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# Integrating biocrystallography into traditional biology and chemistry curricula

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New academic courses for teaching protein crystallography to biology and chemistry students have been developed. The general aim of the lecture courses is to introduce crystallographic terminology and modern diffraction methodology, to discuss the principles of macromolecular structure, to develop confidence in assessing macromolecular models, and to develop skills in extracting biostructural information from crystallographic literature as well as from bioinformatics resources available on the Internet. Emphasis on structural biology (chemists) or on crystallographic concepts and methodology (biologists) differs depending on the background of the students. In practical classes, the students work with real models of crystals and lattices, and use construction kits to build models of protein secondary structure. Diffraction images of oriented protein crystals are used for determination of unit-cell parameters. An Internet-based multiple-choice quiz is used for tests, self-assessment and practice.

## 1. Introduction

This paper describes recent experience of introducing protein crystallography into academic curricula in Poland. The experience is derived from our teaching at A. Mickiewicz University in Poznan. There is a very good tradition of small-molecule crystallography in Poland, which has earned respect at the international level. As an academic subject, it is offered mainly in schools of chemistry. In this context it is quite striking that protein crystallography was first offered (at least in this university) in the school of biology, where it was actually an important element of curriculum modernization, while chemistry curricula included it after some delay. Obviously, the programs that are offered to biology and chemistry undergraduates are quite different, though almost complementary. The biology student has a very good understanding of the biological matter and of its fundamental properties, but tends to think in 'one dimension', *i.e.* is mostly concerned with primary structure. What is lacking is good training in three-dimensional thinking and basic understanding of crystallographic concepts and methodology, at least at a level that is necessary for comprehension of protein crystallographic literature and exploitation of the information contained in structural databases. In the case of chemistry students, the general crystallographic knowledge is usually good. Much effort is needed, however, to explain the fundamental properties of biomolecules as well as the principles of their structure and folding. Because of the difference in the programs, there are also differences in the practicals offered to those students. The biology practicals are more of classroom-type, while chemistry students are offered laboratory exercises, by which quite a few of them have their first opportunity to work with real proteins.

Our effort to develop biocrystallography teaching began practically from scratch. One aspect of this development has been the creation of a database of examination questions for multiple-choice tests. Recently, these questions have been turned into a computerized quiz that is accessible *via* the Internet. This PXQuiz is very useful, not only for mid-term tests and for final examinations, but also as an educational aid, available to the students at any time.

## 2. Biocrystallography for biologists

As an obligatory subject, protein crystallography was first introduced at this university about a decade ago, in the syllabus offered to biotechnology students. The original decision to teach it as a lecture course only, soon proved to be misjudged. Today, our biology students majoring in biotechnology and molecular biology are offered a 30 hour block of laboratory practicals and only a short 15 hour lecture course.

Protein crystallography forms the structural foundation of molecular biology and in our experience should be taught early in the curriculum, preferably during the second year. Crystallographic instruction has several important functions in the formation of modern biologists (Table 1). We start by telling them about symmetry. This is of dual advantage: the notion of symmetry is crucial for understanding many other crystallographic concepts, and, equally important, it helps to train the students in three-dimensional thinking. The latter aspect should not be underestimated as, traditionally, the biology students view biological problems from a one-

**Table 1**
Objectives of the biocrystallography course taught to biology students.

| | |
|---|---|
| 1 | Understanding symmetry |
| 2 | Three-dimensional thinking |
| 3 | Principles of three-dimensional structure of biomolecules |
| 4 | Understanding the process of protein crystal structure determination |
| 5 | Understanding the information in crystallographic literature |
| 6 | Ability to assess three-dimensional models of biomolecules |
| 7 | Familiarity with modern computer graphics |
| 8 | Familiarity with global bioinformatics resources |
| 9 | Exploiting the information in the Protein Data Bank |
| 10 | Correlation between biotechnology and protein crystallography |
| 11 | Protein crystallization |

