

# From rare to routine

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The growing importance of synchrotron radiation for structural biology can be charted from the construction and use of an X-ray beam line at DESY in Hamburg, Germany in 1970, to the completion of the three third generation synchrotrons in France, the USA and Japan in the 1990s.

Two techniques have been the pillars of our burgeoning understanding of biology at the molecular level: recombinant DNA technology and X-ray crystallography. Much as crystal structure determination became a handmaid of chemistry so now we see structure determination as important for underpinning molecular biology. Furthermore, during the last decade — a result of its excellent collimation, brilliance, and polychromaticity — synchrotron radiation has become an indispensable X-ray source for protein crystallography and therefore an integral part of modern biological research.

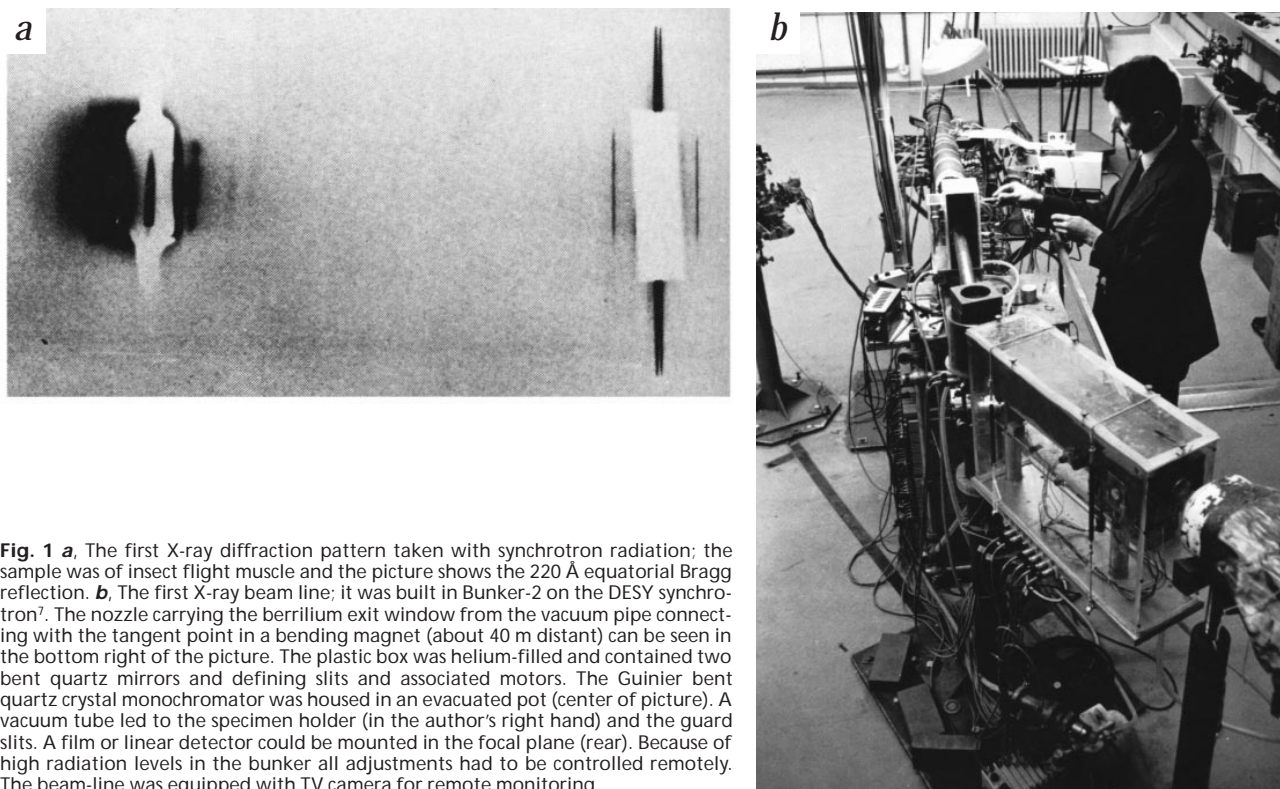
However, muscle contraction, not protein crystallography motivated the use of synchrotron radiation as a source for X-ray diffraction. Skeletal muscle contracts by the mutual sliding of thick myosin and thin actin filaments. H. E. Huxley first noted that a detailed low angle X-ray fiber diffraction

pattern could be obtained from living frog muscle. Later it was shown that the low angle pattern was responsive to the conformation of the myosin 'cross bridges'<sup>1</sup> which protrude from the thick filaments and move the thin filaments (the 'swinging cross-bridge hypothesis'<sup>2</sup>). Efforts to observe the cross bridges in different conformations during a contraction<sup>3</sup> showed that much stronger X-ray sources than could be obtained from rotation anode X-ray tubes would be required.

In 1963, a group measured the soft X-rays that were emitted from the 1 GeV synchrotron at Frascati<sup>4</sup>. At this time a number of more energetic electron synchrotrons were being commissioned, and Schwinger's theoretical studies<sup>5</sup> allowed one to calculate that a 6 GeV machine would be a powerful X-ray source. In 1964 I contacted the DESY (Deutsches Elektronen-Synchrotron) with a view to

using DESY as an X-ray source. However, it was only after moving to the Max Planck Institute in Heidelberg, Germany in 1968 where I was joined by Gerd Rosenbaum that this idea became a reality. Rosenbaum contributed essential hands-on experience, acquired at the vacuum-UV synchrotron radiation facility (F41) at DESY. Together with Jean Witz we set up a curved quartz monochromator on the DESY vacuum-UV beam-line and in August, 1970 we used the resulting monochromatic beam to obtain a smudgy diffraction picture of the 220 Å equatorial Bragg reflection from a sample of insect flight muscle<sup>6</sup> (Fig. 1a).

