of Synchrotron Radiation on Structural Biology and Biotechnology* Keith Watenpaugh (USA)
	Future
11:30 - 12:30 p.m.	*Synchrotrons and Biological Complexity: "The Action Is in the Interaction"* Paul Sigler, Panel Chair (USA)
	Synchrotron Radiation Protein Crystallography in the Genomics Era John Helliwell, Panel Member (UK)
	X-ray Absorption Spectroscopy in the Era of Synchrotron-based Crystallography: Is It Obsolete? James Penner-Hahn, Panel Member (USA)
	The Potential of X-ray Free-electron Lasers Based on Superconducting Linear Accelerators Jochen Schneider, Panel Member (Germany)
12:30 - 2:00	**Lunch** Bldg. 402 Lower-level Patio, under the Tent

Session V

Apparatus and Techniques (*Afternoon, first half session*)
Bldg. 402, Lecture Hall

2:00 - 2:20	*A Pixel Array Detector for Time-resolved X-ray Diffraction* Eric Eikenberry, Session Chair (USA)
2:20 - 2:40	*Cryocrystallography: Present Highlights and Future Prospects* Elspeth Garman (UK)
2:40 - 2:55	*Design and Testing of X-ray Fluorescence Detectors Using Synthetic Multilayers* Ke Zhang (USA)
2:55 - 3:15	*Diffraction Enhanced X-ray Imaging* Dean Chapman (USA)
3:15 - 3:30	*The Design and Prototype of a Detector for Protein Crystallography* Nguyen-Huu Xuong (USA)
3:30 - 5:30	**Poster Session** (*Afternoon, second half session*) Bldg. 438, Pentagons A and B Andy Howard, Poster Co-Organizer (USA)

SESSION VI

8:00 - 10:00 **"MEET THE EXPERTS"** (*Evening*)
Argonne Guest House

Several small groups of senior scientists, each expert on a particular style of experiment (e.g., virus crystallography, time-resolved solution scattering, soft x-ray microscopy), apparatus (e.g., CCD detectors, ultra-small-angle scattering cameras), systems (e.g., ribosomes and their subunits, muscle proteins, light-sensitive systems) or sources, will be available to meet casually with students and others who would like to chat about their own experiments or any topic related to synchrotron-based research. The emphasis is on informality and topicality.
See also Session IX.

Thursday, August 6, 1998

8:00 a.m. - 5:00 p.m. **VENDOR EXHIBITS**
Bldg. 402, Gallery

SESSION VII

SCATTERING FROM NON-CRYSTALLINE SYSTEMS (*Morning, full session)*
Bldg. 402, Lecture Hall

9:00 - 9:30 a.m. *Time-resolved Techniques for Muscle Diffraction with Synchrotron Radiation*
Hugh Huxley, Session Chair (USA)

9:30 - 9:55 *Structural Studies of Model Metalloprotein Maquettes Vectorially Oriented at a Soft Interface*
Kent Blasie (USA)

9:55 - 10:20 *The Mechanisms of Self Assembly and Polymorphic Switching of the Bacterial Flagellar Filament*
Keiichi Namba (Japan)

10:20 - 10:50 **REFRESHMENTS**
Bldg. 402, Gallery

10:50 - 11:15 *A Fibre Diffraction and Atomic Modeling Study of the Actomyosin Complex*
Katrina Poole (Germany)

11:15 - 11:40 *Structural Dynamics of Actomyosin in Muscle Contraction by X-ray Diffraction / Scattering*
Katsuzo Wakabayashi (Japan)

11:40 - 12:05 p.m. *Solution Scattering Studies of Protein Conformations and Interactions in Biochemical Regulation*
Jill Trewhella (USA)

12:05 - 12:30 *Biophysics and Synchrotron Radiation: When the Marriage Fails*
Martin Caffrey (USA)

12:30 - 2:00 **LUNCH**
Bldg. 402 Lower-level Patio, under the Tent

SESSION VIII

MICROSCOPIES AND MEDICAL RESEARCH (*Afternoon, full session*)
Bldg. 402, Lecture Hall

MICROSCOPIES

2:00 - 2:20 *X-ray Microscopy: Prospects and Current Developments*
Günter Schmahl, Chair (Germany)

2:20 - 2:45 *Cryo Samples with a Scanning Transmission X-ray Microscope: Imaging, Tomography, and Spectromicroscopy*
Chris Jacobsen (USA)

2:45 - 3:05 *High-resolution Soft X-ray Microscopy of Medically Important Protozoa*
Cathleen Magowan (USA)

3:05 - 3:30 *High-resolution X-ray Imaging of Frozen Hydrated Samples in Amplitude and Phase Contrast*
Gerd Schneider (Germany)

3:30 - 3:45 **REFRESHMENTS**
Bldg. 402, Gallery

MEDICAL RESEARCH

3:45 - 4:05 *Synchrotron Radiation in Research and Clinical Medicine*
William Thomlinson, Chair (USA)

4:05 - 4:30 *Intravenous Coronary Angiography with Synchrotron Radiation: Experience in 366 Patients*
Rainer Dix (Germany)

4:30 - 4:50 *Microbeam Radiation Therapy: Principle and Current Status of Pre-clinical Studies*
Per Spanne (France)

4:50 - 5:15 *Recent Topics of Synchrotron X-ray Imaging for Medical Research in Japan*
Tohoru Takeda (Japan)

SESSION IX

8:00 - 10:00 **"MEET THE EXPERTS"** (*Evening*)
Argonne Guest House

See Session VI.

Friday, August 7, 1998

8:00 a.m. - 12:00 p.m. **VENDOR EXHIBITS**
Bldg. 402, Gallery

SESSION X

X-RAY, VUV, AND IR SPECTROSCOPIES (*Morning, full session*)
Bldg. 402, Lecture Hall

9:00 - 9:20 a.m. *Whither Biological XAFS?*
Grant Bunker, Session Co-Chair (USA)

9:20 - 9:45 *X-ray Spectroscopy of Metal Sites in Proteins*
Steve Cramer (USA)

9:45 - 10:10 *X-ray Absorption Spectroscopy of Mn Enzymes*
James Penner-Hahn (USA)

10:10 - 10:35 *Crystallographic and XAFS Studies of Copper Proteins*
Samar Hasnain (UK)

10:35 - 11:05 **REFRESHMENTS**
Bldg. 402, Gallery

11:05 - 11:25 *Spectroscopy with Synchrotron Radiation: New and Not-so-new*
John Sutherland, Session Co-Chair (USA)

11:25 - 11:45 *Recent Advances in Fluorescence XAFS Using High-brilliance Photon Sources*
Hiro Oyanagi (Japan)

11:45 - 12:10 p.m. *Applications of Synchrotron Infrared Microspectroscopy to the Study of Biological Cells and Tissues*
Lisa Miller (USA)

12:10 - 1:45 **LUNCH**
Bldg. 402 Lower-level Patio, under the Tent

SESSION XI

MACROMOLECULAR COMPLEXES: THE FRONTIER WITH CELL BIOLOGY
(*Afternoon, first half session*)
Bldg. 402, Lecture Hall

1:45 - 2:05 *Macromolecular Complexes: The Frontier with Cell Biology*
Chairman's Introductory Remarks
Louise Johnson, Session Chair (UK)

2:05 - 2:20 *Drug Design against Shifting Targets: A Structural Basis for Drug Resistance to an Influenza Virus Neuraminidase Variant*
Joseph Varghese (Australia)

2:20 - 2:40	*Bacteriorhodopsin: Crystallography under Extreme Conditions* Eva Pebay-Peyroula (France)
2:40 - 2:55	*Redox-coupled Structural Changes in Bovine Heart Cytochrome c Oxidase* Tomitake Tsukihara (Japan)
2:55 - 3:15	*Crystals of Ribosomes, Exhibiting Severe Non-isomorphism, Extreme Radiation Sensitivity, and No Internal Symmetry, as Subjects for Synchrotron Radiation Crystallography* Ada Yonath (Israel)
3:15 - 5:00	**Poster Session** (*Afternoon, second half session*) Bldg. 438, Pentagons A and B Grant Bunker, Poster Co-Organizer (USA)
5:15	**Buses leave for banquet at the Field Museum** Buses will board in front of the APS Conference Center
6:15	**Guests arrive at the Field Museum**
6:15 - 7:15	**Social Hour**
7:30 - 9:30	**Dinner**
9:45	**Buses depart for Argonne**

Saturday, August 8, 1998

Session XII

Macromolecular Complexes: the Frontier with Cell Biology
(Morning, full session)
Bldg. 402, Lecture Hall

9:00 - 9:15 a.m.	*Synchrotron Radiation as a Tool for Investigating Virus Structure: Future Horizons and Past Successes* Michael Rossmann, Session Chair (USA)
9:15 - 9:40	*The Stereochemistry of Chaperonin Assisted Protein Folding* Paul Sigler (USA)
9:40 - 10:05	*Crystallographic Analysis of Eukaryotic Signaling Proteins* John Kuriyan (USA)
10:05 - 10:30	*EGF Receptor Trafficking and Viral Infection Resolved in Live Cells Using Synchrotron Radiation (SR) Microfluorimetry* M. Martin-Fernandez (UK)
10:30 - 11:00	**Refreshments** Bldg. 402, Gallery

11:00 - 11:25	*Structure and Function of the Mitochondrial ATP Synthase* John Walker (UK)
11:25 - 11:50	*Structural Studies on a dsRNA Virus* David Stuart (UK)
11:50 - 12:15 p.m.	*The Structure and Dynamics of Protein and Nucleoprotein Assemblies Determined with Synchrotron X-ray Radiation* John Johnson (USA)
12:15 - 1:45	**LUNCH** Bldg. 402 Lower-level Patio, under the Tent
	CONFERENCE CLOSES AFTER LUNCH

HOT TOPICS AWARDS

The following abstracts, submitted by graduate students and postdoctoral appointees, were selected by a panel of judges for their quality and scientific merit. These awards were sponsored by the International Union of Crystallography. Congratulations!

Crystal Structure of the Ferric Enterobactin Receptor from E. Coli
Susan Buchanan

Approach to Determine the Distribution of a Contrast Agent in the Brain by MRI and X-ray Synchrotron Radiation Computed Tomography (SCRT)
Geraldine Le Duc

Complete Structure of a Bifunctional 11-subunit Membrane Protein Complex from Bovine Heart Mitochondria, the Cytochrome bc1 Complex
Joong W. Lee

High-resolution Structure of Titin Kinase from Crystals with Very Thin Plate Morphology
Olga Mayans

Signal Transduction on the Nanosecond Time Scale: Early Structural Events in the Photocycle of a Xanthopsin
Benjamin Perman

Structural Biology of RNA Folding Using Synchrotron Footprinting
Corie Ralston

Ultra-fast MAD Data Collection on the 19ID Beamline at the Advanced Photon Source
Martin Walsh

Scattering Contrast in Diffraction Enhanced Imaging
Zhong Zhong

General Information

Concierge Desk

The Concierge Desk, located in the Bldg. 401 Atrium, will be available daily during conference breaks. Information about local and Chicago restaurants and attractions is available, and Concierge desk staff will be glad to make reservations for dinners and other activities for you.

Meals

The conference fee includes refreshment breaks, continental breakfasts each day (available in the Gallery at the Conference Center), lunches on Tuesday through Saturday, and the conference banquet at The Field Museum in Chicago on Friday evening. The Argonne Guest House, which is a one-block walk from the APS Conference Center, contains a full-service restaurant. A list of local restaurants is included in the conference packet.

Communications

Pay telephones are located in the Bldg. 401 Atrium and on the lower level of Bldg. 402, the APS Conference Center. Messages can be left for you at the registration desk; the telephone numbers there are 630.252.9580 and 630.252.9581. The messages will be posted on a bulletin board by the entrance to the auditorium. Messages for guests of the Argonne Guest House can be left at the front desk of the hotel by dialing 5, then 0 from on-site telephones, or by calling 630.739.6000 from off-site. If you need to send or receive a fax, a fax machine is located in Bldg. 401, B1154 (the APS User Office). The number of this machine is 630.252.9250. Computer terminals are located in Bldg. 401, User Lounge (located just off the Atrium), for you to use for access to your home computer.

Transportation

Shuttle buses will run between the conference hotels and the APS Conference Center in the morning, at mid-day, and in the evening. Bus transportation will also be provided for the conference banquet. Limousine reservations can be made for you during the meeting by conference staff.

Tour of the APS

You must sign up at the Tour Desk in the Conference Center Atrium to participate in the tours of the APS and the facilities of the biophysics-oriented Collaborative Access Teams. Tours will begin at 4:00 p.m. on Tuesday, August 4, 1998.

Poster Sessions

Set-up for the poster sessions will begin Monday, August 3 in pentagons A and B of laboratory/office module (LOM) 438, and all posters can be left up throughout the conference. Posters numbered in the 100 series will be presented on Wednesday, August 5, and those in the 200 series will be presented on Friday, August 7.

Social Events

Reception

Monday, August 3, 1998; 5:00 - 7:00 p.m.

Meet your old and new friends in the spacious **Argonne Guest House Dining Room** for a welcoming reception.

"Meet the Experts"

Wednesday, August 5, 1998; 8:00 - 10:00 p.m. and
Thursday, August 6, 1998; 8:00 - 10:00 p.m.

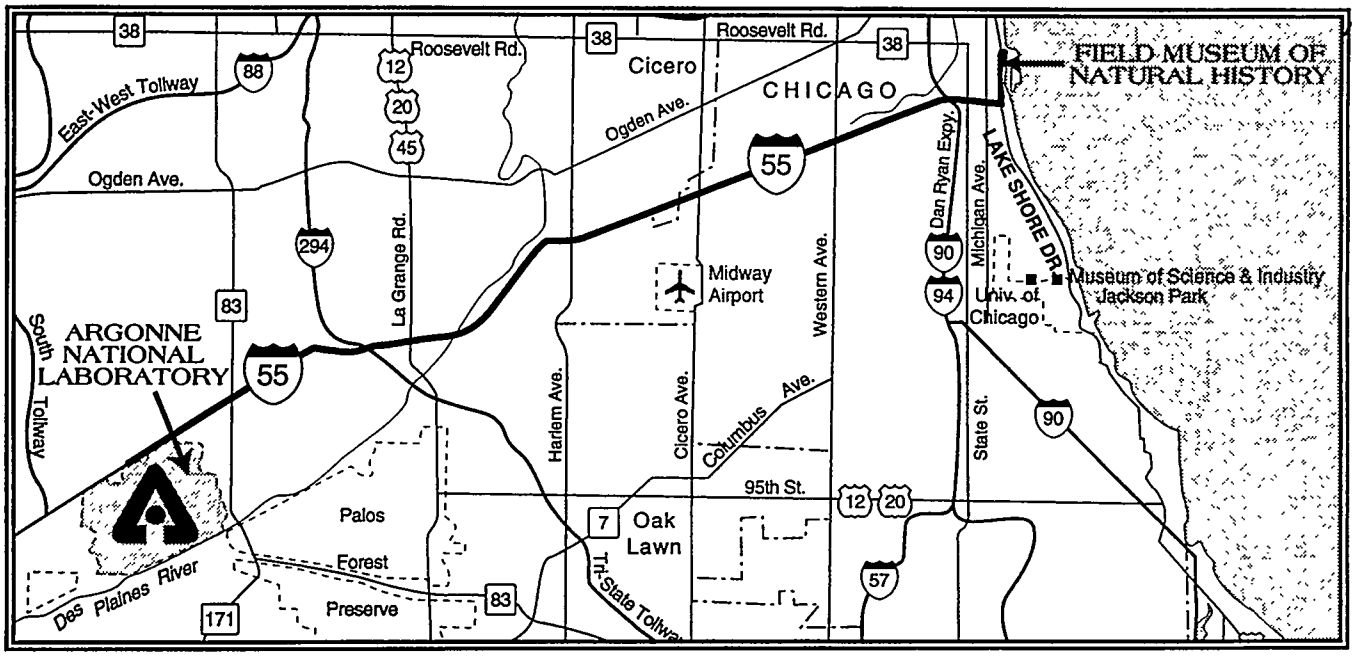
Join colleagues in the **Argonne Guest House Dining Room** for an evening of informal panel discussions on topics related to synchrotron-based research. The specific discussion topics will be posted on the day of the session and will depend on the interests of the participants and the availability of experts.

Conference Banquet

Friday, August 7, 1998; 6:15 - 9:45 p.m.