dimensional perspective. After this introduction, the lecture course and the practicals are concerned with three-dimensional structure of biomolecules: first proteins then nucleic acids. The proteins are discussed along the lines of anatomy and taxonomy as introduced by Richardson (1981), but with illustrations including more recent examples. The discussion of the structure of nucleic acids includes topological problems connected with supercoiling and the conservation of the linking number. The structural block also covers the principles of spherical virus architecture with elements of icosahedral triangulation (Caspar & Klug, 1962). Without going into technical details, the principles of protein crystal structure determination are then outlined. Emphasis is placed on quality indicators to enable the students to assess three-dimensional models of macromolecules. They are also expected to be able to understand the information in crystallographic publications and in structural databases, primarily in the Protein Data Bank (Berman *et al.*, 2000). In conjunction with macromolecular databases, the students also learn how to use the Internet to exploit the global bioinformatics resources. They are introduced to simple but efficient computer graphics tools, such as *RasMol* (http://www.umass.edu/microbio/rasmol/) and the *Kinemage* technology (Richardson & Richardson, 1992, 1994; Sokolik, 1995; http://www.faseb.org/protein/kinemages/kinpage.html), that allow them to deepen the appreciation of three-dimensional aspects of molecular biology. The course ends with a discussion of special biocrystallographic techniques, like protein crystallization, use of Se-Met proteins, cryocrystallography, synchrotron radiation, Laue techniques, and neutron diffraction. In conclusion, the mutually beneficial links between crystallography and biotechnology are summarized (design and production of proteins for crystallography, structural information in rational drug design, *etc*.).

The topics discussed in the practical class meetings and the assignments/problems that the students have to solve are listed in Table 2. The sequence of these topics reflects the program of the lecture course.

## 3. Biocrystallography for chemists

The chemistry students at this university are offered a course in biocrystallography as a facultative (optional) subject when they are approximately halfway through the syllabus. It includes a lecture course (30 hours) and two blocks of practicals, (i) protein handling and crystallization (45 hours) and (ii) diffraction techniques and molecular modelling (30 hours), which can be chosen in any combination.

In contrast to the biologists, the chemistry students come with a thorough knowledge of basic crystallography and diffraction theory, but they are almost completely ignorant of structural biological problems. The introduction to the lecture course and the entire block of 'wet-lab' practicals (i) are devoted to physicochemical characterization, purification, handling and crystallization of proteins (Table 3). The lecture course then turns to diffraction methods, but only to emphasize the most advanced techniques, including synchrotron radiation, modern area detectors, Laue diffraction and special aspects of the phase problem in protein crystallography, particularly the multi- and single-wavelength anomalous diffraction (MAD and SAD) approaches. Electron density maps, their variants, interpretation and modification are then discussed. With regard to structure refinement, the need for stereochemical restraints is stressed and elements of molecular-dynamics simulation are introduced. The methodological part is concluded with a discussion of structure validation and quality indicators. The structural part begins with a description of protein structure hierarchy and the

**Table 2**
Practical classes offered to biology students.

| | Topics | Practical assignments |
|---|---|---|
| 1 | Symmetry elements, point groups | Find symmetry elements and determine crystal class for a crystal model (solid) |
| 2 | Screw axes, enantiomorphism, space groups | Determine the space group symbol for a model of a Bravais lattice |
| 3 | *International Tables for Crystallography*, fractional and orthogonal coordinates | Transformation of atomic coordinates. Calculation of bond lengths from atomic coordinates |
| 4 | Hydrogen bonding | Analyse proton-donor and proton-acceptor capabilities of amino acids and nucleotides |
| 5 | Torsion angles, Ramachandran plot | Construct models of $\beta$ sheets |
| 6 | The $\alpha$ helix | Construct a model of the $\alpha$ helix |
| 7 | Triangulation of icosahedral viruses | Polio virus – video presentation |
| 8 | DNA structure | The double helix – video presentation |
| 9 | Diffraction of X-rays by crystals | Description of X-ray laboratory. Determination of a lattice parameter from a protein oscillation image |
| 10 | Bioinformatics resources on the Internet | Browsing the Internet for biostructural information (ExPASy) |
| 11 | The Protein Data Bank | Structural characterization of a selected protein using *RasMol* |
| 12 | Advanced molecular graphics tools | Analysis of protein structure using the *Kinemage* technique |

