The SER-CAT SPECTRUM

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Director's Message

Bi-Cheng Wang

Welcome to the Winter 2008 issue of *The SER-CAT Spectrum*. Thanks to the diligent work of our members and staff, SER-CAT continues to be a very productive facility with over 102 publications in 2008! In this issue, we review our past and current activities during 2008.

The 5th Annual SER-CAT Symposium and SER-CAT Board Meeting were held in Charleston, South Carolina in March 2008. Please read the summary of the symposium from Prof. Christopher Davis, the local host, for this successful event.

It has been a long-term goal of SER-CAT to provide a virtual home synchrotron source to SER-CAT members. See the update by Dr. John Chrzas, SER-CAT Beamline Manager, for the status of this development.

SER-CAT has launched a facility upgrade. In December 2008, an NIH Award for the purchase of a microdiffractometer was received; installation expected in Fall 2009. Also, the original silicon 220 crystal monochrometer on 22 ID was recently replaced by a 111 crystal. This replacement should avoid unwanted harmonic contamination for longer wavelength X-rays.

Please check the announcement for the upcoming SER-CAT Symposium and Board Meeting being held in Huntsville, AL, hosted by Prof. Joe Ng and the University of Alabama in Huntsville. Again, this year's events promise to be both interesting and successful.

We extend a warm welcome to Ms. Elizabeth (Beth) Kolbusz as our new User Support Coordinator and Roderick (Rod) Salazar as a new beamline engineer and user support person.

As always, we welcome your suggestions and comments on how SER-CAT may best serve its members and users.

Thanks!

SER-CAT Meeting at UAH

The 6th Annual SER-CAT Symposium will be held at the University of Alabama in Huntsville (UAH) on Friday, March 20, 2009. All SER-CAT users and others interested in X-ray crystallography are welcome to participate in the program to discuss new crystal structures, improved methods for structure determination and productive use of the SER-CAT facility. The symposium will take place in the new Shelby Science Center on

the UAH campus. Huntsville, AL is known as the rocket city, home of Marshall Space Flight Center, NASA. The city holds the second largest research park in the U.S. and is a growing center for Biotechnology. Details of the meeting and registration information can be found at:

http://sercat-symp2009.org/

The SER-CAT board meeting will also be held on Saturday, March 21, 2009.

Important Date:

Registration Deadline March 6, 2009

INSIDE THIS ISSUE:

Fifth Annual SER-CAT Symposium

Christopher Davies

The 5th Annual SER-CAT Symposium was hosted by the Medical University of South Carolina (MUSC) and held at the Charleston Marriott in Charleston, South Carolina. In common with previous meetings, its theme was "Interesting structures, methods and advances in SER-CAT facilities" and it attracted over 65 participants (see group photograph) from a wide geographical area, including Texas, Illinois, Florida and Pennsylvania.

2008 SER-CAT Symposium Participants

This symposium showcases the diverse and often outstanding science emanating from the use of the SER-CAT facility and this year's meeting was no exception.

The oral program was divided into four sessions. The theme of the first session (chaired by Prof. Bill Furey, University of Pittsburgh) was new methods and developments at the SER-CAT beamlines. In the first talk of the day, **Prof. Zhen Huang** (Georgia State University) presented details of a new method to derivatize nucleic acids with selenium, which promises to expand the scope of phasing crystal structures. This presentation was followed with talks by **Prof. John Rose** (University of Georgia) who updated SER-CAT members on using 'soft' X-rays to determine crystal structures using the anomalous signal of sulfur phasing and **Dr. John Chrzas** (Sector Manager, SER-CAT, APS) who described how a mini-beam capability has been developed at both SER-CAT beamlines. This session ended on a somewhat somber note when **Dr. John Quintana**, (Associate Director, Engineering Support Division, APS) brought news of the fiscal difficulties facing the APS that might lead to a reduced production of available X-rays to the users at the APS.

Session 2 (chaired by Prof. Steve Lanier, MUSC Associate Provost) was opened by the Keynote speaker, **Prof. John Sondek** (University of North Carolina at Chapel Hill), who presented a fascinating talk explaining how the activation of human phospholipase C-η2 by Gβγ may be governed by the interaction of the protein with the membrane surface. **Dr. Darcie Miller** (St. Jude Children's Research Hospital) hit on a similar theme of membrane interaction when she described the structure of diacylglycerol kinase from *S. aureus*. This session also included a talk from **Mr. Graham Solomons** (MUSC) who described efforts to decipher the molecular mechanism underlying cooperativity in pyruvate kinase.