On the basis of this successful trial we were strongly encouraged by the directors of DESY to build a laboratory on the synchrotron DESY (Bunker-2) for X-ray diffraction. This took place in 1971. It was clear that the constant-current storage



**Fig. 1 a**, The first X-ray diffraction pattern taken with synchrotron radiation; the sample was of insect flight muscle and the picture shows the 220 Å equatorial Bragg reflection. **b**, The first X-ray beam line; it was built in Bunker-2 on the DESY synchrotron<sup>7</sup>. The nozzle carrying the berrillium exit window from the vacuum pipe connecting with the tangent point in a bending magnet (about 40 m distant) can be seen in the bottom right of the picture. The plastic box was helium-filled and contained two bent quartz mirrors and defining slits and associated motors. The Guinier bent quartz crystal monochromator was housed in an evacuated pot (center of picture). A vacuum tube led to the specimen holder (in the author's right hand) and the guard slits. A film or linear detector could be mounted in the focal plane (rear). Because of high radiation levels in the bunker all adjustments had to be controlled remotely. The beam-line was equipped with TV camera for remote monitoring.

ring DORIS (at this time still being built) would be a much better source than the synchrotron, which dumped its beam 50 times a second, but in the mean time much useful experience was gained using DESY. The first X-ray beam line (Fig. 1b) was operational by the summer of 1972<sup>7</sup>, allowing a number of experiments on insect muscle and other biological materials to be performed<sup>8</sup>. The long awaited time-resolved experiments on frog muscle needed the strength of the storage ring and were finally carried out by H.E. Huxley and his team on DORIS in Bunker-4 in 1980<sup>9</sup>.

While the high energy physics community was very supportive one could not overlook the fact that they were paying for the machines and we were using them, almost parasitically. They had their own priorities concerning energy and modes of operation, which often led to long periods with no useful beam. Furthermore, the machines were neither particularly stable nor reliable. Initial attempts by our laboratory to use the X-ray beam line in Bunker-2 for X-ray crystallography led to a distinct improvement over a rotating anode tube (~10-fold gain) but when the working conditions were considered it almost did not seem worth the bother<sup>10</sup>. However, experiments carried out about the same time at Stanford on the constant-current storage ring SPEAR showed a ~50-fold gain and demonstrated the power of the method<sup>11</sup>. Moreover, the fantastic collimation of the synchrotron beam began to make its impact: accurate data could be obtained from large unit cells and small crystals.

From its inception the European Molecular Biology Laboratory under its director general John Kendrew was very supportive of the synchrotron radiation enterprise and in 1975 Bunker-2 and a second laboratory on the storage ring DORIS (Bunker-4) became the EMBL outstation at DESY. X-ray crystallography in the outstation grew slowly but steadily. Low angle scattering and fiber diffraction were still supported but lost their pre-eminence. Furthermore, in the course of time the high energy physicists moved on to bigger machines, leaving the synchrotron radiation user community in sole charge. Synchrotron radiation had become important enough to contemplate building dedicated machines that would be run for the con-

venience of synchrotron radiation users rather than high energy physics. The use of synchrotron radiation as an X-ray source for macromolecular crystallography took off and even the small molecule crystallographers began to take an interest.

In the last decade a number of technical advances have helped make synchrotron radiation widely available as a standard technique for macromolecular crystallography (see articles by Keith Wilson and Robert Sweet in this supplement). Clearly, the great improvements in stability and brilliance of synchrotron radiation sources have played an important role. Since our first experiments at DESY the brilliance has risen by more than five orders of magnitude. Additionally, cryo-crystallography has made it much easier to use synchrotron radiation for experiments. In 1934 J.D. Bernal founded protein crystallography by photographing pepsin crystals wet sealed in a capillary tube. However, when X-rays hit the mother liquor it becomes a soup of free radicals and radiation damage takes out even the hardest protein crystal in time. A number of groups experimented with non-aqueous environments and liquid nitrogen temperatures. It was established that frozen crystals could survive if the freezing was very rapid<sup>12</sup>. In 1990 Teng<sup>13</sup> combined this notion with a traditional mounting: catch the crystal in a film in a thin wire loop and plunge it into liquid nitrogen, a method which is particularly good for small crystals, and yields crystals which are nearly immortal. The use of area detectors, particularly imaging plates, instead of photographic film to collect accurate data marks another advance in the field. Finally Hendrickson's multi-wavelength anomalous diffraction (MAD) technique<sup>14</sup> (see the article by Craig Ogata), particularly when used with selenomethionine, is changing the way crystallographic experiments are performed — by providing an almost universal method of experimentally determining phases (that can be carried out only on a synchrotron source).

Initially, synchrotron X-ray sources were considered suitable only for 'difficult' problems such as determining the structures of viruses. Furthermore, as described above they could be very frustrating to use. However, the accuracy of the data coupled with the unique ability to vary wavelength has led to the routine use of these sources for protein structure

determinations. Moreover, protein structures are no longer solved solely by specialists but rather are increasingly determined by cell biologists and biochemists. Thus protein crystallography using synchrotron radiation is now experiencing rapid growth: the annual publication of new structures has grown to a flood of 800 per year (Brookhaven Protein Data Bank 1996) of which most depend on synchrotron radiation. Given the impact of the genome projects, the magnitude of future growth and the character of future research at synchrotron facilities around the world will for some decades be limited by the funding of biological science and availability of beam lines rather than by scientific need, which is virtually open-ended.

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