Synchrotron Radiation in Pharmaceutical Research

A new UK-based synchrotron light source is expected to help advance drug discovery by offering advanced research capabilities in structural biology.

By Louise N. Johnson at Diamond Light Source, Harwell Science and Innovation Campus

Professor Dame Louise Johnson was appointed Director of Life Sciences at Diamond Light Source in October 2003 – an appointment which takes up 50% of her time as she retains her David Philips Professorship in Molecular Biophysics within the Biochemistry Department of the University of Oxford (UK). Over the past 35 years, Professor Johnson has been involved in research, teaching and administration at the University of Oxford, and has served on various international and national committees and boards. Her research interests are in understanding the structural basis of cellular control processes using protein crystallography complemented by electron microscopy and various other biophysical and biochemical methods. Her current research focuses on protein kinases and ubiquitin-mediated interactions involved in regulation of the cell cycle. She has published nearly 200 scientific papers in the field of structural biology, and in 1990 was elected Fellow of the Royal Society for her work with glycogen phosphorylase and the understanding of its mechanism through control by phosphorylation.

The UK's next generation synchrotron, Diamond Light Source, is being built on the Chilton campus in the Oxfordshire countryside, just 15 miles south of Oxford city. This new science facility is housed in a striking doughnut-shaped building over half a kilometre in circumference, covering the size of five football pitches (see Figure 1) and is on schedule to welcome its first users in January 2007. Funded 86% by the UK Government through the Office of Science and Technology, and 14% by the Wellcome Trust, Diamond is the largest single investment in science in the UK for over 30 years. Ultimately it will host as many as 40 research stations, supporting the life, physical and environmental sciences.

This article reviews the Diamond Light Source project, and looks at some of the ways in which synchrotron radiation can be used in drug discovery and development.
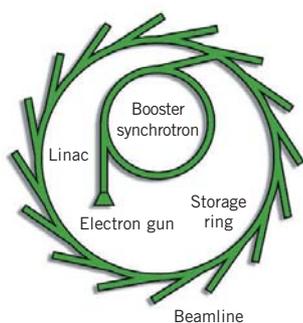
THE DIAMOND SYNCHROTRON

A synchrotron uses properties of accelerated electrons to produce an intense beam of photons in the wavelength range from hard X-rays to infrared radiation (see Figure 2, page 18). By taking advantage of new developments in accelerator technology and insertion devices for the production of X-rays, Diamond will produce a beam that will equal – if not surpass – in brightness the beams produced by other synchrotrons around the world. The quality of the photon beam will be complemented by purpose-built experimental stations supported by scientific and technical staff. Visiting researchers will also benefit from Diamond's proximity to complementary research facilities on the Chilton Harwell science campus, including the ISIS neutron facility, the Central Laser Facility, computing and networking facilities located at the Rutherford Appleton Laboratory, secure data handling and conveniently located accommodation. In future years, a research complex will be built alongside

Figure 1: An aerial view of Diamond Light Source in February 2006

The view shows the doughnut-shaped synchrotron building which houses the 562m in circumference machine generating the light required for the experiments (Figure 2). The main building is linked to a square shaped office block – Diamond House – at the front, which houses staff meeting rooms in support to the facility.

Figure 2: A schematic diagram showing the generation of synchrotron radiation

Electrons are fired from a gun into a linear accelerator (Linac) where they are accelerated up to energies of 100 MeV. They are injected into a Booster ring where they are further accelerated to 3GeV energy and are travelling close to the speed of light. They are then injected as bunches into the Storage ring, where their energy is maintained at 3 GeV. The electrons are travelling within high vacuum and steered with the help of magnets. At various points around the storage ring the electrons are curved with specially designed magnets and they emit radiation from the visible to the hard X-ray region. The specially designed magnets are called undulators and wigglers and they enhance the brightness of the beams. This radiation is selected and channelled in experiments on the beam lines where it is filtered and refined, ready to be used in experiments.

Diamond House to promote and exploit research at the synchrotron and other facilities on site.