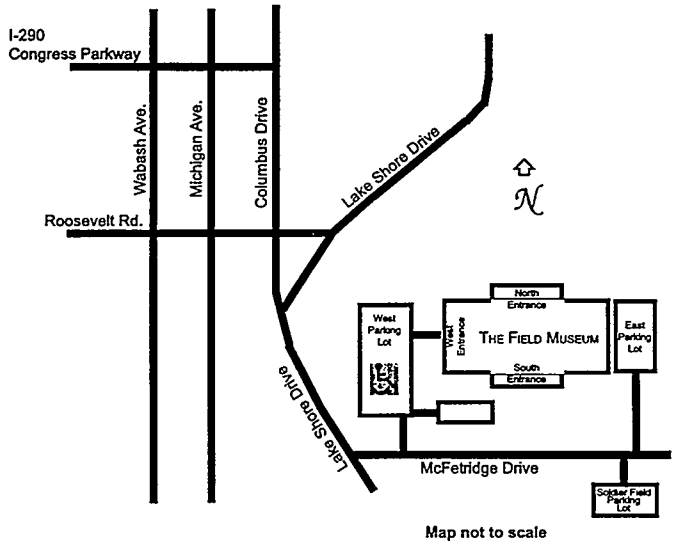
Chicago's world renowned **Field Museum** is the site of this year's conference banquet. The museum has a rich history dating back to the 1893 Chicago Columbian Exposition held to commemorate the 400th anniversary of the discovery of America. During this world fair, a citizens' committee launched the effort to establish the museum. Now, more than a century later, the Field Museum contains a natural-history collection encompassing more than 19 million artifacts in more than nine acres of exhibit space.

During the pre-dinner reception, which will be held on the Stanley Field Hall balcony, guests will have the opportunity to visit museum attractions. The museum is currently featuring several exciting exhibits including "Living Colors: A Butterfly Garden"; the state-of-the-art laboratory where "Sue," the largest, most complete *Tyrannosaurus rex* ever found, is being prepared; the classic Hall of Gems; the ancient Egypt collection; and the actual "Lions of Tsavo," recently depicted in the popular movie *The Ghost and the Darkness*. The banquet itself will be held in the majestic Stanley Field Hall rotunda.



Transportation to Banquet

Bus transportation is being provided from the APS Conference Center and the Argonne Guest House to the Field Museum. If you prefer to drive, please be aware that parking near the museum campus is very limited and can be costly. To drive to the Field Museum from Argonne, take Interstate 55 (the Stevenson Expressway) north (toward Chicago) to Lake Shore Drive. Exit north, drive past Soldier Field to McFetridge Drive, and turn right. The Museum will be on your left. Parking lots are indicated on the map below.

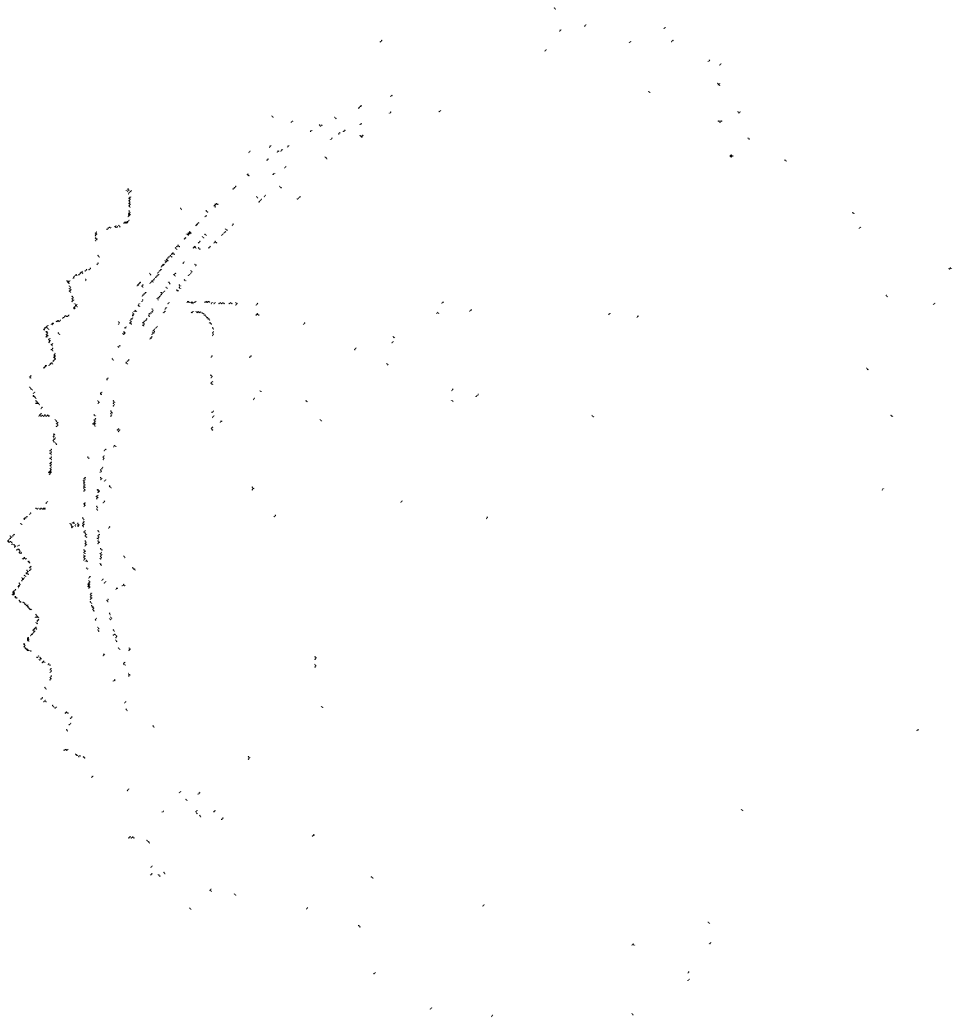


Map not to scale


BIOPHYSICS & SYNCHROTRON RADIATION
BSR 98
ARGONNE, IL • AUGUST 4-8, 1998

Notes

Abstracts for Invited Talks



Advances in Macromolecular Structure Determination

Wayne A. Hendrickson
Columbia University, New York, New York, USA, and the Howard Hughes Medical Institute

The determination of atomic structures by x-ray crystallography dominates among the applications of synchrotron radiation in biology, if not in all of science. Increasingly complex structures are being determined in ever increasing numbers. Advances that fuel this progress include cryopreservation, MAD phasing, selenomethionyl proteins, CCD detectors, and undulator sources. These developments reinforce one another and have greatest power when brought together in concert. As this is now happening, one can expect a further surge in macromolecular crystallography at synchrotrons and perhaps new-found applications such as in structural genomics.

MAD Becoming Sane?*

Janet L. Smith
Purdue University, West Lafayette, Indiana, USA

Multiwavelength anomalous diffraction (MAD) is a highly successful new phasing method in macromolecular crystallography. MAD exploits physical changes to a sample crystal during the data collection experiment to derive phase information. The anomalous scattering factors of selected atoms in the crystal change with the incident x-ray energy in the vicinity of an atomic resonant frequency. The attractiveness of MAD is due to the possibility of deriving accurate phase estimates from data measured from one sample crystal in one experiment. The advent of cryogenic sample protection and specialized beamlines has resulted in an explosion of successful MAD experiments. MAD phasing depends critically on determination of the partial structure of anomalous scatterers. In cases where their number is small or the crystal symmetry is simple, Patterson methods are sufficient for establishing the partial structure of anomalous scatterers. However, this is often not the case. Much of the success of MAD is due to the Se label in selenomethionine (SeMet) because of its biological incorporation in place of the amino acid methionine. Thus, for SeMet problems, the complexity of the anomalous-scatterer partial structure is proportional to the size of the protein, on average one SeMet for every 50-60 amino acids. A bottleneck in solving large Se partial structures has been eliminated recently by application of statistical direct methods to structures having up to 80 Se sites in the crystallographic asymmetric unit. Despite the additional challenge of MAD problems, there is no indication that the upper limit has been reached in size of protein or anomalous-scatterer partial structure to which direct methods can be applied successfully. MAD is rapidly becoming the method of choice for solving new protein crystal structures, limited only by availability of beam time.

*This work has been supported by NIH grant DK 42303.

Anomalous Dispersion and the Phase Problem in Biocrystallography

R. Fourme, W. Shepard, M. Ramin, and M. Schiltz
LURE, Université Paris-Sud, Orsay, France

The anomalous dispersion of x-rays is an essential tool for the determination of structure factor phases in macromolecular crystallography. Multiple wavelength methods (MAD and MASC), applied to a cryocooled crystal, put into actual practice the ideal scheme of *ab initio* phase determination where the waves diffracted by the unknown structure should remain invariant. MAD on crystals of purposely bio-engineered proteins is a rather systematic approach to the solution of the phase problem, and might become the method of choice for structural genomics. MASC may provide information on the macromolecular envelope and low-resolution phases. We have shown that reliable values of amplitudes of the envelope structure factors can be derived from MASC experiments, but phasing these structure factors is still an open question. Single wavelength experiments will be powerful when isomorphism between a native and a heavy atom derivative can be preserved, which is the case in SIRAS experiments with noble gas (Xe, Kr) derivatives. The development of anomalous dispersion methods from pioneering experiments to the present situation results from a number of experimental advances (in synchrotron radiation sources, instrumentation, and molecular biology) and theoretical and computational advances. The impact of maximum likelihood methods is especially noteworthy. They have provided a rigorous and common frame to all *ab initio* phasing methods. Unbiased phase information produced by maximum likelihood, combined with solvent flattening, produces high-quality results and has allowed solution of structures from minimal information (for instance SAD). Current developments aim at parameterizing the anisotropy of anomalous scattering by a label atom (e.g., selenium). These improvements will remove remaining systematic errors in the MAD phase estimates and can ultimately be exploited to give additional phase information.

Protein Crystal Structures at Atomic Resolution*

Keith S Wilson
University of York, York, UK

Victor S Lamzin
EMBL, Hamburg, Germany

Zbigniew Dauter
Brookhaven National Laboratory, Upton, New York, USA

Thomas Schneider
University of Göttingen, Göttingen, Germany

The structural chemistry of the last 50 or more years was underpinned by small-molecule crystallography, which provided a complete description of the molecule at atomic (roughly 1 Å) resolution. Crystallography could reveal the positions of all atoms in the unit cell and provide a detailed 3-D geometry of the whole molecule. No other technique gives comparable information. Protein crystallography is now playing a comparable role for biological chemistry and a full description of a protein is no longer complete without a 3-D structure. This is made even more important by advances in molecular genetics which mean that most proteins are potentially available in quantities suitable for crystallisation. X-ray analyses of crystals of macromolecules pose special problems due to the nature of the molecules themselves and of the way they pack into crystals. That they have large molecular weights means that the unit cells are large, resulting in large numbers of reflections, all of which are weak relative to the small-molecule case, with a low signal-to-noise ratio. This in recent years has been alleviated by the advent of efficient detectors, and most importantly, high-intensity synchrotron radiation (SR). However, this is not enough. In addition, the crystals contain on average about 50% disordered aqueous solvent and the molecules themselves betray substantial disorder especially at the solvent interface. Hence the intensities of the high-resolution data are even more weak or indeed absent. Furthermore, the crystals at room temperature are subject to substantial radiation damage. The latter problem has been greatly alleviated in the last decade by the use of cryogenic freezing, which is now used for the majority of synchrotron radiation (SR) experiments. Taken together, the use of SR, 2-D detectors, and cryogenics have meant that for an increasing number of proteins, atomic resolution data (to 1.2 Å or better) can be recorded. This allows much more accurate models to be analysed with a full anisotropic model. Results on a representative set of examples will be described and implications for the future discussed. Most of the results have come from work carried out at EMBL, Hamburg.

*The EC is thanked for financial support through BIOTECH contract CT96-0189.

Time-resolved Crystallographic Studies of Isocitrate Dehydrogenase Using Intermediate Trapping, Photolytic Triggering, and Synchrotron Radiation*

Barry L. Stoddard, Jill Bolduc, and Dave Dyer
The Fred Hutchinson Cancer Research Center, Seattle, Washington, USA

Andrew Mesecar, Bruce Cohen, Mike Brubaker, and Daniel E. Koshland, Jr.
University of California at Berkeley, Berkeley, California, USA

Time-resolved crystallography uses a group of related techniques for reaction trapping and data collection. The experiments are designed to accumulate a specific catalytic intermediate throughout the crystal for a short period of time during which diffraction data is collected. In this context, three parameters can be defined: the method used to isolate the intermediate, the longest exposure time allowed for data collection (which is limited either by the lifetime of the intermediate or by the lifetime of the crystal during the experiment), and the method of data collection. At one end of the spectrum is a single-turnover experiment, triggered by a photolytic event, in which the lifetime of the rate-limited species is short, necessitating rapid data collection using a synchrotron light source and Laue diffraction. At the other extreme is a reaction intermediate trapped by chemical and physical techniques to produce an extended lifetime in the crystal, usually visualized by the use of a slower method of data collection. The enzyme isocitrate dehydrogenase catalyzes the decarboxylation of isocitrate to α-ketoglutarate and exhibits a catalytic mechanism with several rapid steps prior to the rate-limiting dissociation of products. In order to accumulate the initial ES complex and the subsequent enol intermediate, site-directed mutants were used to impose specific kinetic barriers, and steady-state accumulation of the rate-limited species was used in conjunction with Laue data collection. Molecular dynamics simulations provided significant additional details of the structure of the ES complex and of the factors that contribute to an efficient hydride transfer reaction. A subsequent development, demonstrating the importance of comparative studies using multiple strategies of intermediate trapping, is the use of small structural perturbations in the active site ES complex to evaluate the contribution of precise substrate alignment to catalytic rate enhancement during hydride transfer. Steady-state, freeze-trapping methods were used to visualize and compare the resulting complexes. Most recently, the structure of the rate-limited product complex formed during a single synchronized round of turnover has been determined using photolytic liberation of caged substrate and Laue x-ray data collection. The experiment was conducted with three different caged compounds, each possessing a unique mechanism leading to the formation of the ES complex. Photoreaction efficiency and subsequent substrate affinities and binding rates in the crystal are critical parameters for these experiments.

*Work supported by NIH grant GM49857, and conducted at the NSLS (Brookhaven National Laboratories) and the ALS (LBNL, Berkeley, CA).

Crystal Structures of Intermediates Occurring along the Reaction Pathway of Cytochrome P450cam

Ilme Schlichting
Max Planck Institute, Dortmund, Germany

Joel Berendzen and Kelvin Chu
Los Alamos National Laboratory, Los Alamos, New Mexico, USA

Robert M. Sweet
Brookhaven National Laboratory, Upton, New York, USA

Dagmar Ringe and Gregory A. Petsko
Rosenstiel Center, Brandeis University, Waltham, Massachusetts, USA

Steven Sligar
Department of Biochemistry & Beckman Inst. for Advanced Science and Technology, University of Illinois, Urbana-Champaign, Illinois, USA

Cytochrome P450cam from *Pseudomonas putida* catalyses the 5-exo-hydroxylation of camphor. Although many structures of different p450.cam complexes have been determined by Poulos and coworkers, the structure of the biochemically important oxygen complex of p450.cam has not yet been solved as it is unstable (half-life ~3 min at 4°C) due to autooxidation. This instability requires collection of the diffraction data of the short-lived complex either very quickly (e.g., by the Laue method) or by prolonging its life time. Only the latter approach was successful for us: We used cryocrystallography to determine the structures of unstable intermediates occuring along the reaction pathway of p450.cam. We chemically reduced ferric p450.camphor crystals to generate the ferrous complex. Subsequently, oxygen was diffused into the crystals to generate the ternary p450.camphor.O_2 complex; x-ray radiolysis was used to produce a state that we identify as an oxyferryl species. This intermediate can be transformed to the product 5-hydroxy-camphor by rapid thawing and refreezing of the crystals. The methods used and the structures obtained will be discussed.

Optics and Special Techniques: Advances and Trends

Glaucius Oliva
University of Sao Paulo, Sao Paulo, Brazil, and The Howard Hughes Medical Institute

The advent of third-generation synchrotron radiation sources has opened up the scene for the conception and realization of several new experiments with a unique impact in many areas of science, structural biology in particular. The titanic science and engineering effort in the construction of such facilities is now flourishing with the wealth of information derived from experiments that make use of the available photons. The challenge is now creative innovation in optics and complementary techniques that will allow the full use of the unique features of the synchrotron light in a wide spectrum of applications. One example is the development of optical elements based on mirrors, Fresnel zone plates, capillaries, and waveguides to form highly focused microbeams that could be extremely useful in imaging, diffraction, and fluorescence. Another area of interest has been the use of the unique time structure of synchrotron radiation, with developments that have made feasible sub-nanosecond time-resolved crystallography experiments, which allow the elucidation of the dynamics of the molecular mechanisms involved in biological catalysis. The possible use of the polarization of the synchrotron beam in the direct measurement of phases in multiple-beam x-ray diffraction is also a new challenge. Although not reviewed in this panel due to the natural limitations in the program, many other new developments are being made in monochromators, mirrors, detectors, and other instrumentation, which will certainly make for a much more effective use of the full spectrum of photons available at different facilities around the world.