The theme of Session 3 (chaired by Prof. Jeff Hansen, MUSC) was nucleic acids and featured talks by the recipients of the two SER-CAT awards. **Dr. Da Jia** (UT Southwestern Medical Center at Dallas) won the SER-CAT Young Investigator Award for work conducted in the lab of Prof. Xiaodong Cheng at Emory University on DNA methyltransferases. Dr. Jia is to be particularly commended for making it to the meeting (just 10 minutes prior to his talk) after enduring a series of flight cancellations and delays during his journey from Texas! **Prof. Dmitry Vassylyev** (University of Alabama at Birmingham) won the SER-CAT Outstanding Science award for his work on the mechanism of substrate loading in RNA polymerases. **Prof. Hong Li** (Florida State University) ended this session with an illuminating talk on how the H/ACA ribonucleoprotein complex, which comprises four proteins and one guide RNA, captures and places ribosomal RNA in its active site.

Dr. Jia is presented the SER-CAT Young Investigator Award by Dr. Wang

Dr. Vassylyev receives the SER-CAT Outstanding Science Award from Dr. Wang

The final session (chaired by Prof. Christopher Davies, MUSC) included two more local talks: one by **Dr. Yaroslav Tsybovsky** (MUSC) about how an expected covalent bond between enzyme and cofactor was identified in folate dehydrogenase using structural and spectroscopic methods, and one by **Dr. Leslie Lovelace** (University of South Carolina), who presented the crystal structure of the MACPF domain of human complement protein C8α in complex with C8γ. The session ended with a glimpse of the future when **Dr. Leighton Coates** (Oak Ridge National Laboratory) updated the participants on the progress to develop a neutron crystallography beamline (MaNDi) at the Spallation Neutron Source.

At the end of the oral sessions at the Marriott Hotel, participants boarded a trolley bus for a tour of historic Charleston. This included a stop at White Point Gardens to view Fort Sumter at the mouth of Charleston Harbour, the site where the Civil War began in 1861. The meeting then continued with a poster session and reception at the South Carolina Aquarium, where participants viewed posters and enjoyed some excellent Low Country cuisine set among some of the aquatic life found in the South Carolina coastal region.

EDITOR'S NOTE: The meeting was sponsored by the South Carolina EPSCoR/IDeA program, the MUSC Provost's Office, the MUSC Center for Structural Biology, Qiagen, Inc., and Rigaku, Inc. **SER-CAT thanks all the above sponsors and Prof. Christopher Davies for another outstanding meeting!**

Virtual Synchrotron Update

JOHN CHRZAS

Hello everyone. I thought it would be a good time to provide an update on the status of the SER-CAT virtual synchrotron program. The concept of the virtual synchrotron was proposed by B.C. Wang a number of years ago to provide our membership with "Light When YOU Need IT!" and the operations team has been working diligently towards this goal. The concept of remote access to a synchrotron beamline is a complex combination of beamline hardware and software whose ultimate objective is to provide a reliable and stable environment for a user to collect their data from the comfort of their home lab or family room. This process may be divided into four categories: beamline performance, sample robotics, remote access environment, and automation. While I do not have the space to fully describe all aspects of each of these projects, I hope to provide enough information to peak your interest and welcome suggestions and questions on any beamline topic (after all, you are the ones that use the facility the most and we depend on your feedback for improvements).

Beamline Performance The SER-CAT beamlines are now 7 years old and have been thoroughly tested over the years. The performance of 22ID and 22BM has been well documented by the number of PDB and research publications that have been produced over the years. Outside of the few mechanical failures experienced, both beamlines work well.

Sample Robotics The sample robotics problem was solved by adopting (and heavily modifying) the ALS style AutoMounter design. Both beamlines have fully functional robots and are heavily used by our membership. A conservative estimate would be that 40% of our member beamtime is conducted by using a robot for sample mounting. The staff is diligently looking for ways to improve the robots by adding new capabilities such as automated annealing and washing capabilities. The bending magnet robot has been the test bed for all of our development and is currently at a higher level of performance, providing such capabilities as reliable cap detection (did the sample mount? Is there a sample already on the goni?), improved sample visualization, reliable loop centering and automated sample screening and data collection (see **Automation** below). The software improvements were installed on 22ID during the winter shutdown and are currently being commissioned.