In 2007 – year one of commissioning – Diamond will provide eight experimental stations or beam lines (see Phase I (2007) Beam Lines at Diamond Light Source). In the life sciences, these will comprise three macromolecular crystallography (MX) beam lines for determination of the 3D structures of proteins and macromolecular complexes. Described as 'state of the art', these beam lines and will comprise robotic sample changers, automatic data collection, an intense X-ray beam whose wavelength can be optimised for the experiment and cryo facilities. One of

these beam lines will have Category 3 containment so that pathogenic samples (for example, intact viruses) can be examined. Diamond plans to have rapid access for users and to provide fast high throughput for data collection and structure determination. In subsequent years, a microfocus MX beam line, a fixed wavelength MX side-station and a long wavelength MX beam line will be added.

The Wellcome Trust's input into Diamond stems from the great advances in structural biology that synchrotron radiation has made possible, and the implications of this for medical science. It is anticipated that it is this area of structural biology and macromolecular crystallography that will have the greatest impact on pharmaceutical research.

MACROMOLECULAR CRYSTALLOGRAPHY AND STRUCTURE-BASED DRUG DESIGN

Once a target molecule has been identified as a component of a disease, then knowledge of the structure of the target can be enormously informative in the development of new therapeutic agents. To date, there are seven compounds in the clinic where knowledge of the target protein structure was the main inspiration for design of the compound (Table 1) (1). Comparison of the involvement of zinc and peptide recognition properties of carboxypeptidase and angiotensin converting enzyme (ACE) led to the development of captopril for the treatment of hypertension in the late 1970s. More recently, a structure of ACE has shown that most of these ideas were correct but some needed revision (2). Carbonic anhydrase controls the intra-ocular pressure in the eye and, by inhibiting this enzyme, an effective treatment for glaucoma was developed. The structure of the enzyme helped guide the manipulation of ligand stereochemistry to give a compound that was both a potent inhibitor and lipid- and water-soluble.

The start of the AIDS epidemic led to an intense period of research to combat HIV that resulted in several compounds that are potent inhibitors of the HIV protease – a specific protease that is composed of a symmetrical dimer. The challenge was to create a non-peptidic compound that could mimic the peptide recognition properties of the dimer, and it was in this aspect that the structure was most useful. Finally the influenza virus neuraminidase structure determination presented a unique target for the development of an anti-flu compound. The neuraminidase allows the virus to be released from the host cell receptor. Based on knowledge of how sugars that resembled the transition state of the catalysed reaction bound, modifications were designed
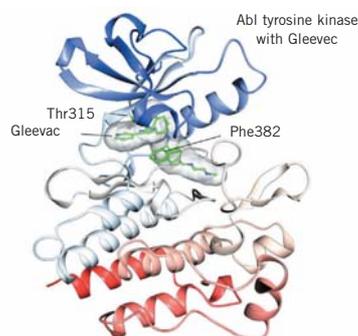
Table 1: Successful drugs developed on the basis of structure-based drug design (adapted from (1))

Target	Compound	Therapeutic activity	Date when approved and marketed	Company
Angiotensin converting enzyme	Captopril	Hypertension	1981	Bristol-Myers-Squibb
Carbonic anhydrase	Trusopt (dorzolamide)	Glaucoma	1995	Merck
HIV protease	Viracept (nelfinavir)	HIV	1999	Pfizer
HIV protease	Agenerase (amprenavir)	HIV	1999	Vertex
HIV protease	Aluviran (iopinavir)	HIV	2000	Abbott
Influenza neuraminidase	Relenza (zanamivir)	Influenza	1999	GlaxoSmithKline
Influenza neuraminidase	Tamiflu (oseltamivir)	Influenza	1999	Roche

taking into account conformational flexibility to produce the potent inhibitors Relenza and TamiFlu.