Picosecond Snapshots of Chemical and Biochemical Reactions Using Pulsed Synchrotron Radiation

Michael Wulff, F. Schotte, S. Techert, and D. Bourgeois
European Synchrotron Radiation Facility, Grenoble, France

C. Bressler
Harvard University, Cambridge, Massachusetts, USA

V. Srajer, T.-Y. Teng , B. Perman, K. Moffat, and W. Schildkamp
Consortium for Advanced Radiation Sources, The University of Chicago, Chicago, Illinois, USA

P. A. Anfinrud
Harvard University, Cambridge, Massachusetts, USA

All proteins undergo structural changes while carrying out their biological function. While the "before" and "after" structures are known for some proteins, the pathway connecting these limiting structures is largely unknown and, until now, unexplored. The ability to watch macromolecular structural changes as they occur has recently been developed on beamline ID09 at the ESRF. A pump-probe set-up has been built for time-resolved studies of photoactive molecules.[1] A "two-colour" interaction point at the sample position is produced which consists of 100-femtosecond optical pulses and 100-picosecond x-ray pulses which run at up to 900 Hz and where the relative phase can be set with 2.5-picosecond resolution. The set-up comprises a synchronous x-ray chopper and a Ti:sapphire laser with a large tunability between 400-850 nm. The beamline has so far been used for single shot Laue diffraction of photo-reversible systems such as ligand release in heme proteins and studies of intermediates in the photo cycles of bacteriorhodopsin and the photoactive yellow protein, PYP. The potential for time-resolved studies of protein kinetics in solution using EXAFS and diffuse scattering will be discussed.

[1]"Time-resolved Structures of Macromolecules at the ESRF: Single-pulse Diffraction, Stroboscopic Data Collection and Femtosecond Flash Photolysis", M. Wulff, F. Schotte, G. Naylor, D. Bourgeois, K. Moffat and G. Mourou, Nuclear Instruments and Methods in Physics Research A 398 (1997), 69-84.

Nanosecond Time-resolved Macromolecular Crystallography: Probing Photo-initiated Protein Relaxation

Vukica Srajer, T. Y. Teng, B. Perman, Z. Ren, C. Pradervand, W. Schildkamp, and K. Moffat
The University of Chicago, Chicago, Illinois, USA

T. Ursby
Lund University, Lund, Sweden

W. Royer
University of Massachusetts, Amherst, Massachusetts, USA

R. Kort and K. Hellingwerf
University of Amsterdam, Amsterdam, Netherlands

D. Bourgeois, F. Schotte, and M. Wulff
European Synchrotron Radiation Facility, Grenoble, France

Structural changes in biological macromolecules are very fast at physiological temperatures and often occur on the sub-microsecond time-scale. Recent technological, instrumentation, and software developments[1] allow investigation of structural changes in protein crystals on the nanosecond (ns) (and in the near future, sub-ns) time-scale at the third-generation synchrotron sources such as ESRF (France), APS (USA), and SPring8 (Japan). We present here results of ns time-resolved crystallographic experiments conducted at the ID9 beamline of the European Synchrotron Radiation Facility (ESRF) in Grenoble, France, on carbonmonoxy complexes of two heme proteins: sperm whale myoglobin (Mb) and dimeric hemoglobin (HbI) from the clam *Scapharca inaequivalvis*. We also report our results on ns structural events in the photocycle of photoactive yellow protein (PYP).[2] The goals of these studies are to understand the evolution of the photo-induced structural changes and their propagation from the active site through the protein; to determine the trajectories and the docking sites of the photo-dissociated ligands in case of heme proteins; and to investigate how these trajectories are affected by mutations of side chains known to affect ligand binding properties. We use intense, focused, white, 150 ps and 1-microsecond x-ray pulses in pump-probe type of measurements to investigate structural changes that are photo-initiated by 10 ns laser pulses. The reversibility of the reaction allows us to signal average (typically 10-30 fold) and to obtain complete and even multiple data sets from one crystal. Data are typically 70% complete to 1.7 Å resolution, with Rmerge = 12%. Departure of the CO ligand upon photolysis and its subsequent rebinding and the iron atom displacement from the heme plane are clearly observed in both heme proteins. We identified a possible docking site for the photodissociated CO molecule in the MbCO heme pocket and compared this site with the sites observed at low temperatures[3] and with the sites predicted by computational methods.[4] Our ns PYP investigations yielded the structure of the short-lived, red-shifted intermediate state that develops within 1 ns after photoexcitation of the PYP chromophore.[2]

[1]Bourgeois D. et al., J. Sync. Rad. 3, p. 65 (1996); Srajer V. et al., Science 274, p. 1726 (1996); Chen, Y. et al., Rev. Sci. Instrum. 65, p. 1506 (1994); Ren, Z. and Moffat, K., J. Appl. Cryst. 28, p. 461 (1995), ibid. p. 482 (1995); Ren, Z. and Moffat, K., J. Appl. Cryst.29, p. 246 (1996).
[2]Perman, B. et al., Science 279, p. 1946 (1998).
[3]Teng et al., Nat. Struct. Biol. 1, p. 701 (1994); Teng et al., Biochemistry 36, p. 12087 (1997).
[4]Vitkup et al., Nat. Struct. Biol. 4, p. 202 (1997).

X-ray Microprobe Applications to Life Science

Wenbing Yun
Argonne National Laboratory, Argonne, Illinois, USA

X-ray fluorescence microscopy and microspectroscopy with 0.15-μm spatial resolution and unprecedented capabilities have been developed and applied to problems in life sciences. In addition to the intrinsic advantages of x-rays for elemental mapping, chemical state imaging, and phase contrast imaging, the unprecedented capabilities result mainly from the combination of the high-brilliance synchrotron radiation sources and high-performance x-ray microfocusing optics. In this presentation, we report experimental results in the study of several important problems in life science, such as the function of metallic ions in cells, the symbiotic relationship between mycorrhizal plant roots and fungi, the evaluation of anticancer agents, the determination of mesoscopic structure of DNA-membrane self-assemblies, and the dynamics of blood platelets in solution.

Protein Micro-crystallography at the ESRF Microfocus Beamline

Christian Riekel
European Synchrotron Radiation Facility, Grenoble, France

One feature of the high brilliance of third-generation synchrotron radiation sources is the possibility of reducing sample volume in single-crystal and fiber-diffraction experiments. Experiments in this area were initially developed at ESRF for the study of inorganic crystals down to sub-μm^3 sample volumes, but have also recently been applied to protein crystallography. The talk will give an overview on instrumentation developed for this purpose at the ESRF microfocus beamline (ID13) with beam sizes down to a few micrometers based on an ellipsoidal mirror and a Si-111 double monochromator. Emphasis will be given to single-crystal diffractometry and fiber diffraction, although scanning diffractometry will be also mentioned for the study of more complex biological objects. Single crystal experiments are currently not so much limited by sample volume as by sample handling, alignment, and radiation damage. It appears that the quality of data that can be obtained with a microbeam can be at least as good as that obtained on a larger crystal.

Present State of Experimental Phase Determination by Three-beam Diffraction from Macromolecular Crystals

Edgar Weckert, K. Hoelzer, R. Mueller, K. Schroer, and J. Zellner
Universitaet Karlsruhe, Karlsruhe, Germany

The phase of the structure factors is lost during a normal x-ray diffraction experiment. Therefore, a direct calculation of electron density from the measured intensities is not possible. Experimental methods to obtain phases require, in most cases, an insertion of heavy atoms into the structure. Then isomorphous replacement techniques (MIR) or the MAD technique can be applied. It has been shown that phase information in form of triplet phases can also be obtained by means of three-beam interference experiments. During these experiments, two strong reflections will be excited simultaneously. The wavefields of these two reflections will interfere with each other via a coupling provided by a third reciprocal lattice vector. Due to this interference, the intensity of the reflections will be changed in a characteristic way that depends on the phase difference (triplet phase) of the structure factors of the three reciprocal lattice vectors involved. A convenient method for generating these three-beam interferences are psi-scan experiments. The prerequisite for these experiments is crystals of low mosaic spread, since otherwise the interference effects of different mosaic blocks overlap and an interpretation is not possible. However, the crystals do not have to be perfect. Three-beam interference experiments have been observed for a number of crystals of small- and medium-size macromolecular structures (e.g., lysozyme, trypsin, proteinase K).[1,2] However, there are some restrictions on the structure factor moduli of those reflections for which triplet phases can be measured. In general, only reflections with large structure factor moduli that give rise to significant interference effects are accessible. Compared to intensity data collection, the speed of an interference experiment is slow (e.g., about 4-6 triplet phases can be determined in one hour from tetragonal lysozyme at an ESRF bending magnet beamline). Undulator radiation might speed up the experiment but then radiation decay will be even more severe. It has recently been shown that enough triplet phases (>800) can be measured to calculate a first electron-density map that can be interpreted. The mean-phase error of these measurements compared with the known model is about 20 degrees. Therefore, in principle it is possible to solve the structure of a small protein using the phase information from three-beam interferences. Present investigation is aimed at speeding up the experiment. The first three-beam interference experiments with crystals from an unknown protein structure have already started.

[1]Huemmer, K., Schwegle, W., Weckert, E., Acta Cryst. A42, 60-62.
[2]Weckert, E., Huemmer, K., Acta Cryst. A53, 108-143.

Impact of Synchrotron Radiation on Biology and Biophysics

Gerd Rosenbaum
Argonne National Laboratory, Argonne, Illinois, USA

In a span of 28 years since the first application of synchrotron x-radiation,[1] the number of users has exploded from a half dozen to several thousands. Flux, compared to pre-synchrotron levels, has increased 20-million times–outpacing by a factor of 1000 the much publicized increase in speed of microprocessors. Time-resolved muscle diffraction, high-speed protein crystallography, MAD phasing, and EXAFS would be unthinkable without synchrotron radiation. The first part of this session will outline these historical advances. Biotechnology is starting to invest in synchrotron radiation, as witnessed by the building of a beamline at the Advanced Photon Source by a consortium of drug companies. The state of biotechnology's involvement in synchrotron radiation is the topic of the second part of this session. As we explore the opportunities of third-generation (undulator) sources, new, tremendously more powerful sources are planned. The third part of this session will discuss the promise of undulators and the potential–and limits–of the fourth-generation sources for biology and biophysics.

[1]Rosenbaum, G., Holmes, K. C., Witz, J. 1971 Nature, 230:434-437.

Synchrotron Radiation and Muscle Contraction

Kenneth C. Holmes
Max Planck Institute for Medical Research, Heidelberg, Germany

Gerd Rosenbaum
Argonne National Laboratory, Argonne, Illinois, USA

Synchrotron radiation has played an absolutely essential role in elucidating the molecular mechanism of muscle contraction. Conversely, muscle contraction was the driving force for the development of synchrotron radiation as an x-ray source. Muscle fibers give detailed semi-crystalline low-angle x-ray fibre diagrams which yield information about the molecular movements during a contraction. To interpret these data requires millisecond time resolution, which is not possible using conventional x-ray sources - hence synchrotron radiation. The first synchrotron x-ray diffraction pattern ever obtained was from a slice of insect flight muscle at the DESY synchrotron in Hamburg in 1970.[1] In the following two years, a laboratory was built on DESY with a fully remotely controlled x-ray beam line.[2] A curved quartz crystal was combined with a curved mirror to focus and monochromatize the beam. Data obtained allowed a time-resolved study of the strong equatorial reflexions.[3] The intensity, however, was inadequate for the time-resolved registration of the meridional reflexions. These crucial measurements[4] were carried out later on the DORIS storage ring. The main components of muscle are the proteins actin and myosin. The atomic structures of actin and the fragment of myosin involved in contraction (the cross-bridge) have been elucidated by protein crystallography (using synchrotron radiation). By combining these structures with electron microscopy data, it has been possible to arrive at an atomic model of the actin-myosin complex. Electron microscope studies and crystal structures show that the outer end of the cross-bridge moves as a lever arm during contraction.[5] The lever arm movement can be followed by fiber diffraction (see K. Poole, this meeting).

[1]Rosenbaum, G., K. C. Holmes, and J. Witz. 1971., Nature. 230:434-437.
[2]Barrington Leigh, J. and G. Rosenbaum. 1974. J. Appl. Cryst. 7:117-121.
[3]Barrington Leigh, J. and G. Rosenbaum. 1976. Ann. Rev. Biophys. Bioeng. 5:239-270.
[4]Huxley, H. E., et al. 1981. Proc. Natl. Acad. Sci. USA. 78:2297-2301.
[5]Holmes, K. C. 1997. Current Biology. 7:R112-R118.

The Evolution of Synchrotron Radiation as a Key Enabling Technology in Structural Biology and Biophysics

Keith Hodgson
Stanford University and the Stanford Synchrotron Radiation Laboratory, Stanford, California, USA

Synchrotron radiation in the x-ray region became accessible for research in the early 1970s in Europe and in the USA. Early experiments on bending-magnet sources demonstrated that remarkable advances could be made in applications which included x-ray scattering and diffraction and x-ray crystallography. This talk will primarily focus on the first protein crystallography experiments and trace the experimental evolution, including MAD phasing and important technical advances, that coupled to continue to push the forefront and develop the basis for synchrotron science as we know it today.

Early Days of Biologically Oriented XAS at SSRL

Dale E. Sayers
North Carolina State University, Raleigh, North Carolina, USA

From the first days of operation at the Stanford Synchrotron Radiation Laboratory (SSRL) (then known as SSRP) in 1974, it was evident that the new understanding of XAFS proposed by Sayers, Stern, and Lytle in 1971, coupled with the tunability of intensity of the x-rays from a synchrotron source, would make XAS an important tool for studying complex structures. One of the initial experiments using XAS was on a Cu-etioporphyrin, which demonstrated the potential application to metalloproteins. Shortly thereafter, several groups began experimental programs on a variety of biologically oriented problems. These groups not only provided new science but also demonstrated the capability of doing XAS on dilute systems. This talk will review some of the early contributions of several of the groups working at SSRL in the mid to late 1970's, particularly in the context of the subsequent development of this field.

Synchrotron Radiation and Industry

Tony Kossiakoff
The University of Chicago, Chicago, Illinois, USA

Research in the industrial sector has been impacted, in a major way, by the availability of synchrotron sources. The direct effects are obvious: throughput and higher resolution for structure-based design initiatives. However, the availability of these resources has spawned a much more open attitude in the industrial community leading to new efforts in fully basic research applications. The resulting work is published and presented in the public domain. A challenge for the both the academic and industrial sectors is how to best co-partner to make efficient use of structural genomics.

The Impact of Synchrotron Radiation on Structural Biology and Biotechnology

Keith D. Watenpaugh
Pharmacia & Upjohn, Inc., Kalamazoo, Michigan, USA

The impact of synchrotron radiation on structural biology and biotechnology must be considered in the context of developments in molecular biology, cell biology, genomics, and macromolecular crystallography in general. During the past 10-15 years, cloning, expression, purification, and crystallization of proteins have made impressive advances. Genomics and the general understanding of biological processes have opened up vast new opportunities to explain biology at continually more complex molecular structural levels. Software to process the data, solve structures, and analyze structural information has grown from poorly organized individual programs to comprehensive software libraries. Crystallography has continued to be the dominant method of determining the three-dimensional structure of increasingly larger and more complex molecular problems. Complexes of multiple proteins and tens of thousands of atoms are being undertaken and solved. Alternatively, proteins can be broken into their functional domains to study their mechanisms or substrate binding at the atomic resolution level. Not only does 3-D structural biology help in understanding biological processes and molecular mechanisms, it allows for the development of methods to rationally modify biological processes to control them. Pharmaceutical research is focusing increasingly on molecular targets involving understood biological processes. Crystallography is an increasingly important tool in the development of new therapeutic agents in a broad range of diseases, but new factors are influencing structure-based drug design, including combinatorial chemistry, rapid assaying of structural interactions by NMR, structural genomics, high throughput screening, and informatics.

The impact of synchrotron radiation on structural biology and molecular-targeted drug development relies on the broad band width of x-radiation, high collimation, and brilliance. These properties allow for (1) data collection on very small crystals; (2) data collection on crystals with very large unit cells; (3) structure solution using multiwavelength anomalous scattering; and (4) rapid data collection for time-resolved crystallography, and for rapid determination of large numbers of structures for protein engineering, and study of mutations or protein/inhibitor complexes. Reliance of macromolecular structure scientists on synchrotron radiation has increased dramatically. This is quantified in the 1990 and 1997 surveys used in the BioSync reports on the evaluation of resources and needs[1,2] and on data derived from the Brookhaven Protein Data Bank. During this period, the number of new structures determined involving the use of synchrotron radiation has increased from 18% to 44%, and over 90% of macromolecular crystallographers are using synchrotron radiation. Synchrotron usage has tripled during this period and data collection rates have increased dramatically as a result of improved detector technology and radiation brilliance.

[1]"Structural Biology and Synchrotron Radiation: Assessment of Resources and Needs," Structural Biology Synchrotron Users Organization (1991).
[2]"Structural Biology and Synchrotron Radiation: Evaluation of Resources and Needs," Structural Biology Synchrotron Users Organization (1997).