Remote Access Environment Our first attempt to provide a remote access portal into the APS was by use of a program called Access Grid, which was difficult for the remote user to install and became impossible to manage with the required APS firewall penetrations. A few years ago, we started using the freely distributed NX (www.nomachine.com) software package. NX is easy to manage from the facility side and extremely easy for the remote user to download and install. NX works well, but we do have some problems with bandwidth limitations from the remote user's side of the connection. When the connection is good, life is good, but when the connection is slow, life can become very tedious. We can address the connection speed problem in at least two ways: (1) the remote user can make sure they have dedicated bandwidth from their home institution and (2) the facility can provide more automation.

Automation The objective of beamline automation is to provide the user with simple and reliable automated methods for performing various functions. Early in the project, automation was limited to "mundane" functions such as moving the energy (i. e., changing the wavelength), beamline optimization (optimize tune), fluorescence measurements and analysis (Benny and Chooch) and data archival (ssbackup). When we moved into robotic sample mounting, the scope of the automation program became much larger. Theoretically, we should be able to mount a sample, collect data, process the data though scaling, and then, using SGXPro, take the scaled data and perform structure solution or molecular replacement calculations using the local Linux cluster. I want to take a moment while we catch our breath to say that the purpose of the automation program is not to eliminate the need for highly trained crystallographers, but to try and ensure that the data collected will provide them with the information to answer the question that brought them to the beamline in the first place.

The automation program may be divided into the following categories: **(a) mounting, (b) alignment, (c) collecting** and **(d) processing.**

(a) Mounting: The sole function of the robots is to mount samples; both robots perform very well. The ID robot will have cap detection enabled for next run, which will allow us to safely perform the automation described below.

(b) Alignment: We are currently using a new loop centering system on the BM line that has over a 99% success rate and takes less then 1-minute to align most samples. While this system works well, there are cases that present problems with this type of approach, such as needles and samples not matching their loop size. In these cases, while the loop is "aligned" the sample may not be in the beam. There are more complicated optical solutions, such as c3d, for aligning a sample, but they only claim an 80% success rate; we are looking for near 100%. In an attempt to implement a reliable sample centering system, we are looking into the possibility of using a UV sample detection system in collaboration with one of the commercial suppliers.

(c) Collecting: Once the sample is aligned, collecting data is very simple – we do it all the time. The trick was the development of the Screen page within SERGUI. The objective was to provide the user with a spread sheet of possible experiments and then let the automated systems do their thing. Currently, the automated systems can screen your sample (index and develop a data collection strategy) and collect data using a supplied set of parameters. The next step is to provide the capability to identify projects and desired feedback metrics such as resolution and anomalous signal strength, which will be used to automati-

Virtual Synchrotron Update, from page 3...

cally change samples, if needed, to meet the specified criterion (i.e. maintain at least 2.5Å resolution).

(d) Processing: The cornerstone of data processing is the program you choose to use. Currently, we have implemented Denzo (indexing), D*TREK (index/strategy/integration/scaling) and XGEN (index/strategy/integration/scaling). While we are not averse to looking at other software options, we are limited in the amount of effort we have, so if anyone wants a different option, they may be asked to provide support to make it happen. Part of this project is to determine the reliability of these programs for a good indexing. Note that D*TREK and XGEN can use space group input to help with the indexing.

Three capabilities that we are currently developing are an *in situ* feedback on resolution as a function of frame number, automated integration and scaling, as well as a quick determination of anomalous signal strength.

Summary Today, we have the capability on 22BM to automatically mount and align samples (to the loop) and to collect and analyze data. We have had two successful runs using the automated system and a snap shot of the Screen page is shown in **Figure 1**.

Failures to date have been when the sample is not aligned near the center of the loop and we miss the sample. If you are interested in trying this capability on your next run, make sure that you match your sample to your loop (until we can get the UV system working) and everything should work fine. We will be working on updating the Beamline User's Guide on our website **(www.ser-cat.org)** to provide a more comprehensive description of these new capabilities.

Figure 1. Screen Shot of Automated Screening Process

Welcome Beth Kolbusz!

SER-CAT extends a warm welcome to Elizabeth (Beth) Kolbusz, our new User Support Coordinator in the Operations office at the APS. Beth recently joined SER-CAT in August 2008 to replace the vacated position of Sharon Granger. Beth is a graduate of Creighton University, with a B.S. in Elementary Education. In addition to working as an elementary and junior high school teacher in Illinois, Beth has worked as a customer service representative in the retail industry. If you would like to contact Beth at SER-CAT, her phone number is 630-252-0648 and her email address is [ekolbusz@anl.gov].

