SSRL Structural Molecular Biology Summer School – 2004

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The fifth annual Structural Molecular Biology Summer School (SMB) was held at the Stanford Synchrotron Radiation Laboratory (SSRL) from August 16-20th. The school focused on three synchrotron-based techniques: small angle x-ray scattering, x-ray absorption spectroscopy, macromolecular crystallography, and the application of these techniques to biological problems. This year's summer school was attended by 23 students and was lead by a team of 18 tutors. It consisted of three days of lectures, followed by a day and a half of rotating practical sessions.

The summer school was opened by Keith Hodgson (SSRL Director), who gave an overview of synchrotron radiation and structural biology at SSRL. It was followed by introductory talks on synchrotron radiation and beam line optics, given by Helmut Wiedemann and Thomas Rabedeau (SSRL), respectively.

Session 1: X-ray Absorption Spectroscopy

The first session of the SMB summer school was dedicated to x-ray absorption spectroscopy (XAS), with the first talk given by Robert Scott (University of Georgia) who provided the students with a general introduction to XAS terminology, the utility of the technique, and elementary aspects of the theory behind it. The second lecture in the session was given by Graham George (University of Saskatchewan), who focused on experimental methods and data collection strategies. This was followed by a talk on XAS theory, given by John Rehr (University of Washington), who discussed development of *ab initio* XAS theory and its utility in obtaining a quantitative interpretation of spectra. Ninian Blackburn (Oregon Health and Sciences University) was the final speaker in this session and discussed specific examples of

the application of XAS in structural molecular biology, including application of XAS to peptidylglycine monooxygenase, the Cu_A site of cytochrome *c* oxidase, and to metallochaperones.

Session 2: Small Angle X-ray Scattering

Pappannan Thiyagarajan (Argonne National Laboratory) introduced the theoretical basis of small angle scattering, including information content, scattering cross-sections in x-ray and neutron scattering, anomalous scattering and how particle morphologies influence the electron pair distribution function and scattering curve. A number of important biological studies on RNA folding, amyloid fiber formation and lipid-peptide interactions were discussed, illustrating complementary aspects of x-ray and neutron small angle scattering techniques. Hirotsugu Tsuruta (SSRL) then talked about experimental aspects of conducting synchrotron solution x-ray scattering, such as instrumentation, tips for successful data collection and time-resolved studies. The third talk in this session was given by Jill Trewhella (University of Utah), who focused on a series of small angle x-ray and neutron scattering studies on calmodulin and its target peptides, as well as cAMP-dependent kinase to show how solution scattering techniques complement crystallographic and biochemical studies. The last talk of this session was given by Kelly Lee (The Scripps Research Institute). He emphasized the combination of time-resolved x-ray scattering, fluorescence and CD kinetics, cryo-EM and x-ray crystallography is a highly effective approach to studying highly cooperative acid-induced maturation of HK97 bacteriophage.

Session 3: Macromolecular Crystallography

The third session was concerned with the experimental aspects of macromolecular crystallography. The first talk was given by Joe Luft (Hauptmann-Woodward Institute, Buffalo), who gave an excellent introduction to protein crystallization and pointed out the important issues that people should be aware of when undertaking these experiments. The second lecture was on crystallographic theory, presented by William Weis (Stanford University), who talked about the fundamentals of x-ray diffraction. This led to the third talk by Paul Adams (UC Berkeley), who discussed the various methods for obtaining phase information and then went on to talk about macromolecular structure refinement. The final lecture in this session was by Irimpan Mathews (SSRL), who gave an introduction to protein structure, talked about model building and structural analysis, and what useful information can be obtained from protein structures.

Session 4: Advanced Applications

The fourth lecture session focused on advanced applications of macromolecular crystallography, XAS, and SAXS. The session opened with a talk by Vittal Yachandra (Lawrence Berkeley National Laboratory), who discussed the application of polarized and high-resolution XAS to the Mn cluster in photosystem II. This was followed by a talk by Samar Hasnain (Daresbury Laboratory), who discussed the use of a

combination of XAS, SAXS, and crystallography for studying structure-function relationships in blue copper and SOD proteins. Dan Herschlag (Stanford University) gave a comprehensive talk on RNA folding, primarily addressed by time-resolved studies using a micro-machined continuous flow mixer and the role of counter ions in stabilizing DNA structure, as revealed by anomalous (resonant) x-ray scattering near the Rb and Sr K edges. The last talk was given by Ashley Deacon (SSRL), who talked about structural genomics and described the ways in which the Joint Center for Structural Genomics is endeavoring to solve some of the problems facing high-throughput structure determination initiatives.

Practical Sessions

The lectures were followed by three rotating hands-on practical sessions for each of the three techniques.

The XAS practical sessions were led by Serena DeBeer George, with additional support from Deanne Jackson Rudd (Stanford). The students were given a general introduction to XAS data analysis using the program EXAFSPAK (developed by Graham George). They learned the basics of calibrating, averaging, and background subtracting data. They were then guided through the use EXAFSPAK as an interface to calculate theoretical phase and amplitude parameters from the program FEFF. The students were given worksheets, which took them through four examples of EXAFS data. The tutors emphasized the importance of exploring the strengths and weaknesses of different XAS analysis software.

The SAXS practical session had a series of tutorials on data interpretation programs developed by the EMBL group and concluded by a visit to the BL4-2 SAXS facility, where a number of practical aspects of conducting experiments were discussed. The participants first obtained the electron pair distance distribution function using GNOM for a set of data recently recorded for a standard protein. An experimental scattering curve of the protein was compared with the one calculated from a PDB file (CRYSOL). Programs SASHA and DAMMIN were run to obtain a low-resolution structure model of a protein, and MASSHA to obtain the orientation of two lysozyme molecules within a “synthetic” dimer of lysozyme whose scattering curve was supplied.

The macromolecular crystallography practical sessions were held at SSRL beam lines BL9-1 and BL9-2 and were led by Clyde Smith, with help from Paul Adams. The students were first shown beam line BL9-1 and the Stanford Automated Mounting robot system (SAM) was demonstrated. They then moved to BL9-2 and were given instruction in mounting frozen protein crystals onto the beam line, and a data collection experiment was then set up. They proceeded to process some diffraction data sets using the programs MOSFLM and HKL2000, and the programs from the CCP4 software suite. Paul Adams then gave a demonstration of his new program (PHENIX) for solving the heavy atom substructure and showed that the positions of 6 sulfur atoms in lysozyme could easily be located from their weak anomalous signal by using a diffraction data set collected with very high redundancy. He then outlined

a macromolecular refinement procedure using the CNS suite of programs and showed the students some electron density maps and atomic models.

Both the lecture and practical sessions were enthusiastically received by the participants. The organizers thank all the tutors for providing a valuable learning experience for everyone involved. This annual event is supported by the National Institutes of Health, National Center for Research Resources, Biomedical Technology Program.