Synchrotrons and Biological Complexity: "The Action Is in the Interaction"

Paul B. Sigler
Yale University, New Haven, Connecticut, and the Howard Hughes Medical Institute

The recombinant DNA era has enabled us to examine the stereochemistry that underlies the mechanisms of a wide variety of macromolecular interactions. The mechanisms of gene regulation, immune response, protein synthesis, signal transduction, viral assembly and infection, and RNA catalysis are just a few exciting examples where the interfaces discovered by high-resolution crystallography have redefined how we think about the chemistry of life processes. While the "blast and saturate" approach of genomics provides a rich context of ground-state structures, it does not address the issues of combinatorial complementarity and the chemical nature of specific functional interfaces. As crystalline asymmetric units required to solve these problems grow in mass and complexity, the crystals themselves often diminish in size, but the bright and tunable synchrotron beam comes to our rescue.

What are the limits/challenges of the single crystal diffraction approach? Are we being lured away from central questions by the ever increasing capabilities of synchrotron-based crystallography to solve bigger but not better complexes?

Synchrotron Radiation Protein Crystallography in the Genomics Era

J. R. Helliwell
Department of Chemistry, University of Manchester, Manchester, UK

Protein crystal structure determination in the context of genome sequencing presents huge challenges and opportunities. The potential for genome level of numbers of protein crystal structure determinations will involve synchrotron radiation (SR) sources and coordination between facilities on a global level, which is practical in the "Internet age." There are at least three levels at which this project [e.g., of a human 3-D genome (proteome) project] can be approached. The first level involves predicting from amino acid sequences where a new protein 3-D fold would be likely. The second involves a systematic, chromosome by chromosome approach of all 100,000 proteins (although 40% are membrane bound, and probably very difficult to "guarantee" crystals). The third approach is where one genome is not enough (i.e., where protein 3-D structure and amino acid sequence comparisons between different human genomes will be pursued; for example, to investigate in detail the genetic basis of relevant diseases). In addition to inter-SR facility coordination, the efficiency of the protein crystallography technique is a critical objective; yet atomic resolution coordinate quality must not be sacrificed. MAD and ultra-high resolution data collection optimisation (including combined protocols), more efficient detectors (like the pixel detector), more automatic crystal sample changing, microfocus beams to work with smaller and smaller crystals, and rapid but accurate structure validation are all needed to increase the rate of determining structures. But can we grow the protein crystals fast enough?

N. E. Chayen and J. R. Helliwell (1998) "Protein Crystallography: The Human Genome in 3-D" Physics World 11, no 5, 43-48.

B. Rost (1998) "Marrying Structure and Genomics" Structure 6, 259-263.

L. Shapiro and C. D. Lima "The Argonne Structural Genomics Workshop" (1998) Structure 6, 265-267.

Sung-Hou Kim (1998) "Taking a Structured Approach to Understanding Proteins" Science 279, 978-979.

X-ray Absorption Spectroscopy in the Era of Synchrotron-based Crystallography: Is It obsolete?

James E. Penner-Hahn
The University of Michigan, Ann Arbor, Michigan, USA

Until recently, x-ray absorption spectroscopy (XAS) was the method of choice for determining the structure of the metal sites in proteins. However, recent dramatic advances in the methodology for protein crystallization and in the capabilities of synchrotron-based crystallographic measurements have led to an explosion in the number of protein crystal structures that are available. In this context, it is appropriate to ask what role remains for x-ray absorption spectroscopy as a tool for structural biology. The future prospects for x-ray absorption spectroscopy, particularly as third-generation synchrotron sources become available, will be discussed. Dramatic improvements in both sensitivity and resolution are likely. These improvements, coupled with an improved appreciation of both the strengths and the weaknesses of XAS, suggest that XAS will continue to play a critical role in structural biology.

The Potential of X-ray Free-electron Lasers Based on Superconducting Linear Accelerators

Jochen R. Schneider
HASYLAB at DESY, Hamburg, Germany

Based on the parameter list of the x-ray free-electron laser (FEL) planned for the TESLA project at DESY, the potential for such facilities to provide coherent radiation with wavelengths down to 1 Å for biology and biophysics will be presented, emphasizing brilliance and time structure. At present, a VUV FEL based on the same SASE principle and the same technology is under construction at DESY, which will offer new opportunities in spectroscopy and imaging.

A Pixel Array Detector for Time-resolved X-ray Diffraction*

Eric F. Eikenberry
Robert Wood Johnson Medical Schoool, Piscataway, New Jersey, USA

G. Rossi, M. W. Tate, and S. M. Gruner
Physics Department, Cornell University, Ithaca, New York, USA

S. L. Barna
Photobit, Inc., La Crescenta, California, USA

P. J. Sellin
Department of Physics, University of Surrey, Guildford, UK

R. L. Wixted
Brunswick, Maine, USA

An all silicon pixel array detector (PAD) with 150-micrometer pixels has been designed and tested as a prototype. The PAD is a two-layer charge-integrating device consisting of a diode layer bump-bonded to a MOS electronic storage and readout layer. The advantages of this design are essentially no x-ray dose rate limitation and no requirement for intermediate conversion of the energy to visible light. The diode layer, fabricated on 300-micrometer thick silicon, converts greater than 99% of 8 keV x-rays and provides full coverage of the active area with no gaps between pixels. The electronic layer stores charge from the diodes for eight successive frames at 1-microsecond (or longer) intervals before readout. The PAD can also be operated continuously. A 4 x 4 pixel prototype was tested and shown to have a well depth of about 20,000 8 keV x-rays, noise corresponding to five 8 keV x-rays, linearity within 0.2%, and no measurable cross-talk between pixels. Operational characteristics and radiation tolerance will be discussed. A 100 x 92 array with 151.2-mm square pixels and a single readout port has been constructed and is currently being tested. A 100 x 92 device with a gallium arsenide diode array has also been fabricated.

*This work is supported by US Department of Energy grants DE-FG02-87ER60522 and DE-FG02-96ER14652.

Cryocrystallography: Present Highlights and Future Prospects

Elspeth Garman
University of Oxford, Oxford, UK

Advances in cryocrystallographic techniques over the last eight years[1, 2, 3] have made a significant impact on the utility of second- and third-generation synchrotron radiation for macromolecular crystallography. In many cases, these techniques are now an essential ingredient for collection of good quality diffraction data and are enabling full exploitation of the intense x-ray beams available. The impetus for the new developments has been the greatly reduced radiation damage observed in crystals flash frozen and held at around 100 K during data collection. However, there are additional benefits of cryo-techniques, resulting in new experimental possibilities (e.g., use of microcrystals and very fragile crystals, ultra-high resolution data, MAD, cryo-enzymology, and virus crystallography), some of which will be highlighted in this talk. The advent of more intense synchrotron beams will involve a dual challenge to contain the inevitable increase in radiation damage: Can we collect data even faster to minimize the time dependent component, and can we achieve any additional protection of crystals from the dose-dependent damage? The new generation of CCD detectors is improving the duty cycle during synchrotron data collection, thus reducing the time for which a crystal must survive after initial irradiation. The recent announcement of a commercially available open-flow helium cryostat for use with single crystals paves the way for serious experimentation down to a temperature of around 30 K. Primary radiation damage is *de facto* unavoidable, but will the spread of secondary damage be affected by reducing the temperature of the cryo-data collection?

[1]T.Y. Teng (1990) J.Appl. Cryst. 23, 387-391.
[2]D. Rodgers, (1997) Methods Enzymol, 276, 183-203.
[3]E.F. Garman and T.R. Schneider, (1997) J. Appl. Cryst. 30, 211-237.

Design and Testing of X-ray Fluorescence Detectors Using Synthetic Multilayers

Ke Zhang
Illinois Institute of Technology, Chicago, Illinois, USA

Gerd Rosenbaum
Argonne National Laboratory, Argonne, Illinois, USA

Grant Bunker
Illinois Institute of Technology, Chicago, Illinois, USA

It is a concern that the 13-element Ge detector, commonly used for x-ray fluorescence detection on dilute systems, will not be compatible with the third-generation sources due to its count rate limitations and the high photon flux from the source. Thus, we are developing energy-resolving x-ray fluorescence detectors using synthetic multilayers. This type of detector can be built with good energy resolution and reasonable solid angle, and is tunable over a wide energy region. We will present the development of multilayer array detectors using graded multilayers. Tests on a prototype multilayer array detector showed superb background rejection and reasonable efficiency. The design and testing of a 20-element multilayer array detector will also be presented.

Diffraction Enhanced X-ray Imaging

Dean Chapman
Illinois Institute of Technology, Chicago, Illinois, USA

Diffraction enhanced imaging (DEI) is a new x-ray imaging method that can be used to independently visualize the refraction and absorption of an object. DEI, developed using a synchrotron x-ray source, produces images that are almost completely scatter-free, which results in an additional source of contrast from the rejection of small-angle scattering. The combination of these two new sources of contrast (refraction and scatter rejection) with absorption has resulted in images of mammography phantoms and tissues that are dramatically improved over standard x-ray radiography. It is potentially applicable to mammography and to radiology in general, possibly for use in non-destructive testing and x-ray computed tomography. The technique and analysis will be presented, as well as recent applications to imaging breast tissue samples, materials imaging (NDT), and tomography. Future research directions and plans to apply the technique will also be addressed.

The Design and Prototype of a Detector for Protein Crystallography*

Nguyen-Huu Xuong
University of California at San Diego, La Jolla, California, USA, and
Lawrence Berkeley National Laboratory, Berkeley, California, USA

Philip Datte
University of California at San Diego, La Jolla, California, USA

Jacques Millaud and Eric Beuville
Lawrence Berkeley National Laboratory, Berkeley, California, USA

The Digital Pixel Array Detector (DPAD), specifically designed for third-generation synchrotrons, is truly a unique photon counting detector with an extremely high throughput (up to 10^{10} photons per second for a 1000 x 1000 pixel detector). Three applications in macromolecular crystallography are as follows: (1) In a standard rotation method, it can easily collect data at a rate of 1° per second, i.e., finish a data set in 90 seconds. (2) In a MAD data collection procedure, one can use the fine slice method (i.e., read out at 0.1° instead of every 1°) without losing time or adding noise due to the read out process. (3) In a time-resolved Laue experiment, DPAD can automatically store eight (or more) successive frames with an exposure of 10 ms and a switching time of a few microseconds.[1]

The room-temperature detector is a high-resistivity N-type Si with a pixel pitch of (150 x 150) microns, a thickness of 300 microns, and is bump bonded to an application-specific integrated circuit (ASIC). The detector event driven readout is based on the column architecture and allows an independent pixel hit rate above one million photons/sec/pixel over the entire detector. The device provides energy discrimination and sparse data readout which yields minimal deadtime. This type of architecture allows a continuous (frameless) data acquisition. For the targeted detector size of (1000 x 1000) pixels, average hit rates of 10 billion photons/sec for the complete detector appears achievable. The detector has almost infinite photon counting dynamic range and exhibits superior spatial resolution when compared to present crystallographic phosphor imaging plates or phosphor-coupled CCD detectors.

An 8 x 8 pixel array x-ray detector prototype has been built and tested. To characterize the analog portion of the readout and the digital characteristics of the detector, the pixel electronics contain only the analog portion of the circuit and are independent of the surrounding cells. The conversion of a photon hit into a pixel address is generated by conventional external electronics. The measured results are very encouraging. The analog electronics demonstrate the capability of processing charge pulses at a rate of 1×10^6 photon/sec/pixel, with an energy resolution of 480 eV (FWHM at 5.9 keV) at room temperature. The detector displays uniform digital behavior and has a very low point-spread function. The full width at 1/100 maximum is less than one pixel width (150 μm), which is less than 1/2 that of CCD and 1/7 that of an imaging plate.[2]

A 16 x 16 pixel array with most of the readout electronics included in the ASIC has been designed and tested. The 16 x 16 device, which includes both analog and digital circuitries on the same chip, continues to display the remarkable low point-spread functions and similar low-noise characteristics of the 8 x 8 analog-only device. More test results will be presented at this conference.

*Work funded from the NIH-RR-10748 and from the Lucille P. Markey foundation.

[1]E. Beuville et. al., IEEE, Transactions of Nuclear Science, 43 (3) (1996) pp. 1243-1247.
[2]P. Datte et. al., Nuclear Instruments and Methods in Physics Research, A391 (1997) 471-480.

Time-resolved Techniques for Muscle Diffraction with Synchrotron Radiation*

Hugh Huxley
Brandeis University, Waltham, Massachusetts, USA

Tom Irving
Bio-CAT, Advanced Photon Source, Argonne National Laboratory, Argonne, Illinois, USA

Alex Stewart
Brandeis University, Waltham, Massachusetts, USA

The x-ray diffraction diagrams from native, functioning muscle can be detailed and informative, but muscle is a very weak diffractor and therefore the intense x-ray beams provided by synchrotron radiation are essential in current research on the mechanism of contraction. The central area of interest is the detailed behavior of the myosin crossbridges as they go through their cycles of interaction with actin, which produce the relative sliding motion of the actin and myosin filaments past each other, which leads to muscle shortening. The various steps in these cycles take place on a millisecond time scale, so the changes in x-ray patterns need to be recorded with this time resolution. Small but very important changes in filament axial periodicity also occur during muscle activity due to filament compliance, and these have a large effect on the interpretation of mechanical transient measurements, which provide crucial evidence about the characteristics of the crossbridge cycle. Thus, spacing changes need to be measured to an accuracy of .05% or less, again with millisecond time resolution.

We have been using imaging plates and CCD detectors (which both give the necessary spatial resolution at convenient camera lengths) in a number of different ways to achieve the required combinations of high spatial and temporal resolution. (1) For experiments in which we require a tetanic contractions of a muscle (at 2-4 min. intervals to allow time for recovery), we use either an imaging plate on which the x-ray pattern is accumulated over 50-100 cycles of contraction, or a CCD array where the whole array is read out and stored between contractions (to avoid the accumulation of dark current noise). In each case, an intermediate speed shutter allows the x-ray beam to fall on the muscle only during the required time interval. Control patterns (either rest or isometric contraction) are recorded during alternate cycles; in the case of the imaging plate by displacing the plate sideways between exposures and arraying a blanking-off plate to move alternately to one side or the other so as to record the two types of image (e.g., fast shortening vs. rest) on the same plate, side-by-side. (2) For higher resolution (say 10 msec), in a succession of up to nine time frames, a 128 pixel wide section at one side of a CCD is used (with a fiber-optic taper) to record the required region of the pattern (e.g., a meridional slice), the images are shifted sideways electronically between each time frame (separated by a rapid acting shutter) so that the nine successive images are temporarily stored on the CCD and then read out between contraction cycles. (We have also used a rotating imaging place to obtain time courses from 'streak patterns'). (3) For the highest time resolution (1-2 msec, during rapid mechanical transients) we have so far used only imaging plates plus a rapid mechanical shutter, so that only one single time point is obtained each contraction cycle. However, we hope to speed up the CCD frame-shifting in the future. A number of new results will be discussed.

*Supported by NIH Grants Nos. AR43733 (H.E.H.) and RR08630 (Bio-CAT).

Structural Studies of Model Metalloprotein Maquettes Vectorially Oriented at a Soft Interface

J. Kent Blasie
University of Pennsylvania, Philadelphia, Pennsylvania, USA

J. Strzalka, X. Chen, P. L. Dutton, and B. Ocko
University of Pennsylvania, Philadelphia, Pennsylvania, USA, and
National Synchrotron Light Source, Brookhaven National Laboratory, Upton, New York, USA

De novo synthetic models or "maquettes" of prototypical electron transport membrane proteins, based on a four-helix bundle motif with selected positions for liganding one or more prosthetic groups (e.g., heme groups), have been designed and synthesized by Prof. P. L. Dutton's research group at the University of Pennsylvania. Correlated structural and electrochemical studies of such maquettes depend on our ability to orient them vectorially at an interface with the axis of the bundle perpendicular to the interface. While sedimentation and NMR methods suggest that the dihelices spontaneously assemble to form a native four-helix bundle in bulk aqueous solution, x-ray reflectivity studies have shown that the bundle is unstable to an air-water interface with both α-helices of the dihelix lying in the plane of the interface irrespective of the surface pressure in a Langmuir monolayer. Subsequently, a palmitic acid hydrocarbon chain (C16) was covalently linked to the amino-terminus of each helix of the dihelix to make the maquette a better amphiphile. X-ray reflectivity studies of this palmitoyl-derivative of the maquette itself and of its binary mixtures with palmitic acid and DMPE have shown that the α-helices of the dihelix are oriented perpendicular to the air-water interface at higher surface pressures within the Langmuir monolayer. X-ray interferometry studies have shown that these Langmuir monolayers can be transferred via the Langmuir-Blodgett technique to an alkylated inorganic substrate retaining their orientation perpendicular to the substrate plane. Such vectorially oriented single monolayers of these maquettes are ideally suited for resonance x-ray reflectivity and interferometry studies of the metal atom locations of their prosthetic groups and for grazing-incidence x-ray diffraction studies of their secondary/tertiary/quaternary structures.