Although the list of compounds whose design was based mainly on structure is small, nearly every major pharmaceutical company can now cite products where knowledge of the protein structure has contributed to the design and synthesis of an effective agent. Once a lead compound has been identified from chemical library screening, physiological response or computational methods, structural information can inform on how the compound can be modified to improve its properties. Structure can also provide information on where the compound should not be modified – information that is often equally important. Pharmaceutical and bioscience companies have been swift to recognise the huge commercial potential that lies behind understanding the structures of biological macromolecules.
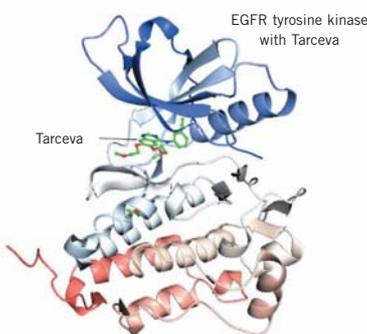
Examples of where structure has helped inform researchers about the mechanisms of drug targeting are given in Figure 3; this details some anti-cancer compounds that target protein tyrosine kinases (reviewed in (3)). The disease of chronic myelogenous leukaemia arises from a chromosomal translocation event that leads to loss in the regulation of Abelson's tyrosine kinase (Abl). Skilful chemistry and rapid effective biological assays led to Gleevec, a powerful inhibitor of Abl developed by Ciba Geigy (now Novartis). If the disease is caught in the early stages, patient treatment with Gleevec can lead to 100% remission; however, chronic treatment of patients has led to mutations in Abl that are Gleevec resistant. Determination of the structure of Abl in complex with Gleevec showed that the compound bound at the ATP binding site of the kinase and, by virtue of its interactions with the protein, bound only to the inactive form of the kinase (4) (Figure 3a). Knowledge of the structure showed why certain mutations – for example, mutation of residue threonine 315 to leucine – would lead to drug resistance. It could also show how more effective compounds might be developed to overcome resistance.

Lung cancer is a leading cause of death, with the so-called non-small cell lung cancers being the most numerous (80%). Tobacco usage is the main cause of lung cancer, but 15% of cases arise in 'never smokers'. Recent clinical trials have shown that lung cancers in non-smokers are the most likely to respond to the new drugs Iressa (gefitinib, AstraZeneca) and Tarceva (erlotimib, OSI). Iressa and Tarceva inhibit the epidermal growth factor tyrosine kinase (EGFR kinase). The EGFR is a receptor

(a)

Abl tyrosine kinase with Gleevec

Thr315
Gleevac
Phe382

(b)

EGFR tyrosine kinase with Tarceva

Tarceva

(c)

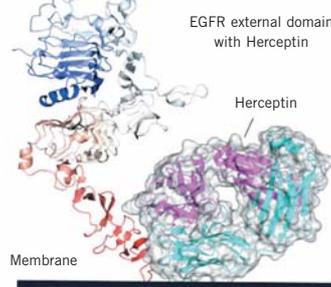EGFR external domain with Herceptin

Herceptin

Membrane

Figure 3: Examples of where structure has contributed to understanding the mechanism of tyrosine kinase inhibitors for compounds that are in the clinic

a) Abl tyrosine kinase in complex with Gleevec. The protein kinase is shown schematically with helices as coils and β strands as arrows, and is coloured blue to red from the N- to C-terminus. Gleevec (shown with carbon atoms green and van der Waals surface outlined) binds at a site between the N- and C-terminal lobes. Thr315 guards the entrance to a pocket that is filled by Gleevec. Mutation of this residue to leucine results in resistance to the drug. Phe382 position is a marker for the inactive conformation of the kinase.

b) The epidermal growth factor tyrosine kinase with Tarceva. Tarceva binds to the kinase in the active conformation at the ATP binding site. The colour scheme is as above.

c) Herceptin, the monoclonal antibody against the extracellular region of the epidermal growth factor receptor binds to the juxta-membrane domain of the receptor.

protein that spans the cell membrane; when bound to its ligand (epidermal growth factor), the receptor is activated and triggers cell proliferation through a signalling cascade. The structure of EGFR kinase in complex with Tarceva showed that the compound bound to the active conformation of the kinase (5) (Figure 3b). Patient response to Iressa has been unpredictable. A favourable clinical response rate is almost three times higher in Japanese patients than in those from the USA. Later results have shown that those patients most likely to respond are those in whom the EGFR kinase carries activating mutations close to the ATP site or catalytic site (6,7). The structure of EGFR kinase can provide information on the likely consequences of these mutations and susceptibility to drug treatment.
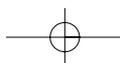
One of the four different forms of the epidermal growth factor receptor, called ErbB2 (or Her2), is over-expressed in a number of different types of cancer – particularly breast cancer – where over-expression correlates strongly with the aggressive form of the disease and poor survival rates. Herceptin (trastuzumab, Genentech) is an anti-ErbB2 monoclonal antibody that is effective in patients that over-express ErbB2. The structure of the extracellular domain of ErbB2 in complex with Herceptin (8) (Figure 3c) has shown that it binds to the region near the cell membrane, where it might interfere with signalling in addition to the cell-killing immune responses triggered by the antibody recognition. Knowledge of the interacting sites and the mechanism of activation of the EGFR in response to growth factor binding, based on structural evidence, has shown how other regions of the receptor may be targeted for more general anticancer treatment.