**Table 3**
Practical classes offered to chemistry students.

| | |
|---|---|
| Protein handling and crystallization | |
| 1 | Preparation of stock buffers and cover slides for protein crystallization |
| 2 | Dissolving the protein. Concentrating protein solutions *via* dialysis and ultracentrifugation |
| 3 | Protein electrophoresis in polyacrylamide gel (SDS-PAGE) |
| 4 | Studying proteins *via* UV–VIS spectroscopy. Determination of protein concentration |
| 5 | Screening for crystallization conditions using the hanging-drop technique |
| 6 | Fine-tuning of crystallization conditions (microseeding, sitting-drop technique) |
| 7 | Mounting crystals in glass capillaries and in fibre loops for flash freezing |
| Diffraction techniques and molecular modelling | |
| 1 | Recording protein crystal diffraction patterns with an imaging-plate scanner |
| 2 | Indexing and integration of a diffraction pattern; determination of the orientation matrix and space group |
| 3 | Calculation and viewing of electron density maps |
| 4 | Analysis of the three-dimensional structure of papain using molecular graphics (*O*) |
| 5 | Bioinformatics resources on the Internet |
| 6 | Exploring the information in the Protein Data Bank |
| 7 | Using *Kinemages* for presentation and analysis of macromolecular structure |

conformation of the main chain, and continues through classification of protein folds. Macromolecular structure and function is then illustrated by selected examples, including hemoglobin (allostery and transport), aspartic proteases (enzymes, retroviral proteins), the polio virus (icosahedral particles), and nucleic acids (types of double helix, supercoiling). The course ends with an overview of bioinformatics resources on the Internet, with special stress on the Protein Data Bank.

The above problems are also focussed upon in the computer-oriented practicals (ii) (Table 3). Here the students begin with computer processing of diffraction images. In the next session they are introduced to computer graphics and familiarize themselves with electron density maps. The remaining exercises are related to molecular modelling and the use of such graphics tools as the *O* program (Jones & Kjeldgaard, 1994), *RasMol* and *Kinemages*.

## 4. Examples of practical exercises

Some of the exercises offered to the students are fairly standard and obvious and do not have to be discussed in detail here. These include the 'wet-lab' practicals in protein handling/crystallization, or the use of standard graphics software like *RasMol*, or the *Kinemages*. However, in some cases, we have developed new exercises that may be less familiar. They have become very popular with the students and it may be worthwhile to describe them in more detail. In one of these practicals, the students are given a molecular construction kit and are asked to build models of protein secondary structure elements. The kit is a selection of construction elements from a standard set for building molecular models that was developed in this department many years ago. The key element of our kit is a planar peptide group with a special oxygen atom, suitable for attachment to a hydrogen bond. The hydrogen bond is a flexible to-scale element (spring) that can connect hydrogen-bond donor and acceptor groups. In addition to C($sp^3$) carbon atoms, the kit also includes a special protractor that allows the students to measure (and set) the $\varphi$ and $\psi$ torsion angles of a growing chain of *trans* peptides. Using several chains in extended conformation, various $\beta$ sheets are then assembled. Construction of the $\alpha$ helix is most instructive because the students quickly learn that without the hydrogen-bond reinforcements, the structure is very flexible and difficult to handle.

We no longer use photographically recorded diffraction patterns in the exercises. Instead, the students are presented with a computer-stored pattern recorded on an image plate. The image can be printed so that the students can carry out the necessary measurements by hand, or else it can be viewed in a program like *CorelDRAW*, where the measurements can be taken off the screen. With specially oriented crystals, it is possible to calculate the unit-cell parameters and estimate the degree of misorientation.

When the students learn the principles of spherical virus architecture, as an extra assignment they are asked to build (from cardboard) a model of an icosadeltahedron. Even though these models are sometimes crude and less elegant than many attractive-looking computer-generated images, we feel (and the students share this opinion) that working with real matter gives a better understanding of structural problems. This applies to several other exercises. For example, without shunning computer-aided instruction, we always use real solids and models of (Bravais or crystal) lattices to introduce symmetry and to encourage three-dimensional thinking.