The Mechanisms of Self Assembly and Polymorphic Switching of the Bacterial Flagellar Filament

Keiichi Namba, Kazuya Hasegawa, Ichiro Yamashita, Yuko Mimori-Kiyosue, Hirofumi Suzuki, and Ferenc Vonderviszt
International Institute for Advanced Research, Matsushita E.I. Co., Ltd., Seika, Japan

The bacterial flagellum is a helical filament, rotated by the motor at its base, that works as a screw tp propel the cell. Yet, it is not a simply rigid propeller. The filament is normally in a left-handed, supercoiled form and several of them form a bundle when bacteria swim. Upon quick reversal of the motor rotation, the filaments switch into right-handed supercoils, making the bundle fall apart, enabling the cell to tumble for its tactic behavior. The filament is a tubular structure formed by self-assembly of single protein flagellin in a helical manner. The supercoiling is thought to involve two distinct subunit conformations or packing, and its mechanism is interesting in terms of conformational distinctness and adaptability of flagellin. X-ray fiber diffraction and electron cryomicroscopy have been used to analyze the structures of various straight filaments. We developed a new method to orient liquid crystalline sols of filamentous macromolecular assemblies, by which the flagellar filaments have been aligned to 0.6-degree disorientation. X-ray diffraction from these specimens allowed us to measure the layer-line spacings, helical symmetries, and layer-line amplitudes accurately. With phases from the EM analysis, we obtained an electron density map at 9-Å resolution, which showed the packing of alpha-helices aligned along the protofilament in the core domain. About 65 N-terminal and 45 C-terminal, residues of flagellin are disordered in the monomeric form, and proper interactions between the termini are essential for correct folding of these regions in the very inner core of the filament. Thus, these disordered portions are responsible for preventing spontaneous filament formation by monomers alone in the self-assembly mechanism. The structures of the L- and R-type straight filaments, which are thought to represent the two states of flagellin subunits that coexist in supercoiled filaments, showed only a small difference in the subunit packing and no appreciable differences in the overall subunit shapes. The intersubunit distance along the 11-stranded protofilaments is 52.7 Å and 51.9 Å for the L- and R-type, respectively; the L-type is longer than the R-type by 0.8 Å, quantitatively explaining the observed forms of supercoils based on a two-state subunit model. The conformational switching between the two states appears to be mutual sliding of the alpha-helical bundles in the core domain. The difference of 0.8 Å is produced by two distinct sliding distances, 1.8 Å and 2.6 Å, at two different intersubunit interfaces, respectively. These sliding movements are triggered by the shear force at the protofilament interface, which is produced by conversion of the twisting force by quick reversal of the motor rotation by the structure and packing of the flagellin subunits.

A Fibre Diffraction and Atomic Modeling Study of the Actomyosin Complex

Katrina J. V. Poole, M. Lorenz, K. C. Holmes, P. Ellison, and M. Furch
Max Planck Institute for Medical Research, Heidelberg, Germany

C. Cremo
Washington State University, Pullman, Washington, USA

A. Szent-Gyorgi
Rosenstiel Center, Brandeis University, Boston, Massachusetts, USA

P. Boesecke
European Synchrotron Radiation Facility, Grenoble, France

G. Evans and G. Rosenbaum
Advanced Photon Source, Argonne National Laboratory, Argonne, Illinois, USA

The molecular mechanism by which motor proteins convert chemical energy into mechanical force or motion is a central question in protein biology. In muscle, it is the globular heads of myosin (subfragment-1 or S1) projecting from the thick filaments that split ATP and interact cyclically with actin filaments to pull them along. Atomic coordinates are now available for the individual proteins actin and myosin-S1 (Kabsch *et al.* Nature 347: 37-44, 1990 & Rayment *et al.* Science 261: 50-58, 1993) but, unfortunately, not for a complex of the two. Cryo-electronmicroscopic studies to date yield 20 to 30-Å resolution density maps of the nucleotide-free skeletal S1-decorated actin (Milligan *et al.* J. Cell Biol. 105: 29-39, 1987 & Whittaker *et al.* Nature 378: 748-751, 1995), and the current standard model of the actomyosin complex represents a fit of the S1 crystal structure and the Holmes *et al.* atomic model of F-actin (Nature 347:44-49, 1990) into such a density map (Rayment *et al.* Science, 261:58-65, 1993). We are using high-angle fibre diffraction data from myosin-decorated actin arrays to further refine this model and to probe different attached states. The experiment is to decorate the thin filaments of stretched rabbit striated muscle fibres with myosin motor fragments and to measure the associated intensity changes in the actin-based fibre diffraction patterns out to 8 Å and better. The diffracting power of the specimen is low, and the experiment is only possible with a "clean," finely focused synchrotron x-ray beam. We used a ~60 cm specimen-to-detector distance, wavelengths of 1-1.3 Å, and a 20 x 25 cm Fuji imaging plate on the NSLS beamline X9B or on the ESRF beamline 4, ID2. So far, we have looked at nucleotide-free chicken skeletal chymotryptic and papain S1 fragments, chicken smooth muscle S1 (phosphorylated and unphosphorylated), scallop fast adductor S1 and Dictyostelium catalytic domain (761). We have refined a model structure against the data from skeletal chymotryptic S1 decorated filaments by allowing the S1 to move with respect to the actin monomer and to bend at appropriate domain junctions until the computed diffraction pattern best matched the data. The result is a structure similar but not identical to the Rayment model. All S1 fragments looked at so far show characteristic x-ray decoration patterns indicating that there are different types of bound head structures in rigor. X-ray patterns from all but the striated muscle chymotryptic S1 decorated fibres show reversible changes on ADP binding. The effect was most striking in the smooth S1 and very slight in the skeletal papain-S1. Like Whittaker *et al.* 1995, we believe that the large structural change in the case of smooth S1 comes primarily from a movement of the lever arm. Third-generation synchrotron beamlines now deliver sufficient x-ray intensity to allow us the fascinating possibility of monitoring the kinetics of such structural changes in bound motor fragments, although the availability of rapid, high-count-rate area detectors is still a problem. The immediate way forward is to use nucleotide analogues and specifically engineered motor fragments in steady-state experiments to search for alternative bound head structures, which may represent different stages of the powerstroke we seek to understand.

Structural Dynamics of Actomyosin in Muscle Contraction by X-ray Diffraction/Scattering

Katsuzo Wakabayashi
Osaka University, Osaka, Japan

The sliding or contractile force in muscle is thought to be generated by the interaction of myosin heads projecting from the thick filaments with actin in the thin filaments, powered by hydrolysis of ATP in the sarcomere. X-ray diffraction has provided a structural basis for the mechanism underlying this process, showing that the interaction between actin and myosin heads occurs in incommensuate periodicities of the two filaments. The excellent x-ray diffraction patterns from a contracting muscle have been recorded by using a high-sensitivity, high-resolution storage phosphor area detector. Our analysis showed that during contraction, the structure of the thin actin filaments was altered and that the alteration was accompanied by the elastic extension and twisting of the helical filaments.[1] The amount of the extension of the actin filaments corresponded to 60-70% of the total extention of the elastic elements in the sarcomere which carries active tension.[2] These findings provide evidence that the thin actin filaments are flexible and very pliant, leading us to make a significant modification of the currently accepted "rotating or tilting crossbridge" model. On the other hand, it has been shown by x-ray solution scattering that the global conformational change is isolated myosin heads (subfragment-1, S1) corresponding to an approximate 5-nm movement of the distal end of the molecule occurred during hydrolysis of ATP.[3] The experiments using ATPase intermediate analogs indicated that such a global change occurred in the state of S1*.ADP.Pi, and returned to the original structure in two steps, on release of Pi and release of ADP. This implication has been tested in active muscle by time-resolved x-ray diffraction in a ~0.2-ms resolution using a high-brilliance beam from the undulator installed at the Tristan main ring.[4] On applying a sinusoidal length perturbation at 500 Hz to a contracting muscle, tension changed in phase with the length changes, and the intensity of the 14.5-nm myosin-based reflection changed in antiphase with tension changes. The results indicated that there exists a coupling between the force generation and structural changes occurring in the distal portion of myosin heads which are interacting with actin. Several important findings stimulate a sophisticated model in a framework of the coordinated structural changes of actin and myosin heads.

[1]Wakabayashi and Amemiya, Handbook Synchrotron Rad., 4, 597 (1987); Ueno et al., submitted for publication (1998).
[2]Wakabayashi et al., Biophys. J., 67, 2422 (1994); Takezawa et al., submitted for publication (1998).
[3]Sugimoto et al., Biophys. J., 68, 29 (1995); Sugimoto et al., submitted for publication (1998).
[4]Yagi et al., J. Synchrotron Rad., 3, 305 (1996).

Solution Scattering Studies of Protein Conformations and Interactions in Biochemical Regulation

Jill Trewhella
Los Alamos National Laboratory, Los Alamos, New Mexico, USA

Joanna Krueger, Jinkui Zhao, and Sharron Francis
Vanderbilt, Nashville, Tennessee, USA

James T. Stull
UT Southwestern Medical Center, Dallas, Texas, USA

Donal A. Walsh
The University of California, Davis, California, USA

Small-angle solution scattering, using both x-rays and neutrons, has provided key insights into the regulation of kinase activity. Small-angle scattering from proteins in solution is particularly sensitive to domain movements, as well as protein-protein associations. We have characterized the conformational transitions and associations in the activation mechanism of the Ca^{2+}/calmodulin-dependent kinase myosin light chain kinase (MLCK), as well as in two cyclic nucleotide-dependent protein kinases. The kinase family, with > 500 members identified to date, has a highly conserved catalytic core that consists of a large mostly alpha-helical domain and a smaller predominantly beta-structured domain. The cleft between the large and small domains encompasses all the elements of the protein required for phosphorylation of protein substrates. The structure of the catalytic subunit of the cAMP-dependent dependent kinase was the first of this class of enzymes to be solved by x-ray crystallography, and this first structure was of the enzyme with a bound peptide pseudosubstrate. We used small-angle x-ray scattering to show that the effect of pseudosubstrate binding was to close the catalytic cleft, thus bringing all of the elements required for catalysis together in close proximity to the substrate binding sites.[1] We further showed that this cleft closure was achieved via a hinge formed by a pair of glycine residues that are highly conserved in the kinase family tree branches that contain the calmodulin-dependent and cyclic nucleotide-dependent protein kinases. Catalytic activity in these kinases is commonly regulated by inhibitory mechanisms that use a pseudosubstrate sequence either as part of an auto-regulatory domain in the case of the calmodulin-dependent kinases, or from a regulatory partner as is the case for the cyclic nucleotide-dependent protein kinases. Binding of Ca^{2+} to calmodulin (CaM) which then binds to the kinase, or cyclic nucleotide binding to a regulatory partner, releases the inhibition. In our solution scattering studies of the Ca^{2+}/CaM/MLCK activation mechanism, we have evaluated the Ca^{2+}-dependent binding of MLCK to the enzyme showing that binding occurs with substoichiometric Ca^{2+} concentrations,[2] determined the conformational transitions undergone by both the kinase and CAM upon complex formation,[3] and the effects of substrate binding on the complex.[4] Our solution scattering studies of the cAMP-dependent protein kinase have revealed the quaternary structure of the kinase, which has two identical catalytic and two identical regulatory subunits, and also revealed information on the conformation of the catalytic subunit in its inhibited state.[5] Our studies of the cGMP-dependent protein kinase have elucidated information on the conformational transitions induced by cGMP binding and subsequent release of this kinase inhibition.[6] Small-angle scattering instrumentation developments

at synchrotron facilities have significantly accelerated the rate at which these types of experiments can be done, and thus have enabled us to systematically track the conformational transitions and associations within specific activation pathways.

[1]Olah, G. A., Sosnick, T. R., Mitchell, R., Walsh, D. A., & Trewhella, J. "The Solution Structure of the cAMP-dependent Protein Kinase Catalytic Subunit and Its Contraction upon Binding of the Protein Kinase Inhibitor Peptide," *Biochemistry 32*, 3649-3657, 1993.
[2]Krueger, J., Bishop, N. A., Blumenthal, D. K., Zhi, G., Beckingham, K., Stull, J. T., & Trewhella, J. "Calmodulin Binding to Myosin Light Chain Kinase Begins at Substoichiometric Ca^{2+} Concentrations: A Small-Angle Scattering Study of Binding and of Conformational Transitions," to be submitted, 1998.
[3]Krueger, J. K., Olah, G. A., Rokop, S. E., Zhi, G., Stull, J. T., & Trewhella, J. "The Structure of $4Ca^{2+}$/Calmodulin and a Functional Myosin Light Chain Kinase in the Activated Complex," *Biochemistry 36*, 6017-6023, 1997.
[4]Krueger, J., Zhi, G., Stull, J. T., & Trewhella, J. "Neutron Scattering Studies Reveal Further Details of the Ca^{2+}/Calmodulin-Dependent Activation Mechanism of Myosin Light Chain Kinase," submitted, 1998.
[5]Zhao, J., Hoyer, E., Boylen, S., Walsh, D. A., & Trewhella, J. "Quaternary Structure of the cAMP-Dependent Protein Kinase by Neutron Contrast Variation," to be submitted, 1998.
[6]Zhao, J., Trewhella, J., Corbin, J., Francis, S., Mitchell, R., Bruschia, R., & Walsh, D. A. "Progressive Cyclic Nucleotide-Induced Conformational Changes in the cGMP-Dependent protein Kinase Studied by Small-Angle X-ray Scattering in Solution," *J. Biol. Chem. 272*, 31929-31936, 1998.

Biophysics and Synchrotron Radiation: When the Marriage Fails*

Martin Caffrey
The Ohio State University, Columbus, Ohio, USA

Anchi Cheng
Scripps Research Institute, La Jolla, California, USA

The damaging effects of synchrotron-derived x-rays on aqueous phospholipid dispersions have been evaluated. The effect of degree of lipid hydration, phospholipid chemical structure, mesophase identity, aqueous medium composition, and incident flux on the severity and progress of damage was quantified using time-resolved low- and wide-angle x-ray diffraction and chromatographic analysis of damage products. Electron-spin resonance measurements of spin-trapped intermediates generated during irradiation suggest a free-radical-mediated process. Interestingly, radiation effects revealed by x-ray diffraction were imperceptible when the lipid was prepared at less than full hydration despite the fact that x-ray-induced chemical breakdown of the lipid occurred regardless of hydration level. Of the fully hydrated lipid systems studied, saturated diacyl-phosphatidylcholines (PC) were most sensitive to radiation damage compared to the ester- or ether-linked phosphatidylethanolamines or the ether-linked PCs. The inclusion of HEPES, Tris/HCl, phosphate buffer, or indeed sodium chloride in the aqueous dispersing medium had only a minor effect in reducing x-ray damage development.

A small, inverse dose-rate effect was found when the x-ray beam intensity was changed 15-fold. These results contribute to our understanding of the mechanism of radiation damage, to our appreciation of the importance of monitoring structure and composition when evaluating biomaterials radiation sensitivity, and to the development of strategies for eliminating or reducing the severity of damage due to synchrotron x-radiation. Since damage is shown to be free-radical mediated, these results also have a bearing on age-related accumulation of free radicals in cells and how these might compromise membrane integrity culminating in cell death.

*Work supported by grants from the National Institutes of Health (DK 36849, DK 46295, GM56969).

X-ray Microscopy: Prospects and Current Developments

Günter Schmahl
University of Göttingen, Göttingsen, Germany

The properties of x-rays that give them their special role in microscopy are short wavelengths – which allow high spatial resolution, high penetrating power, and a near absence of impedance mismatch at interfaces, which is important for imaging of thick specimens. X-ray energies suited for microscopy span K-shell and L-shell resonances of many elements of the periodic chart providing natural contrast mechanisms, as well as chemical bond mapping. Therefore, in several countries in recent years, there have been a considerable number of developments of x-ray microscopes and scanning x-ray microscopes, especially those using zone plate optics and synchrotron-radiation sources. Besides systems working with x-rays at energies between the carbon and oxygen K edges at 284 eV and 534 eV respectively, in the so-called water window, systems are under development for harder x-rays in the keV-region. X-ray microscopy is currently being used for investigations in biology, medical research, colloid physics, and soil sciences, as well as in material research (for example, to study polymer structures or magnetic domain structures in magnetic material). Especially for biological applications, cryo x-ray microscopy is of importance. It has been shown that chemically unfixed biological specimens in a vitrified state are able to tolerate a radiation dose of up to 1010 Gray without observable structural changes. This allows x-ray microscopic tomographic imaging to reveal the 3-D structure of such specimens. To obtain information about the localization of proteins, especially in cell nuclei, metal-conjugated antibody probes can be used. It is a major challenge in the field to combine antibody labeling with cryo and tomography techniques.

Cryo Samples with a Scanning Transmission X-ray Microscope: Imaging, Tomography, and Spectromicroscopy

C. Jacobsen, R. Fliller, J. Kirz, J. Maser, A. Osanna, Y. Wang, M. Weigel, and B. Winn
SUNY, Stony Brook, New York, USA

Cryo x-ray microscopy allows one to study whole, wet, unsectioned cells which have not been modified by the use of chemical fixatives. We describe a cryo scanning transmission x-ray microscope at the NSLS. The microscope allows high-resolution imaging without limitations from radiation damage. Tomographic imaging of fibroblasts will be described, as will efforts toward compositional mapping of cells using near-edge absorption resonances.