Welcome Roderick Salazar!

SER-CAT also extends a warm welcome to Roderick (Rod) Salazar as our new beamline engineer and user support person, beginning February 16, 2009. Rod joins us with over 15 years of beamline hardware experience, primarily gained by working with the APS as a Radiation Safety Systems Engineer and Floor Coordinator. Rod's new position at SER-CAT will greatly benefit the hardware upgrades and usage optimization plans currently in progress, as well as provide additional support to our members.

MD2 Microdiffractometer Funded at SER-CAT

Gary Newton

Congratulations to Prof. John Rose, Dr. John Chrzas and Prof. B. C. Wang who have been funded by NIH for purchase of equipment to provide microdiffraction capabilities on the SER-CAT undulator beamline (22ID). Specifically, a MAATEL/ACCEL MD2 microdiffractometer will be purchased and integrated into the 22ID beamline control system. This MD2 microdiffractometer ultimately will be permanently installed in the 22ID end station hutch replacing the 10-year-old single-axis goniometer and optics system currently in place and will be used for all experiments. The requested instrument is required to meet the growing demands of the SER-CAT membership for microdiffraction capability and for the increase in data quality for data collected from larger crystals afforded by the MD2 optics.

As principal investigator, Prof. Rose will have overall responsibility for the project and insure that its aims and objectives are achieved in a timely manner. Prof. Wang, as SER-CAT Director, will be responsible for ensuring access and will also serve as the program liaison. Dr. Chrzas will act as the Technical Director for the project and will be responsible for issues related to integrating the proposed instrument into the beamline control and hardware systems. Dr. Chrzas will also be responsible for the day-to-day operation of the instrument and will assist with scheduling APS GU's.

This microdiffractometer system has proved to be highly effective for data collection on microcrystals, as illustrated in the structure determination of GNNQQNY cross-β spine of amyloid-like fibrils where the MD2 was used to scan the elongated microcrystals to find high-quality diffraction domains for data collection (See Sawaya, M. R *et al.*, Nature 2007, 446, (7131), 97-101). In another example, data collected using the MD2 on 5-12μM microcrystals of silkworm cypovirus polyhedra, harvested directly from infected silkworms, was used to produce a 2Å MIR structure. (See Cork, C. *et al.*, Acta Crystallogr D Biol Crystallogr 2006, 62, 852-8).

The MD2 is a self contained high-precision goniometer and beam conditioner with the high-powered optics needed to visualize microcrystals based on initial designs from the European Synchrotron Radiation Facility (ESRF) and addresses most, if not all, problems one would encounter in collecting data on microcrystals using a microbeam. These include: (1) Beam shaping to produce a uniform microbeam, (2) Beam visualization to ensure the beam is both uniform and the correct size, (3) High-resolution sample imaging to aid in centering the microcrystal and (4) High-precision goniometry to insure that the microcrystal remains in the microbeam during the experiment.

For full information, see:
www.accel.de/pages/microdiffractometer.html

220 Out, 111 In

John Rose

During the January shutdown, SER-CAT replaced the Si 220 first crystal in the 22ID monochromator with a Si 111 crystal. The decision to replace the Si 220 crystal was based on user demand for data collection at longer wavelengths for SAD/MAD experiments. Although the experimental envelope for 22ID allows for data collection using X-ray wavelengths up to 2.0Å, severe harmonic (n=2) contamination of the diffraction pattern has been a continuing problem. SER-CAT has spent considerable time and effort over the years in trying to mitigate the problem using a variety of methods including (1) detuning the monochrometer, (2) upgrading the thermal shielding of the first and second crystals of the 22ID monochromator and (3) using the adjustable white beam aperture to reduce the power load on the 22ID monochromator first crystal. These attempts, although somewhat successful in reducing harmonic contamination, also had adverse effects on quality of the sulfur anomalous scattering data collected. We are pleased to report that the Si 111 optics are delivering a measured full beam flux increase of 2X, as well as a decrease in the focused beam size (differences in the crystallographic response of 220 and 111 to sagittal focusing) which means that we are measuring an increase of almost 3X the flux in our standard 50 micron pinhole.

