Institute of Molecular and Cell Biology
A*STAR

SEMINAR ANNOUNCEMENT

DATE: 13 March 2012, Tuesday
TIME / VENUE: 11:00AM @ Level 3, IMCB Seminar Room 3-46, Proteos, Biopolis
SPEAKER: Prof. Kenneth Holmes
TITLE OF SEMINAR: **The impact of synchrotron radiation on molecular biology**

On 13th Feb 1960, two very remarkable papers appeared in Nature: "The structure of haemoglobin. A three dimensional Fourier synthesis at 5.5Å resolution" by Max Perutz and collaborators and "The structure of myoglobin, a three dimensional Fourier synthesis at 2Å resolution" by John Kendrew and collaborators. These papers reporting the first atomic structure of a protein were the harbingers of structural molecular biology, which together with the genetic approach founded by Luria and Delbrück became the twin pillars of molecular biology. The first X-ray diffraction pictures from protein crystals (pepsin) were obtained by J.D. Bernal and Dorothy Crowfoot in 1934, by the simple expedient of keeping them wet. This led to Bernal's vision that by solving protein crystal structures you could understand life. Max Perutz took up this challenge but it took another twenty years to solve the first protein structure. The turning point was Max's discovery in 1953 that the contribution to the X-ray scattering from mercury atoms attached to haemoglobin was large enough to provide the missing phase information.

In 1948 Max' group was joined by Hugh Huxley, who discovered that living frog muscles give detailed crystalline diffraction patterns. This observation led to the discovery that muscles consist of interpenetrating filaments made of myosin and actin, which move passed each other during a contraction. Furthermore, Hugh Huxley discovered the myosin cross-bridges that "row" the myosin filaments along. Attempts to register the diffraction from these cross-bridges in real time drove the early application of synchrotron radiation (SR) to biology. In paper in Nature in 1970 Rosenbaum Holmes and Witz described experiments that used a bent crystal monochromator to select and focus a narrow band from a white SR source. The monochromator was then combined with a bent glass mirror to produce the world's first X-ray beam-line, which was operating at DESY Hamburg in 1972. Because the electrons in the synchrotron were dumped 50 times a second, the average beam intensity was only about ten times more than a conventional source but the very good collimation made it ideal for low angle X-ray scattering, particularly for diffraction from muscle. Thus the primary use of this first

beam-line was for obtaining low angle X-ray diffraction patterns from living muscle.

On the basis of these early results, EMBL opened an outstation for biological diffraction on the colliding beam storage ring DORIS (1974), which soon became one of the important centers for protein crystallography using SR. Parallel developments took place at the colliding beam storage ring SPEAR in Stanford. Since on these machines the SR users were parasitic and the machine operated to suite the needs of high energy physicists, early SR users needed to be workers of great resilience. In spite of these difficulties the EMBL outstation became a very important centre for collecting diffraction data from protein crystals.

Other scientists became aware of the usefulness of SR as an X-ray source. This led to the building of dedicated storage rings with optimized electron optics, with wigglers and undulators to produce ever more intensity. Particularly in Europe there is now an abundance of third generation sources. Intensities are now about a million-fold higher than can be obtained with conventional sources. After two decades of instrumental development SR transformed protein crystallography from an esoteric method needing some 10 man-years to solve a structure to a general method able to produce the atomic coordinates of a crystalline macromolecule in a few days or even a few hours. The hard intense radiation available from SR allows one to work with small crystals, which eliminates most systematic errors. Indeed, the superb optical properties and intensity of SR yield data of great accuracy. The ability to vary wavelength continuously enables the exploitation of anomalous scattering at absorbtion edges of heavy atoms for phase determination by MAD (Multi-wavelength Anomalous Diffraction). Thus the method can be successfully applied to very large complexes such as ribosomes. Moreover, frozen crystals, which are resistant to radiation damage, can be used for data collection. Frozen crystals can be transported long distances allowing remote data collection. Even refractory membrane proteins have yielded to protein crystallography. To date, eleven scientists have been honoured with the Nobel Prize for work involving the structures of proteins. Of these nine used SR. Modern cell biology would not exist without the input from SR-derived protein crystallography.

In the near future (2014) a completely novel source of X-ray radiation will be available from an X-ray free electron laser (XFEL) being built at DESY Hamburg. This is based on shooting 30 GeV electrons into a long undulator. In an undulator the electron beam runs a slalom course induced by a periodic arrangement of magnets. At each bend of the trajectory each electron emits X-ray radiation. Because the radiation is faster than the electrons the radiation overtakes the electrons accelerating some and slowing others down. As a result, the electrons organize themselves into a multitude of thin disks. If the undulator is long enough all of the electrons in a given disk emit their light in phase. This produces extremely short and intense X-ray flashes with the properties of laser light. Since the structure of thin disks takes some time to form, free-electron lasers require very long (100m) undulators. The peak intensity will be an amazing factor of 10^{10} higher than present-day synchrotron sources. One proposed use of this intense light is to register diffraction from single molecules before they fly apart. Since the radiation is coherent, it could perhaps be used for holography. Time will tell.

Host: Prof. Robert Robinson