High-resolution Soft X-ray Microscopy of Medically Important Protozoa

Cathleen Magowan, Narla Mohandas, and Werner Meyer-Ilse
Lawrence Berkeley National Laboratory, Berkeley, California, USA

Some of the most pernicious diseases afflicting humankind are caused by parasitic protozoa such as *Plasmodium falciparum*, the parasite that causes malaria. The 1-5 micron malarial parasite spends a large part of its life cycle within a red blood cell, where it metabolizes hemoglobin in its digestive vacuole; elaborates a tubulo-vesicular network for protein transport within the red cell cytosol; and inserts neoantigens in the red cell membrane to alter its antigenicity, morphology, and function. The results of these processes have been detected and investigated using the soft x-ray microscope developed by the Center for X-Ray Optics at the Advanced Light Source in Berkeley. Our earliest studies established the morphology and structural development of parasites in normal erythrocytes and enabled us to then study aberrations in parasites that developed either in abnormal erythrocytes or in the presence of antimalarial drugs.[1] We made an important advance recently by utilizing an immuno gold labeling technique that is compatible with soft x-rays for localizing antigens on the red cell surface.[2-4] We are evaluating whether this method can be used quantitatively, and we are developing novel labeling techniques to examine the alterations of phospholipid asymmetry in sickle cells infected with malarial parasites.[5] Our progress over the past two years and the promise of recent innovations in soft x-ray microscopy of intraerythrocytic malarial parasites demonstrates the value of this approach for biomedical research.

[1]Magowan, C., Brown, J. T., Liang, J., Heck, J., Coppel, R. L., Mohandas, N., and Meyer-Ilse, W. 1997. Intracellular structures of normal and aberrant *Plasmodium falciparum* malaria parasites imaged by soft x-ray microscopy. Proc. Natl. Acad. Sci. USA 94:6222-6227.
[2]Chapman, H. N., Fu, J., Jacobsen, C., Williams, S. 1996. Dark-field x-ray microscopy of immunogold-labeled cells. J. Micro. Soc. Am. 2:53-62.
[3]Chapman, H. N., Jacobsen, C., Williams, S. 1996. A characterization of dark-field imaging of colloidal gold labels in a scanning transmission x-ray microscope. Ultramicroscopy. 62:191-213.
[4]Yeung, J., Brown, J. T., Nair, A., Meites, E., Coppel, R. L., Narla, M., Meyer-Ilse, W. and Magowan, C. 1998. X-ray microscopic visualization of specific labeling of adhesive molecule CD36 and cytoadherence by *Plasmodium falciparum* infected erythrocytes. Res. Commun. Mol. Pathol. Pharmacol. 99: 245-258.
[5]Kuypers, F. A., Lewis, R. A., Hua, M., Schott, M. A. Discher, D., Ernst, J. D. and Lubin, B. H. 1996. Detection of altered membrane phospholipid asymmetry in subpopulations of human red blood cells using fluorescently labeled annexin V. Blood 87:1179-1187.

High-resolution X-ray Imaging of Frozen Hydrated Samples in Amplitude and Phase Contrast

G. Schneider, B. Niemann, P. Guttmann, and G. Schmahl
Forschungseinrichtung Roentgenphysik, Georg-August-Universitaet Goettingen, Goettingen, Germany

X-ray microscopy provides higher resolution than optical microscopy and higher penetration power than electron microscopy. Therefore, x-ray microscopy allows high-resolution imaging of thick hydrated samples. The two dominating processes determining the contrast in x-ray microscopy are photoelectric absorption and phase shift. For this reason, x-ray microscopy can be performed in amplitude and phase contrast. The Goettingen x-ray microscope at the BESSY electron storage ring in Berlin is operating in both contrast modes and is used for different application fields, for example in biology, biophysics, medicine, colloid chemistry, and soil sciences. Biological objects are especially sensitive to ionizing radiation. Theoretical investigations show that x-ray images of frozen hydrated specimens can be obtained without radiation-induced artifacts. Therefore, an object stage for cryogenic specimens was developed and implemented on the Goettingen transmission x-ray microscope (TXM) at the electron storage ring BESSY. It allows objects to be imaged at temperatures below 120 K in cryogenic nitrogen gas at atmospheric pressure. This system was used to perform experiments with initially living biological objects, e.g., cells, chromosomes, and algae. Amplitude and phase contrast x-ray images show details inside the frozen hydrated objects as small as 30 nm with high contrast, and the cryogenic samples show no structural changes. The preparation of the samples was accomplished by shock freezing in liquid ethane to cryogenic temperatures. Furthermore, it was demonstrated that at cryogenic temperatures the structural stability of hydrated biological objects is increased by about four orders of magnitude compared to unfixed wet specimens at room temperature. It is now apparent that multiple imaging of a frozen hydrated sample done at different viewing angles will reveal images of the three-dimensional morphology, e.g., of whole cells, which are close to the natural, functional state.

Synchrotron Radiation in Research and Clinical Medicine*

William Thomlinson
National Synchrotron Light Source, Brookhaven National Laboratory, Upton, New York, USA

The application of synchrotron radiation to research and clinical medicine generates a lot of interest because it deals with some of today's most fundamental and important medical problems. Thus, it is easy to relate to the research, which has exciting potential for advancements in the field of medicine. This talk will give an overview of significant developments in the field from the pioneering Transvenous Coronary Angiography projects to computed tomography, micro-planar beam radiation therapy, and diffraction enhanced imaging (DEI) for mammography. Most of the medical programs use or have used phantoms and animal models. However, there have been human coronary angiography studies at SSRL, NSLS, Photon Factory, and an on-going major pre-clinical trial at HASYLAB. Operation of the ESRF Medical Research Facility and the mammography beamline at ELETTRA promise a significant increase in efforts over the next few years. Medical research is also planned at SPring-8. During the past year, interest has developed at a number of laboratories in mammography imaging using diffraction enhanced imaging. Originally developed at the NSLS, DEI experiments have already been carried out at APS, ESRF, and ELETTRA, and a program is being planned at Daresbury Laboratory. All of these efforts are developing new techniques using synchrotron radiation as a "gold standard" x-ray source. Independent of success as research programs at synchrotrons, some of the techniques may never advance to routine clinical use. Synchrotrons, unless available in truly compact size, are too costly and access is very limited. The synchrotron medical community has to find the balance between unique utilization of the large machines for research and clinical applications and the need to expand the techniques into clinical and hospital environments. Efforts are underway to develop compact sources based on synchrotron and other technologies.

*Work supported by the US Department of Energy under Contract # DE-AC02-CH10886.

Intravenous Coronary Angiography with Synchrotron Radiation: Experience in 366 Patients

W. R. Dix, T. Dill, M. Jung, M. Lohmann, B. Reime, and R. Ventura
HASYLAB at DESY, Notkestrasse, Hamburg, Germany

Worldwide efforts have been made to image coronary arteries by non-invasive or minimally invasive techniques. One method under development is dichromography. It represents a digital subtraction angiography method based on the subtraction of two images at different energies. Dichromography allows imaging of small, fast-moving objects like the coronary arteries, inclusive distal parts, and sidebranches after intravenous injection of contrast media down to a diameter of less than 1 mm. Two images with monochromatic x-rays just below and above the absorption K-edge of the iodine-containing contrast agent at 33.17 keV are simultaneously obtained and logarithmically subtracted. Monochromatic x-rays of sufficient intensity to visualize coronary arteries of 1-mm diameter with an extremely low iodine mass density of 1 mg/cm^2 are only provided by synchrotron radiation.

At the Hamburger Synchrotronstrahlungslabor HASYLAB at DESY in Hamburg, Germany, the system NIKOS was developed for dichromography. This line scan system consists of six main parts: a wiggler beamline, a two-beam monochromator, a safety system, a scanning device with a seat for the patient, a two-line detector with low noise and high dynamic range, and a computer system.

After experimental studies in dogs, patient studies have been conducted since 1990. Results of 136 patients in pre and pilot studies demonstrate the feasibility and safety of the method, as well as high diagnostic accuracy. In all cases, follow up investigations after bypass surgery or interventions like angioplasty, rotablation, and/or stent implantation were performed.

From June 1997 to June 1998, a large scale study with 230 patients was performed. The aim was to validate diagnostic sensitivity and specificity compared to selective coronary angiography. Thirty milliliters of contrast agent were injected into the brachial vein via an introducer sheath (94% of the cases) or into the superior vena cava (7% of the cases), respectively. Two series with two to four images each were taken under different projection angles. The scan speed was 50 cm/s. A preliminary evaluation gives a diagnostic imaging quality between 80% and 95% depending on the target vessel. Comparison with angiograms from selective coronary angiography shows concordance in 82% of available 121 cases. A great advantage of the method compared to competitive ones (MRI, EBT) is that restenoses in stents become clearly visible.

The acceptance of the method by the patients is very high. Nevertheless, widespread use of the method will be possible only if compact sources for hospitals become available. A design study is in progress at DESY.

Microbeam Radiation Therapy: Principle and Current Status of Pre-clinical Studies

Per Spanne, M. DiMichiel, C. Nemoz, and M. Renier
European Synchrotron Radiation Facility, Grenoble, France

J. A. Laissue, N. Luybimova, and H. P. Weber
University of Bern, Switzerland

H. Blattmann
Paul Scherrer Institute, Villigen, Switzerland

J.-O. Gebbers
Kantonspital Luzern, Switzerland

Microbeam Radiation Therapy (MRT) is a proposed radiotherapy technique accomplished by cross-firing a lesion with arrays of parallel, microscopic x-ray beams. The spatial micro-fractionation of the absorbed dose outside the target area that results from such an irradiation has been shown to considerably reduce the damage to normal tissues, even though the absorbed dose in each individual micro-beam was much higher than the threshold absorbed dose for tissue necrosis from continuous-field irradiation. From this observation it was hypothesized that MRT could be useful for radio-therapy of brain tumors in small children. Synchrotron x-ray sources are today the only sources that can produce microbeam arrays with sufficient energy and photon flux rate to allow MRT. Following experiments on 9L gliosarcoma-bearing rats at Brookhaven National Laboratory, a proposal to build an MRT irradiation facility was therefore submitted to the directorate of the European Synchrotron Radiation Facility (ESRF). As a result, a small animal microbeam irradiation facility has been commissioned and is in use for preclinical MRT studies. We will present the principle for MRT, in particular with regard to normal tissue tolerance, the small animal irradiation facility at the ESRF, and the present status of preclinical MRT studies.

Recent Topics of Synchrotron X-ray Imaging for Medical Research in Japan

Tohoru Takeda, T. Doi, S. Ohtsuka, Y. Sugishita, T. Saito, and Y. Itai
University of Tsukuba, Ibaraki-ken, Japan

A. Momose and K. Umetani
Hitachi Ltd.

K. Hyodo and M. Ando
KEK, Tokyo, Japan

F. Toyohuku and K. Tokumori
Kyushu University, Fukuoka-shi, Japan

C. Yuama
National Cardiovascular Center, Osaka, Japan

Q. Yu, T. Yuasa, and T. Akatsuka
Yamagata University, Yamagota, Japan

Various types of medical research using x-ray imaging with synchrotron radiation (SR) such as angiography, monochromatic x-ray computed tomography (CT), and radiography are being developed in Japan.[1] Here, I introduce recent topics of SR x-ray imaging performed in Japan.

SR coronary arteriography (CAG) is quite an important medical application. By the use of two-dimensionally (2D) spread SR beam and a 2D x-ray detector, the first human intravenous CAG was carried out in four patients at KEK in May, 1996. Significant coronary stenosis was observed in two patients; however, further improvement of the image quality is required for clinical practice. We have also carried out aortographic CAG on seven dogs. On 2D aortographic CAG, stenosis of canine coronary arteries was detected clearly, and coronary arteries less than 0.2 mm in diameter were imaged. In this 2D method, delayed coronary flow was also observed in a coronary artery with 99% stenosis. Image quality of 2D aortographic CAG was quite similar to that of conventional selective CAG because of less contract dilution and enough sensitivity of the imaging system for such situations. With prospective future application of SR, several studies have been carried out on the synchrotron x-ray CT, phase-contrast x-ray CT, fluorescent x-ray CT, and 3D x-ray CT with a fluorescent x-ray source. Phase-contrast x-ray CT could reveal various structures of human pathological specimens. Following preliminary experiments, a new system to image slightly larger objects (more than 20 mm) is being constructed. By fluorescent x-ray CT, the human thyroid gland and carcinoma could be described, and iodine content was evaluated quantitatively. 3D x-ray CT with a fluorescent x-ray source has clearly demonstrated coronary arterial images of a phantom.

Thus, x-ray imaging technique with SR might be the new powerful tools for biomedical research.

[1]Takeda, T., et al.: Medical applications with Synchrotron radiation in Japan. J. Synchrotron Rad. 5;326-332, 1998.

Whither Biological XAFS?

Grant Bunker
Biophysics Collaborative Access Team, Illinois Institute of Technology, Chicago, Illinois, USA

The first biological XAFS experiments were carried out approximately 25 years ago, and the utility of XAFS for studying metalloproteins in solution has always been evident. Despite significant progress and important applications, the promise of XAFS in biology has not been fully realized. This presentation will describe some recent technological advances that may change all that. Emphasis is on the new potential of third-generation sources.

X-ray Spectroscopy of Metal Sites in Proteins*

Stephen P. Cramer
Lawrence Berkeley National Laboratory, Berkely, California, USA, and UC Davis

EXAFS measurements constitute the vast majority of biological x-ray spectroscopy measurements. However, other x-ray techniques are now possible thanks to the continuing increase in flux available from synchrotron radiation sources. Soft x-ray absorption, x-ray magnetic circular dichroism, and high-resolution x-ray fluorescence can all provide information about electronic structure that complements the molecular structures determined from EXAFS. Over the past few years, we have developed instrumentation to enable us to make these measurements on the dilute metals sites found in biological systems.

Sum rules are powerful tools for the interpretation of these x-ray spectra. The integrated intensities of L-edge spectra can be used to quantify the number of d-vacancies on enzyme metal centers. The integrated MCD intensity can be used to characterize the spin and orbital moments on these centers. Finally, the integrated intensity of certain fluorescence features can be used to characterize the filled valence orbitals. This talk will illustrate the application of alternative x-ray spectroscopies with recent results on Ni, Cu, and Mn enzymes.

*Supported by DOE Office of Biological & Environmental Research and NIH GM44891-5.

X-ray Absorption Spectroscopy of Mn Enzymes

Eileen Y. Yu, Pamela J. Riggs-Gelasco, Timothy L. Stemmler, Charles F. Yocum, and James E. Penner-Hahn
The University of Michigan, Ann Arbor, Michigan, USA

Manganese redox enzymes are crucial in a variety of biological systems including the photosynthetic oxidation of H_2O to O_2, the disproportionation of hydrogen peroxide, and the disproportionation of superoxide. The Mn sites in these systems have been characterized using x-ray absorption spectroscopy. The photosynthetic oxygen evolving complex (OEC) shows approximately two 2.7-Å Mn-Mn interactions characteristic of di-μ-oxo bridged Mn dimers. These binuclear-like sites can be selectively reduced by the appropriate choice of reductant, leading to formation of two spectroscopically distinct reduced species. A similar di-μ-oxo bridged binuclear site is found in superoxidized Mn catalase, although with nearest neighbor ligation that is clearly distinct from that in the OEC. The OEC and reduced Mn catalase contain EXAFS detectable Mn-Mn interactions at 3.3-3.4 Å; similar features are not found in other binuclear Mn enzymes. The similarities and differences between these binuclear sites will be discussed. Recently, we have found that treatment of the OEC with fluoride and turnover of Mn catalase in the presence of fluoride both lead to the reversible formation of species in which the Mn site has been reduced, as judged by the XANES energy. Possible interpretations of these observations will be discussed.

Crystallographic and XAFS Studies of Copper Proteins

S. Samar Hasnain
CCLRC Daresbury Laboratory, Warrington, UK

Metalloproteins are an important class of proteins that perform a variety of fundamental biological processes and in doing so exploit the redox and ligand chemistry of biological metals. In order to understand how these metalloproteins utilize the chemistry of metals to perform a particular function, it is imperative to know the three-dimensional structure of these proteins, in general, and of the metal site, in particular, to a very high resolution. Perhaps nowhere in the determination of molecular structure is precision more at a premium than in the case of metalloproteins. A combined approach based on high-resolution (~1.5Å) crystallographic studies and the very-high-resolution (~0.1Å) XAFS studies of the metal centre in aqueous/crystalline state is thus most powerful in elucidating mechanisms of metalloenzyme catalysis and regulation. We note that XAFS is also a diffraction technique but of electrons which are selectively generated from a single type of atoms using the x-ray absorption edge of the element. Here, we will concentrate on the combined use of XAFS and crystallography for two copper proteins, namely the blue copper containing nitrite reductase and bovine superoxide dismutase. Crystallographic data for nitrite reductase[1] have provided information about the mode of substrate binding, changes in the water structure, and a communication channel between the two types of copper, while the XAFS data[2] have provided information suggesting that an ordered mechanism is operative in this enzyme. For superoxide dismutase, XAFS provides[3] evidence for cyclic oxidation/reduction of copper–key for substrate utilization–while our crystallographic data shows that the reduced state is highly sensitive to the crystal form.[4] The complementary information provided by the two techniques has thus helped advance our current understanding of the biological mechanism operative in these two cases. We believe that it is only through the rigor of quality structural data that the chemistry of this class of proteins can be underpinned.