SER-CAT Crystal Shipping Kit

James Tucker Swindell II

SER-CAT has developed a set of low cost tools to allow more of its members the ability to access the beamlines remotely for crystal screening and data collection. The price of the kit is approximately $1500. For more information, please contact John Rose [rose@bcl4.bmb.uga.edu] who may arrange for the purchase of the kit directly from the UGA instrument shop.

EDITOR'S NOTE: This kit was developed and tested by UGA graduate student James Tucker Swindell II with assistance from Profs. John Rose and B. C. Wang (both at UGA), Mr. Lewis Fortner (UGA Instrument Shop), Dr. John Chrzas and Mr. John Gonczy, (both at SER-CAT).

Ms. Julia Goalby, Chicago Regional Director, UGA Alumni Association, visits SER-CAT

B. C. Wang

Ms. Julia Goalby, the University of Georgia's Chicago Regional Director of Leadership and Major Gifts of the Alumni Association visited SER-CAT on February 12, 2009. This was a very pleasant experience for all of the staff and we sincerely express our appreciation for her interest in learning more about SER-CAT. Also, I would like to take this opportunity to extend a warm welcome to the alumni associations of other SER-CAT members who may wish to visit in the future.

Ms. Goalby with Prof. Wang at SER-CAT

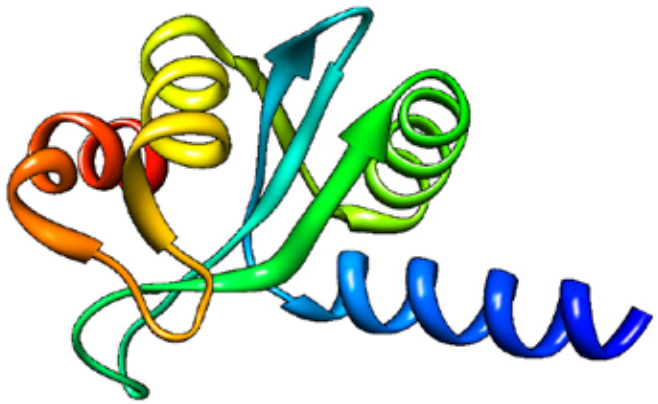
Recent Interesting Structures at SER-CAT

Gary Newton & John Rose

Three structures recently deposited to the PDB (2JLH, 2JLI and 2JLJ) were determined by single wavelength anomalous dispersion data collected at SER-CAT 22ID. This work has been recently published in Protein Science under the title "Atomic resolution structure of the cytoplasmic domain of *Yersinia pestis* YscU, a regulatory switch involved in type III secretion" by authors Lountos, Austin, Nallamsetty and Waugh. The *Yersinia pestis* organism is the causative agent of plague or "Black Death". This current work is part of an effort to develop countermeasures against this organism and is very timely in view of national security.

These crystallographic studies provide structural insights into the conformational changes induced upon auto-cleavage of the cytoplasmic domain of YscU. The structures indicate that the cleaved fragments remain bound to each other. The conserved NPTH sequence that contains the site of the N263-P264 peptide bond cleavage is found on a beta-turn which, upon cleavage, undergoes a major reorientation of the loop away from the catalytic N263, resulting in altered electrostatic surface features at the site of cleavage. Additionally, a significant conformational change was observed in the N-terminal linker regions of the cleaved and noncleaved forms of YscU which may correspond to the molecular switch that influences substrate specificity. The YscU structures determined here also are in good agreement with the auto-cleavage mechanism described for the flagellar homolog FlhB and *E. coli* EscU.

Congratulations to Dr. David Waugh *et al.* and the Macromolecular Crystallography Laboratory, National Cancer Institute at Frederick, Frederick, Maryland 21702-1201, for their interesting and significant contribution to protein crystallography. The authors thank Dr. Waugh for his input in the preparation of this note.



Structure of 2JLI
(drawn with Chimera)
http://www.cgl.ucsf.edu/chimera/

Wang Wins Patterson

Gary Newton

The American Crystallographic Association's (ACA) Patterson Award, named for Lindo Patterson of Patterson Map fame, is awarded every three years and is one of the ACA's most prestigious awards. At the 2008 Knoxville ACA Meeting, the Patterson Award was presented to **Prof. Bi-Cheng (BC) Wang** by ACA President Marvin Hackert, for "significant contributions to the methodology of structure determination from single isomorphous replacement or single-wavelength anomalous scattering data and for its impact on structural biology". An ACA symposium was organized in honor of BC's Patterson Award with BC as the lead speaker. The symposium was titled "Advances in Macromolecular Phasing and their Impact to Molecular Biology" and included eight speakers in the afternoon session.