In recent years automated genome sequencing has changed the face of biology. The human sequence encodes some 20,000-25,000 proteins, and with recombinant DNA technology, these proteins can now be made to order. In the past, people looked at proteins that were easy to obtain in large amounts and structural biologists were restricted to studying naturally abundant proteins. Modern methods allow researchers to be more pro-active; it is possible to identify important targets, clone relevant genes, and express and purify the key proteins to support crystallographic investigations. Growing crystals is still the rate-limiting step towards a successful structure determination. The increased brightness of new sources – such as Diamond – means that the crystals need only be a fraction of a millimetre across (as small as 10 μm). This has opened up a whole new series of target proteins for structural investigation.

Almost 50% of drug compounds in clinical use are targeted at membrane proteins; consequently, structural studies in this area are becoming increasingly important. Of the 35,000 structures in the Protein Data Base (as of March 2006), less than 100 are membrane proteins, reflecting the difficulty in determining these structures. There are now several UK Research Council and EU funded initiatives that aim to speed up the difficult steps in the expression, purification and crystallisation of membrane proteins. As a step in this direction, Diamond Light Source in collaboration with the Wellcome Trust will set up a membrane protein laboratory under the direction of Professor So Iwata, with the aim of facilitating diffraction studies on small weakly diffracting crystals of membrane proteins. This is an example of Diamond's aim of creating a strong research environment associated with the synchrotron source.

SMALL MOLECULE CRYSTALLOGRAPHY AND POWDER DIFFRACTION

In Phase II, Diamond will commission beam lines for small molecule crystallography and for powder diffraction in 2008. In addition to basic research, these beam lines will help address some of the problems that occur in the processing of drugs. During production, pharmaceutical compounds undergo a range of processes including drying, granulation, milling and compression. Exposure to changes in temperature, pressure and relative humidity may affect the properties of these compounds, such as the creation of polymorphs – substances with the same chemical composition but different crystal structures. The process of compressing drugs into tablet form can create new polymorphs. Understanding the forces within the crystal structure can help predict the properties of the polymorphs, and can guide the creation of new polymorphs with desirable characteristics, for example improved solubility.

In future, new polymorphs of pharmaceutical compounds may be developed using these techniques, to produce more effective drugs and improve understanding of production processes. Diamond will provide a bright beam so that small samples can be rapidly characterised, either by crystallography or by powder diffraction. Recent advances in small molecule powder diffraction mean that changes in conformation of a known structure can be rapidly determined, and *ab initio* structure determination is also possible.

NON-CRYSTALLINE DIFFRACTION AND CIRCULAR DICHROISM

The non-crystalline diffraction beam line, the first Phase II beam line which will be commissioned in 2007, will allow powerful small-angle and wide-angle scattering studies on proteins in solution. Such methods give information on the overall shape of a molecule and changes in shape in response to ligand- or protein-binding. This beam line will also be set up with state-of-the-art facilities for recording diffraction patterns of fibre specimens such as muscle.

In Phase II in 2008 a beam line for recording circular dichroism spectra will be commissioned. This will enable the analysis of protein secondary structure in solution, and give clues on protein stability and folding. Some 30-50% of the proteins of eukaryotic cells are predicted to have natively disordered structures that cannot be crystallised. These proteins are important in signal

transduction of normal and tumour cells, and so 'in solution' studies are crucial to investigate these systems. In addition this beam line will have a Raman optical activity station, which will be important for the study of proteins, carbohydrates and glycoproteins. Circular dichroism can also be used to monitor ligand binding to proteins (9). The brightness of the light source at Diamond will mean that lower concentrations can be used and more rapid data collection can be achieved than with sources in the home laboratory.

DISCo

Diamond will continue its outreach to new potential users, thereby ensuring that once opened the facility and various techniques on offer are fully maximised. In order to develop best practice for industrial engagement with Diamond and to increase awareness of its potential for industry, Diamond has set up DISCo – the Diamond Industrial Science Committee. The aims of DISCo are to identify industrial research priorities, to help develop the case and requirements for future beam lines, and to advise on opportunities for industry to be engaged. Membership includes representatives from the pharmaceutical and other industries.

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