[1]Dodd, F. E., Beeumen, J. V., Eady, R. R. & Hasnain, S. S. (1998; In press) JMB.
[2]Strange, R. W., Murphy, L. M., Dodd, F. E., Abraham, Z. H. L., Eady, R. R., Smith, B. E. & Hasnain, S. S. (to be submitted to JMB).
[3]Murphy, L. M., Strange, R. W. & Hasnain, S. S. (1997), Structure 5, 371.
[4]Hough, M. A. & Hasnain, S. S. (1998, submitted) Structure.

Spectroscopy with Synchrotron Radiation: New and Not-so-new*

John C. Sutherland
National Synchrotron Light Source, Brookhaven National Laboratory, Upton, New York, USA

Two trends affecting the synchrotron spectroscopy community are the rapid emergence of infrared experiments at several facilities and the increasing availability of polarized x-ray sources where the polarization can be switched at a reasonable rate, typically greater than 10 Hz. Periodic modulation of the polarization of an x-ray beam should greatly increase the sensitivity of experiments such as circular dichroism and magnetic circular dichroism that measure the difference in absorption of two opposite polarizations. The spectral extensions provided by high-brightness infrared beams from synchrotrons are particularly dramatic as the spectral region of greatest interest is four decades broad, i.e., wavelengths from 1 micron to 1 cm. However, neither polarization modulation or infrared applications of synchrotron radiation are really "new" methods. Rather, both are emerging from a period of 10 to 25 years of research and exploratory development and entering a period of rapid deployment. On the other end of the electromagnetic spectrum, the ability of accelerator-based light sources to generate well-collimated, nearly monochromatic beams of gamma rays is an exciting new opportunity, albeit several years from widespread deployment.

The evaluation of light sources is frequently discussed in terms of brightness or brilliance, both of which have the attraction of combining a number of important parameters into a single figure-of-merit, hence facilitating comparisons. Over reliance on such metrics may, however, not provide the most appropriate means of evaluation in some situations.

The great success being enjoyed by synchrotron sources in certain areas of biomedical research is promoting the welcome influx of new users and the resources to support them. These developments also pose new challenges in quality assurance that the synchrotron community should address proactively.

*Supported by the Office of Biological and Environmental Research, United States Department of Energy.

Recent Advances in Fluorescence XAFS Using High-brilliance Photon Sources

H. Oyanagi
Electrotechnical Laboratory, Tsukuba, Japan

Recent advances in fluorescence X-ray Absorption Fine Structure (XAFS) techniques, in particular, rapid and sensitive measurements using a tunable x-ray undulator, will be reported. One of the advantages of high-brilliance photon sources (i.e., x-ray undulator-based sources) is that one can study the local and dynamical atomic arrangements around optically excited atoms within ~8 Å using "pump and probe" XAFS techniques. A snapshot of local lattice distortion, relaxation, and, in some cases, bond alteration as a result of electronic excitation can be obtained from a fluorescence yield *in situ* at low temperature using a tunable undulator and a densely packed solid-state detector array. In this paper, the recent advances in fluorescence XAFS techniques and studies of atomic rearrangements caused by optical pumping at low temperature are described. One of the serious limitations in optical pumping experiments used to be the "depth mismatch" between x-ray probing and optical excitation. Sample concentrations have been therefore severely limited in order to achieve an optimum absorption of pump photons in the visible wavelength region. If a highly parallel x-ray beam is available, however, the extinction length can be reduced by orders of magnitude to match that of optical excitation. Utilizing a grazing-incidence fluorescence excitation technique, one can probe the local structure of optically excited region using a concentrated specimen. For example, in the photosynthetic water oxidation catalyzed by the tetranuclear Mn cluster in the photosystem II (PS II), stable intermediate reaction states induced by four photons were studied by X-ray Absorption Near Edge Structure (XANES) using dilute specimens (200-300 mM). It is expected that more concentrated specimens can be studied using a flat x-ray/optical window. Applications to chalcogenide glasses have demonstrated the potential of pump and probe XAFS providing the evidence of photo-induced local melting phenomena. In this paper, we describe the design and performance of the high-brilliance XAFS station BL10XU of SPring-8. An undulator gap and a fixed-exit double crystal monochromator with a rotated-inclined geometry were successfully controlled to cover a wide energy range (5-25 keV). Although asymmetric Si(111) reflections in a (+,-) configuration with a "rotated-inclined" geometry broaden the rocking curve by a factor of two, because of an advantage of ultra-low emittance, the observed energy resolution is expected to be less than 1 eV at 9 keV. Such a high energy resolution is advantageous for XANES studies of heme proteins in relation to spin states, such as oxy-forms of cytochrome P-450, horseradish peroxidease (HRP), and myoglobin (Mb). With the support of spin-dependent multiple scattering theoretical calculations, one can now quantitatively study the local structure of heme irons including the ligand orientation. For high efficiency fluorescence data acquisition, a 100-element monolithic solid state detector array and digital signal processors are used. In a monolithic approach, a packing ratio around 90% is expected. In this design, each element is 2 mm thick and has an effective area of 22 mm^2. A typical energy resolution is 240 eV at 5.9 keV for 1-msec peaking time. Although this novel detector array is currently under development, preliminary results using undulator gap tuning are presented and the feasibility of pump and probe experiments will be discussed.

Applications of Synchrotron Infrared Microspectroscopy to the Study of Biological Cells and Tissues*

Lisa M. Miller and Mark R. Chance
Albert Einstein College of Medicine, Bronx, New York, USA

Cathy S. Carlson
Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, North Carolina, USA

David Hamerman
Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, New York, USA

Jean-Louis Bantignies
Elf Atochem Company

Paul Dumas
LURE, University of Paris-Sud, Orsay, France

Nadege Jamin
CEA-DIEPE, Saclay

Jean-Luc Teillaud
Institut Curie, Orsay, France

G. Lawrence Carr and Gwyn P. Williams
Brookhaven National Laboratory, Upton, New York, USA

Synchrotron infrared light is an ideal source for infrared microspectroscopy due to its high brightness and broadband nature. These characteristics permit the collection of high signal-to-noise spectra through small apertures (3-5 mm in the mid-infrared region) and optically dense samples (0.1 % transmission). Using the advantages of the synchrotron infrared source, we are able to *chemically* image biological samples that are too small and/or too thick to examine with a conventional globar source. At Beamline U4-IR at the National Synchrotron Light Source, we have imaged the protein (Amide I, 1650 cm^{-1}; Amide II, 1545 cm^{-1}) and lipid (2850 cm^{-1}) components of a living cell in the process of cell division. By imaging the lipid components, we observe a high concentration of lipids in the center of the dividing cell, in the region where the contractile ring responsible for the cleavage furrow is located. We have also chemically imaged dying cells and found significant broadening of the protein (Amide I and Amide II) bands and the formation of a sharp, carbonyl ester peak near 1740 cm^{-1}. This feature most likely signifies protein oxidation, which occurs during necrosis. A second biological application of synchrotron infrared microspectroscopy is the study of hair and the effects of chemical treatment on the structure of hair. For the first time, the high spatial resolution achievable with the synchrotron infrared source allows us to study the three components of hair *individually*, i.e., the medulla, cortex, and cuticle. By imaging the lipid region, we find that the medulla contains a higher CH_2/CH_3 ratio than the cortex, suggesting a higher average lipid chain length in the medulla. In addition, we observed keratin oxidation in the cortex upon bleaching,

represented by the appearance of an S=O feature at 1040 cm^{-1} in bleached hair. Bleaching also results in hydration of the cuticle, which we observe as an increase in the bound-water concentration at 3400 cm^{-1}. A third biological application that will be presented is the study of bone chemical composition and bone disease. In this case, the synchrotron infrared source is necessary due to the highly absorbing mineral components in bone. In osteoarthritis, it has been demonstrated that the bone underlying the joint cartilage (subchondral bone) becomes thickened prior to cartilage breakdown. Thus, using synchrotron infrared microspectroscopy, we have examined the chemical composition of the subchondral bone in histologically normal and osteoarthritic monkeys. We find that the bone crystallinity, i.e., average crystal size, is similar in osteoarthritic and normal bone. However, the subchondral bone of osteoarthritic monkeys is significantly more mineralized than the normal bone, primarily due to an increase in carbonate concentration in the osteoarthritic bone.

*The NSLS is supported by the Department of Energy under contract DE-AC02-98CH10886. The work on cells is supported by NATO Grant CRG 972179. The work on hair is done in collaboration with Yves-Rocher, Inc. The work on bone is supported by the NIH Grants 5T32-AG00194 (L.M.M.), RR01633 (M.R.C.) and RR08562 (C.S.S.).

Macromolecular Complexes: The Frontier with Cell Biology
Chairman's Introductory Remarks

Louise Johnson
University of Oxford, Oxford, UK

Order in nature appeals to the orderly mind of the protein crystallographer. Many cellular processes are not the result of random collisions between freely diffusing molecules but the result of protein molecules acting in concert in macromolecular assemblies to produce a coordinated response. There is the expectation that once structures of these complexes are known, deeper insights into specificity and mechanisms may be obtained, similar to those that have been achieved for individual enzymes and recognition proteins. Already there have been spectacular examples such as the structure of the *S. cerevisiae* 20S proteasome (28 subunits), the mitochondrial F1 ATPase ($\alpha_3\beta_3\gamma\delta\varepsilon$, molecular weight 372 kDa) and both eukaryotic and prokaryotic cytochrome c oxidases, the protein complex located in the inner membrane of mitochondria and many bacteria. Principles of protein-protein recognition and how such interactions can modulate biological activity have been obtained from complexes such as spherical viruses with their beautiful icosahedral symmetry; multidomain structures (e.g., src kinase); and multi-component complexes (e.g., heterotrimeric G proteins and the complex of G$s\alpha$ with adenylcyclase, or the HIV gp120 envelope protein in complex with CD4 receptor and a neutralising antibody, or the GroEL/GroES/ADP chaperone complex). The structure of the Blue-tongue virus (BTV) has demonstrated power to elucidate not only the topology of the structural protein subunits and their principles of self-assembly based on the quasi-equivalence principle and variations of this principle, but also to reveal the packaging of the genomic dsRNA and the multi-enzyme transcriptase complexes, in a model of a transcriptionally active compartment.

What remains to be done? Undoubtedly there are a number of complexes where structural studies are demanded in order to illuminate biology, especially in the areas of DNA replication and transcription, in control of cell cycle processes, in motility, and in membrane transport. Securing sufficient soluble material for crystallisation trials is a major problem. How can we use heterogeneous recombinant DNA expression systems for the assembly of particles that may contain more than a dozen proteins? Advances in single particle imaging in the electron microscope give rise to the expectation that low-resolution images can be achieved with relatively little material and without the need for crystals and provide an image that can be the starting point for a high resolution interpretation, as for example in the structural studies on the topology of the core protein of the hepatitis B virus (a complex that did reassemble from proteins expressed in bacteria). Structural studies on the ribosome, both the individual proteins and the whole complex, are moving forward rapidly with high-resolution images from electron microscopy and promising diffraction quality crystals. In general, crystals of macromolecular complexes are likely to be small and weakly diffracting. The recent structure determination of bacteriorhodopsin from micro-crystals grown in lipidic cubic phases using the microfocus beam line at ESRF has shown that data can be obtained with crystals as small as 30μ x 30μ x 5μ. The introductory remarks will review some of the achievements to date and lead into the papers in the session that will address some of the problems for the future.

Drug Design against Shifting Targets: A Structural Basis for Drug Resistance to a Influenza Virus Neuraminidase Variant

Joseph N. Varghese
Biomolecular Research Institute, Parkville, Australia

Paul W. Smith and Steven L. Sollis
Glaxo-Wellcome Research and Development Limited, Stevenage, Herts, UK

Tony J. Blick, Anjali Sahasrabudhe, Jennifer L. McKimm-Breschkin, and Peter M. Colman
Biomolecular Research Institute, Parkville, Australia

Inhibitors of the influenza virus neuraminidase have been shown to be effective antiviral agents in man. Several studies have reported the selection of novel influenza strains when the virus is cultured with neuraminidase inhibitors *in vitro*. These resistant viruses have mutations either in the neuraminidase or in the viral haemagglutinin. Inhibitors, in which the glycerol side chain at the 6-position of Neu5Acen has been replaced by carboxamide-linked hydrophobic substituents, have recently been reported and shown to select neuraminidase variants.

The neuraminidase variant R292K modifies one of three arginyl residues which encircle the carboxylate group of the substrate. The structure of this variant with the carboxamide inhibitor used for its selection, and with other Neu5Ac2en analogues, is reported here at high resolution. Structural consequences of the mutation correlate with altered inhibitory activity of the compounds compared to wild-type neuraminidase.

The R292K variant of influenza neuraminidase affects the binding of substrate by modification of the interaction with the substrate carboxylate. This may be one of the structural correlates of the reduced enzyme activity of the variant. Inhibitors which have replacements for the glycerol at the 6 position are further affected in the R292K variant because of structural changes in the binding site, which apparently raise the energy barrier for the conformational change in the enzyme required to accommodate such inhibitors. These data provide evidence that a general strategy for drug design when the target has a high mutation frequency is to stay as close as possible to the natural ligands of the target.

Bacteriorhodopsin: Crystallography under Extreme Conditions

Eva Pebay-Peyroula
Institut de Biologie Structurale and Université J.Fourier, Grenoble, France

Hassan Belrhali
European Synchrotron Radiation Facility, Grenoble, France

Juerg Rosenbusch and Ehud Landau
Biozentrum, University Basel, Swizzerland

Bacteriorhodopsin (BR) is a light-driven proton-translocating machine, which converts the energy of photons into an electrochemical potential across the cell membrane. The pioneering studies of Henderson and others using electron microscopy and image reconstruction provided the first insight into the structural organization of a membrane protein at a resolution of 7 Å. During the past 20 years, major advances in electron crystallography have allowed significant improvements of the resolution, which has reached 3.5 Å,[1] and more recently 3.0 Å.[2] The results revealed that the structure of bacteriorhodopsin consists of seven membrane-spanning alpha-helices. The pigment retinal, bound to lysine 216, is buried in the interior of the protein. The mechanism of action of BR has been studied extensively by investigations of the photocycle, using several spectroscopic and structural methods, as well as dynamical modeling. It could thus be concluded that the absorption of a photon causes the release of a proton into the medium, and that the isomerization of the retinal from the all-trans to the 13-cis configuration is followed by a series of intermediate states that include the reprotonation of the Schiff base by a proton from the cytoplasmic compartment. A re-isomerization to the ground state completes the photocycle. In order to understand the mechanistic details of this process, atomic resolution is required. In particular, it was shown that water molecules inside the putative proton channel are essential for the proton translocation. A novel concept that exploits bicontinuous lipidic cubic phases for the crystallization of membrane proteins was recently developed.[3] Lipidic cubic phases provide a three-dimensional bilayer matrix that facilitates growth of bacteriorhodopsin microcrystals. The BR crystals are thin hexagonal plates [20 (50) x 20 (50) x 5 micron^3]. Despite their small dimensions, they diffract isotropically to 2.0 Å in a highly focused monochromatic x-rays (beamline ID13, ESRF). The structure was solved to a resolution of 2.4 Å by molecular replacement using the electron microscopy structure as a starting model.[4] Some water molecules could be positioned in the proton pathway. This structure reveals the atomic positions of BR, the retinal in the ground state of the photocycle. Nevertheless, the consequence of the retinal isomerization during the photocycle on the BR structure and the water molecules bound, as well as its role in the proton translocation, are still open questions.

[1]T. Grigorieff, T. A. Ceska, K. H. Downing, J. M. Baldwin, R. Henderson, *J. Mol. Biol.* **259**, 393-421 (1996).
[2]Y. Kimura, D. G. Vassylyev, A. Miyazawa, A. Kidera, M. Matsushima, K. Mitsuoka, K. Murata, T. Hirai, Y. Fujiyoshi *Nature,* **389**, 206-211 (1997).
[3]E. M. Landau, J. P. Rosenbusch, *Proc. Natl. Acad. Sci. USA* **93**, 14532-14535 (1996).
[4]E. Pebay-Peyroula, G. Rummel, J. P. Rosenbusch, E. M. Landau, Science **277**, 1676-1681 (1997).

