SSRL Structural Molecular Biology Summer School

The Stanford Synchrotron Radiation Laboratory held a Summer School on the scientific applications of synchrotron radiation to the field of structural molecular biology September 9-14th. This year's School was co-chaired by SSRL staff scientists Serena DeBeer George, Clyde Smith and Thomas Weiss, and focused on the application of x-ray absorption spectroscopy, small angle x-ray scattering and macromolecular crystallography to biological systems. The six-day School was attended by 34 students, representing four countries, and involved a team of 20 tutors.

The School opened with an introduction to the role and impact of synchrotron-based structural biology and the Structural Molecular Biology program at SSRL, which was followed by introductory talks on synchrotron radiation and beam line optics. The focus then shifted to three days of lectures on each of the methods with another three days of rotating practical session on each topic.

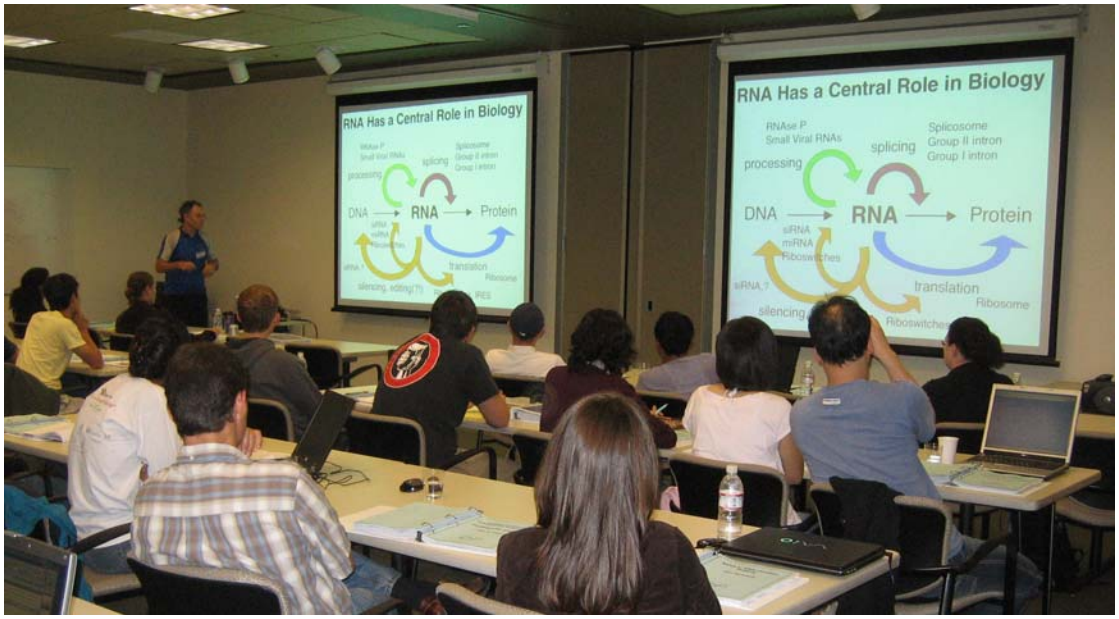

Figure Caption. Summer School students listen intently as Prof. Dan Herschlag (Stanford University) explains the central role of RNA in biology.

The first session of lectures focused on x-ray absorption spectroscopy. The lectures covered basic theory, experimental considerations and applications. The speakers emphasized the utility of XAS as a probe of metalloproteins' active site structure, and its complimentarity to both protein crystallography and small angle x-ray scattering. In addition, the ability to use XAS to enable element specific imaging of biological tissues was discussed. Participants were instructed on common experimental pitfalls and how to avoid making mistakes during data collection.

The following morning opened with a series of lectures on small angle x-ray scattering, which introduced the general theory and applications, as well as an in-depth discussion of experimental aspects and data collection strategies. The students were given an overview of the capabilities of synchrotron-based small angle scattering to investigate a variety of problems, ranging from fiber and muscle diffraction to time resolved solution SAXS on viruses.

The third session was a series of four talks on macromolecular crystallography, describing the crystallization experiment, diffraction theory, data collection strategies, and structure solution and refinement. The talks focused primarily on practical aspects of the discipline and gave the students a good introduction to the topic prior to the practical sessions. During the crystallization talk, students were given the opportunity to set up lysozyme crystallizations, which yielded crystals after 30 minutes.

The lecture portion of the School closed with a series of talks on the interplay of different methods. Here the speakers focused on the utility of using a combination of methods to arrive at more detailed solutions to questions in biology. After three full days of lectures, the students were well-prepared to begin the hands-on practical portion of the Summer School. The students were divided into three groups and had the opportunity to spend a full day learning hands-on data analysis in each of the three methods.

During the XAS practical session, the students were given a general introduction to XAS data reduction and analysis using the programs PYSPLINE for background subtractions and EXAFSPAK. 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. Both single and multiple scattering EXAFS analysis were discussed. The students then worked through a series of example EXAFS data sets of increasing difficulty. The tutors emphasized the importance of exploring the strengths and weaknesses of different XAS analysis software.

In the practical SAXS session, which focused specifically on biological solution scattering, the participants were taken through the entire data analysis process, from the raw data to a final shape model of the protein, using an example data set previously measured at SSRL. The students were shown how to obtain an integrated one-dimensional buffer subtracted curve from the two-dimensional CCD-image using the program MarParse. Special emphasis in this session was placed on identifying potential problems in the experimental data (e.g. radiation damage and protein aggregation) and how to prevent this by changing the data acquisition protocol. The remainder of the time was spent on modeling an example data set using the ATSAS analysis suite.

The PX practical session included an introduction to data processing and scaling using the program MOSFLM along with programs from the CCP4 suite. The students solved the structures of two relatively simple proteins, myoglobin and lysozyme, using the programs SOLVE, SHELX and RESOLVE. This introduced them to structure

determination using MAD and SAD methods. Data sets from several more difficult examples including an esterase and a phosphotransferase, were also used. The students were given the opportunity to process data using a second program (HKL2000) and to work with additional example data sets. During the practical session, the students were taken on a brief tour of the SSRL macromolecular crystallography beam lines, where they watched the robotic sample mounting system at work and analyzed the results of their structure solutions using the program COOT running on 3-D interactive graphics computers.

The Summer School was organized by the SSRL Structural Molecular Biology program, which is funded by the U.S. DOE BER, NIH NCRR BTP and NIH NIGMS.