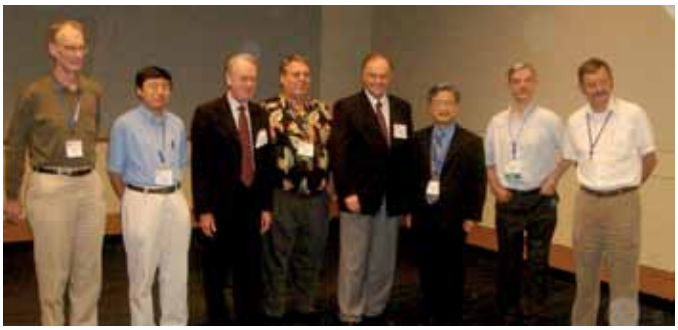
BC's presentation, "Resolution of Phase Ambiguity in Macromolecular Crystallography: 25 Years Later" recalled the development of the "solvent flattening" procedure which resulted in ISIR and ISAS techniques for protein structure phasing. These phasing procedures were ultimately encoded in an easy-to-use program, which was generally distributed to anyone who requested a copy in the mid-1980s. BC then related more recent developments which included (1) anomalous phasing procedures using naturally occurring elements in proteins, such as metals, and the use of the small anomalous scattering signal from sulfur, (2) the use of longer wavelength X-radiation to enhance anomalous signals and (3) development and use of "Signal Based Data Collection".

The second speaker was **Prof. Wayne Hendrickson**, the first Patterson Award winner in 1981, who spoke about "Evolution of Phase Evaluation from MAD and SAD Measurements". Wayne recalled the development of MAD and SAD phasing techniques, the incorporation of Se into protein structures and the gradual change from MAD to a current predominance of SAD phasing in PDB depositions. The next speaker was **Dr. Zbigniew Dauter** who talked about the 'Wang Limit'; the conclusion was "There is no such thing as the 'Wang Limit'. Everything depends on the diffraction data accuracy". Next, **Prof. Emil Pai** related his experiences with the use of Cr X-radiation and his successful phasing of some challenging structures using the larger sulfur anomalous signal by use of longer wavelength X-rays.

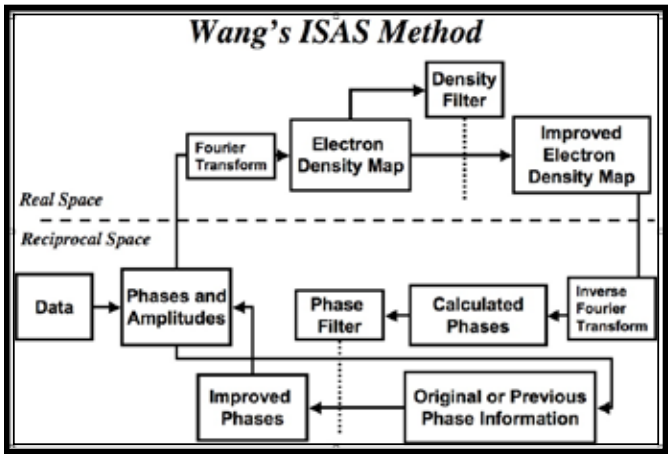
Prof. David Langs recalled some of the developments at HWI that were inspired by BC's 1984 visit there in the development of the SnB phasing program. **Dr. Quan Hao** next told about his experiences with "Direct Methods and Solvent Flattening"; he has applied these ideas in the successful phasing of very impressive examples. The next speaker, **Prof. Wim Hol**, explained his successful phasing of llama-produced antibodies complexed to the protein of interest as an alternative to otherwise problematic crystallizations. Lastly, **Prof. John Rose** reviewed the early attempts to use Wang's ISAS technique for structure solutions: bovine neurophysin derivative using Iodide-SAS, ferrochelatase using Fe-SAS and protein obelin using S-SAS.

This event was also a special family occasion; BC and his wife Johnna were joined by their two sons, their wives and the three grandchildren. As noted by ACA **President Marvin Hackert**, the grandchildren represented the next generation of scientists.

BC Wang receiving the Patterson Award from ACA President Marvin Hackert



Patterson Symposium Speakers (l to r): Hol, Hao, Hendrickson, Rose, Pai, Wang, Dauter, Langs



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