Redox-coupled Structural Changes in Bovine Heart Cytochrome c Oxidase

Tomitake Tsukihara
Institute for Protein Research, Osaka University, Suita, Japan

Shinya Yoshikawa, Kyoko Shinzawa-Itoh, and Ryosuke Nakashima
Department of Life Science, Himeji Institute of Technology, Hyogo, Japan

Rieko Yaono, Eiki Yamashita, Noriko Inoue, Min Yao, Ming Jie Fei, and Tsunehiro Mizushima
Institute for Protein Research, Osaka University, Suita, Japan

Clare Peters Libeu
Department of Life Science, Himeji Institute of Technology, Hyogo, Japan

Hiroshi Yamaguchi and Takashi Tomizaki
Institute for Protein Research, Osaka University, Suita, Japan
Himeji Institute of Technology, Hyogo, Japan

Cytochrome c oxidase, a key enzyme in cell respiration, catalyzes the reduction of dioxygen to water using protons extracted from the matrix side of the inner mitochondrial membrane and electrons from cytochrome c, in a reaction that is coupled with proton pumping. The crystal structures of an eukaryotic and prokaryotic cytochrome c oxidase reported at 2.8-Å resolution in 1995 ushered in a new era for cytochrome c oxidase research.[1,2] In 1996, the protein structure, phospholipids, and possible nucleotide binding sites of the bovine heart enzyme were reported in detail. In addition, the eukaryotic protein structure revealed two possible proton-pumping paths neither of which involves the dioxygen reduction site.[3] In contrast, a scheme of the redox-coupled proton pumping, in which an imidazole ligand of CuB plays a crucial role, has been proposed based on the crystal structure of the bacterial enzyme.[2] For the proton-pumping function of cytochrome c oxidase driven by the dioxygen reduction, an acidic group in the protein must be accessible to only one of the two bulk water phases on both sides of the mitochondrial membrane in a certain oxidation state of the enzyme, and the accessible side must be switched to the other side by change in the oxidation state, concomitantly with a significant change in pK of the acidic group. Here we report crystal structures of the fully oxidized form at 2.30-Å resolution, the fully reduced form at 2.35-Å resolution, and the azide and CO complexes at 2.9- and 2.8-Å resolution, showing significant redox-coupled conformational changes in the segment containing Asp51 of subunit I and at CuB site. The crystal structures of fully oxidized and fully reduced bovine heart cytochrome c oxidase reveal new aspects for the enzymatic mechanism. A covalent link between Tyr244 and His240 suggests an increase in the acidity of Tyr244-OH group. Thus, Tyr244 should be the proton donor to the bound dioxygen to form a hydroperoxo intermediate (Fe-OOH). In the fully oxidized state, Asp51 is completely buried inside the protein and is connected with the matrix surface by a hydrogen bond network. The residue migrates to the cytosolic surface upon reduction, disrupting the hydrogen bond network. The movement indicates a novel proton-pumping mechanism.

[1]T.Tsukihara, et al., Science, 269, 1069(1995).
[2]S.Iwata, C.Ostermeier, B.Ludwig, H.Michel, Nature, 376, 660(1995).
[3]T.Tsukihara, et al., Science, 272, 1136(1996).

Crystals of Ribosomes, Exhibiting Severe Non-isomorphism, Extreme Radiation Sensitivity, and No Internal Symmetry, as Subjects for Synchrotron Radiation Crystallography

Ada Yonath
Weizmann Institute, Rehovot, Israel, and Max Planck Research Unit, Hamburg, Germany

In rapidly growing bacterial cells, the translation of the genetic code into polypeptide chains consumes up to 80% of the cell's energy and constitutes about half of its dry weight. This fundamental life process is performed by more than a hundred components, among which are giant nucleoprotein assemblies called ribosomes, the universal organelles facilitating the sequential polymerization of amino acids according to the blueprint encoded in the mRNA. Bacterial ribosomes (M.W. 2.3 mD) are built of two independent subunits of unequal size which associate upon the initiation of protein biosynthesis. The large subunit (1.45 mD) catalyzes the formation of the peptide bond and provides the progression path of the nascent proteins. The small subunit (0.85 mD) contains the site for the initiation of the process and for the decoding of the genetic information. About one-third of the ribosomal mass comprises some 58-73 different proteins, depending on its source. The remaining two-thirds are three chains of RNA, a total of about 4500 nucleotides.

Crystals have been grown from intact ribosomes and their subunits, despite their unfavorable properties (enormous size, lack of internal symmetry, inherent flexibility, and a surface composed of highly degradable RNA with proteins that may be loosely held). Far beyond the initial expectations, two crystal types, from the large ribosomal subunits of *Haloarcula marismortui* (H50S) and from the small subunit of *Thermus thermophilus* (T30S), diffract to around 0.3 nm. However, high resolution is not necessarily linked to high-quality diffraction. On the contrary, the crystal type diffracting to the highest resolution (H50S), yields the most problematic diffraction data.

The bright synchrotron radiation x-ray beam, necessary for the collection of the high-resolution x-ray diffraction data, causes significant decay even at cryo temperature. Nevertheless, due to the reasonable isomorphism of the T30S crystals, reliable MIR phases were determined. The resulting 0.55-nm electron-density map contains features that can be interpreted as ribosomal proteins, as well as long continuous chains that were traced as the RNA double helices, loop regions and single strands. In contrast, the substantial radiation sensitivity of H50S is accompanied by a low level of isomorphism, instability of the unit cell dimensions, low reproducibility, deformed spot shape, and non-isotropic mosaicity. The 0.85-nm MIR electron density map, constructed to gain insight into this unusual system, may indicate the reasons for the problematic nature of the H50S crystals and provide hints for their improvement.

The progress of ribosomal crystallography (including anomalous and MAD phasing), as well as molecular replacement studies, and the exploitation of images reconstructed from electron micrographs of ribosomal particles embedded in vitreous ice, will be discussed.

Synchrotron Radiation as a Tool for Investigating Virus Structure: Future Horizons and Past Successes

Michael G. Rossmann
Purdue University, West Lafayette, Indiana, USA

An overview will be given of how synchrotron radiation is being used in the structural analysis of biological macromolecules.

The Stereochemistry of Chaperonin Assisted Protein Folding

Paul B. Sigler
Yale University, New Haven, Connecticut, USA

Zhaohui Xu
Howard Hughes Medical Institute

Chaperonins are large double toroidal molecular assemblies that proofread and edit the proper folding of proteins in an ATP-dependent manner. GroEL, the archtype and best studied of the class I chaperononins, is an essential protein in *E. coli*. It is composed of two rings, each composed of seven 60-kD subunits, stacked back-to-back. Non-native polypeptides, but not most native proteins, are bound to the opening of the central cavity. ATP and GroES, a ring-like co-chaperonin of seven 10-kD subunits, bind asymmetrically to one ring (the *cis* ring) creating a large folding chamber into which the non-native polypeptide is released to fold in isolation. During a 15-second folding half-cycle, the seven bound ATP molecules are hydrolyzed, weakening the *cis* assembly. When ATP and GroES bind to the opposite ring, the weakened ADP *cis* complex collapses, releasing ADP, GroES, and a folded polypeptide. Thus, the products formed in one ring are expelled upon loading the reactants in the second ring as in a two-stroke engine. Three crystal structures, unliganded GroEL (2.8 Å), GroEL/ATP-γS_{14} (2.4 Å), and GroEL/GroES/ADP_7 (3.0 Å) reveal the mechanism for (1) the highly cooperative formation of the *cis* ring, (2) the unfolding of a misfolded polypeptide, (3) the release of the polypeptide into the *cis* folding chamber, (4) the disassembly of the *cis* complex and release of folded protein into the environment, and (5) the nearly absolute negative cooperatively that underlies the "two stroke engine" behavior of the double toroid. The size of the unit cell (1.24 x 10^7 $Å^3$) and mass of asymmetric unit (nearly 10^6 Da) require the use of very bright, well-conditioned synchrotron beams such as those provided by 19-ID at the Advanced Photon Source (APS) and X25 at the National Synchrotron Light Source (NSLS).

Crystallographic Analysis of Eukaryotic Signaling Proteins

John Kuriyan
HHMI, The Rockefeller University, New York, New York, USA

We have carried out structural analysis on a number of different components of signaling pathways that rely on tyrosine phosphorylation as the primary molecular switch. The following recent results will be discussed: The mechanism of activation of the small G-protein Ras by the nucleotide exchange factor SOS, the mechanism by which the STAT proteins (signal transducers and activators of transcription) are controlled by tyrosine phosphorylation, and the mechanism of the Src-family of tyrosine kinases.

EGF Receptor Trafficking and Viral Infection Resolved in Live Cells Using Synchrotron Radiation (SR) Microfluorimetry

M. L. Martin-Fernandez, J. M. Oliver, M. J. Tobin, D. T. Clarke, and G. R. Jones
CLRC Daresbury Laboratory, Daresbury, Warrington, UK

Cell proliferation is signaled by the binding of growth factors to their specific receptor tyrosine-kinases, which are embedded in the plasma membrane.[1] Upon binding EGF, the receptor's kinase becomes activated, allowing a signaling cascade that ultimately leads to gene transcription. Growth factor binding to the receptor also triggers rapid sequestration of active EGF-receptor complexes in the cell via a poorly understood, rapid, saturable internalisation pathway.[2] This may play an important role in the regulation of the mitogenic signal.

It is widely accepted that the molecular mechanism for activation of the receptor's tyrosine-kinase is EGF-induced receptor dimerisation. This model is supported by a corpus of evidence from molecular biology techniques, but thus far attempts to test this model in cells using electron microscopy and fluorescence methods have produced contradictory results. One of the major factors limiting the accuracy and time resolution of these data is the need to fix or freeze the specimens. This is done because the speed of data collection is generally much slower than the kinetics of the process under study. For fluorescence measurements, fixing may be also needed to alleviate photobleaching of dyes and cell photodamage because it allows the use of a different sample per data point.

We have developed a SR microfluorimeter capable of continuous measurement of the average intermolecular distances and molecular motions from live cells.[3,4] The pulse structure, tunability, and stability of SR have been crucial for these measurements. The microfluorimeter sacrifices any spatial resolution within the area of illumination to rapidly accumulate fluorescence decays from as little as 10^3 fluorescent molecules at irradiance levels six orders of magnitude smaller than similar instruments. This substantially reduces fluorophore photobleaching and cell photodamage. Using time-resolved fluorescence resonance energy transfer (FRET)[5] the instrument can measure distances in the range of 5 to 100 Å in time slots of about 10 seconds. It is therefore capable of measuring the kinetics of molecular aggregation processes, such as receptor dimerisation. The microfluorimeter has also the option of recording time-resolved fluorescence anisotropy (TRFA) decays, which report on steric constraints of molecules and their modes of rotation.

We have used this instrument to determine the sequence of early EGF-receptor events that follow the binding of EGF to its receptor in the epithelial carcinoma A431 cell line. We are now applying these methods to study the infection of live, cultured Hep2 cells with adenovirus 2. The relevance of this work is that we have been able to follow the viral uncoating process at high time resolution (few seconds) while simultaneously maintaining conditions that mimic the native environment of cells and viruses.

[1]Ullrich, A. & Schlessinger, J. *Cell* **61,** 203-212 (1990).
[2]Lamaze, C. & Schmid, S. L. *J.Cell Biol.* **129,** 47-54 (1995).
[3]Martin-Fernandez, M.L., Tobin, M., Clarke, D., Gregory, C. & Jones, G. R. *Rev. Sci. Ins.* **67,** 10, 3716 (1996).
[4]Martin-Fernandez, M.L, Tobin, M., Clarke, D., Gregory, C. & Jones, G. R. *Rev. Sci. Ins.* **69,** 2, 540 (1998).
[5]Stryer, L. *Ann. Rev. Biochem.* **47,** 819-846 (1978).

Structure and Function of the Mitochondrial ATP Synthase

John Walker
Medical Research Council, Laboratory of Molecular Biology, Cambridge, UK

ATP synthase will be discussed; the presentation will also include some discussion of complex I (NADH-ubiquinone oxidoreductase).

Structural Studies on a dsRNA Virus

David I. Stuart
University of Oxford, Oxford, UK

Jonathan Diprose
Laboratory of Molecular Biophysics, Oxford, UK, and
Oxford Centre for Molecular Sciences, Oxford, UK

Jonathan M. Grimes and Robyn Malby
Laboratory of Molecular Biophysics, Oxford, UK

Nick Burroughs and Peter M. Mertens
Institute for Animal Health, Pirbright Laboratory, Pirbright, UK

Viruses are complex assemblies of macromolecules and the simplest of parasites. They are not simple boxes of genetic information that use the equipment of the host cell for replication. Rather, they are complex bioloigal systems which achieve a variety of functions with considerable economy and elegance. We have studied the structure of Bluetongue virus (BTV). BTV is the prototype virus of the genus *Orbivirus,* and belongs to the family Reoviridae. This is the largest family of double-standed RNA viruses, and as such, these viruses face certain characteristic problems in their life-cycle. They enter the cell, usually shedding an outer capsid layer in the process to leave a smaller particle, termed the core in the case of BTV. This core remains intact in the cytoplasm of the infected cell where it is activated by the presence of nucleotide triphosphate substrates and commences to act as a factory, producing capped mRNA. The genome consists of 10 segments of RNA, which are transcibed independently by transcription complexes that appear to consist of three proteins, thought to possess helicase, polymerase, and capping activities. These are contained within an icosahedrally symmetric shell made up of 780 copies of VP7(T13) and 120 copies of VP3(T2). The structure is, therefore, an intriguing mixture of symmetries, some icosahedral and some not. Since the core contains nearly 1000 protein subunits, its analysis, by single-crystal x-ray diffraction, was a major undertaking. We were fortunate in being able to make use of beamline ID2 at the ESRF, which enabled the complex, crowded, and weak diffraction patterns to be resolved. We have determined the structure of the core of BTV-1 (SA) at approaching 3.5-Å resolution and that of BTV-10 (USA) at about 6.5-Å resolution. The higher resolution structure has revealed the fold and detailed interactions of the proteins which obey icosahedral symmetry whilst the comparison of the two structures at lower resolution reveals something of the positioning of the other proteins (the enzymatic complex) and, surprisingly, a substantial portion of the RNA genome of the virus. We are able to suggest a number of stages in the assembly of the virus and feel that the structure we observe throws light on the way the core acts as a transcriptional machine.

The Structure and Dynamics of Protein and Nucleoprotein Assemblies Determined with Synchrotron X-ray Radiation

John E. Johnson
The Scripps Research Institute, La Jolla, California, USA

William Wikoff and Vijay Reddy
The Scripps Research Institute, Department of Molecular Biology, La Jolla, California, USA

Hiro Tsuruta
Stanford Synchrotron Radiation Laboratory, Stanford University, Stanford, California, USA

Reductionism has been remarkably successful in elucidating the biochemistry and function of individual biological molecules. Our interests, however, are to tackle large structures where association of the "parts" leads to behavior that is not predicatable by examining the individual components. The structure determination of large assemblies like viruses, ribosomes, and transcription complexes often requires the marriage of methods such as crystallography, solution x-ray and neutron scattering, and electron microscopy.[1] In addition, the interpretation of these structures requires a well-defined inventory of biological phenomena.[2, 3] This presentation will address single crystal and solution diffraction studies with synchrotron radiation that have lead to measurement of data that span the range from near atomic resolution to the lowest order resolution diffraction maxima from crystals with unit cells in excess of 500Å.[4] Such complete data sets allow the definition of envelopes defining the accessible volumes of mobile regions of structures and they facilitate the use of models obtained with multiple methods.[5] These data sets have provided insight into RNA organization in viruses and have lead to mechanistic descriptions of large scale virus particle dynamics that result from the assembly of a single type of gene product into a 420 subunit megastructure. Initial assembly of this particle traps a folding intermediate of the subunits in a prohead. The subunits move to their final folded form when the particle expands by nearly 20% into a mature head.

[1]Baker T., Johnson J. 1996. Low resolution meets high: towards a resolution continuum from cells to atoms. *Current Opinion in Structural Biology* 6:585-594.
[2]Duda, R. L., Hempel, J., Michel, H., Shabanowitz, J., Hunt, D., Hendrix, R. W. (1995a) Structural transitions during bacteriophage HK97 head assembly. *J. Mol. Biol.* 247:618-635.
[3]Duda, R. L., Martincic, K., Hendrix, R. W. (1995b) Genetic basis of bacteriophage HK97 prohead assembly. *J. Mol. Biol.* 247:636-647.
[4]Wikoff, W., Duda, R., Hendrix, R., Johnson, J. (1998) Crystallization and preliminary x-ray analysis of the dsDNA bacteriophage HK97 mature empty capsid. *Virology* 243:113-118.
[5]Conway, J. F., Duda, R. L., Cheng, N., Hendrix, R. W., Steven, A. C. (1995) Proteolytic and conformational control of virus capsid maturation: The bacteriophage HK97 system. J. Mol. Biol. 253:86-99.