## 5. Handbooks and visual aids

There is a very good selection of English-language textbooks that can be recommended to biocrystallography students. For the biology students, we usually recommend an easier text, such as that by Rhodes (1999), as the first reading. Those with a better understanding of mathematics will occasionally browse through the books by McRee & David (1999) or by Glusker *et al.* (1994). The latter book and especially the rigorous text by Drenth (1994) can be recommended to the chemistry students who at the outset have a good understanding of general crystallography. For the structural part, we find the handbook by Branden & Tooze (1999) to be very successful, especially in its second edition. It is accompanied by a CD ROM containing the illustrations used in the book. Although it is not a strict structural biology text, we like to recommend the new book by Voet *et al.* (1999) in this context. It is also accompanied by CD ROMs. Of particular interest is that they include illustrations prepared using the *Kinemage* technique, which enable the students to appreciate fully the three-dimensional nature of the macromolecules under study.

There are several useful video films that can be used as aids in macromolecular crystallography teaching. There is, for example, a collection of movies developed by Olson and co-workers at The Scripps Research Institute (http://www.

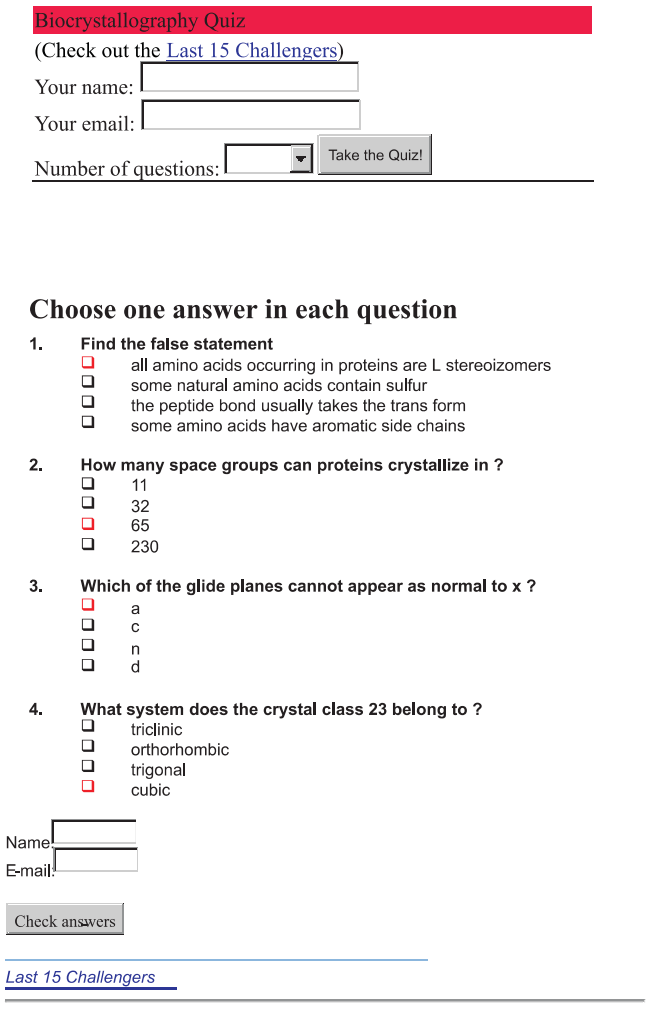


**Figure 1**
The front page (upper part) of the PXQuiz when it is accessed through the World Wide Web. For a particular run of the quiz, different lengths (numbers of questions) can be chosen. A sample set of questions is shown in the lower part. Clicking on the bottom button will return the correct answers.

scripps.edu/pub/olson-web/people/gmm/movies.html). We use two short productions from this collection when we are concerned with protein structure and enzyme action (Cu, Zn superoxide dismutase), and when icosahedral viruses are the subject under discussion (polio virus). For the illustration of the structure of double-helical DNA, the excellent movie by Lucas is used (Lucas *et al.*, 1999). This film, however, is too long for a typical class meeting and we prefer to use it during a specially dedicated session. Another advantage of the videotape by Lucas is that it is accompanied by a diffraction kit that can be used to illustrate how the various structural features of double-helical DNA are reflected in its diffraction pattern. In the same way, we use the popular optical transform kit (Lisensky *et al.*, 1991) to demonstrate the principles of diffraction by regular arrays.

## 6. Web-based quiz

Throughout the years, we have accumulated a rather substantial database of test and examination questions covering different aspects of crystallography, not only biocrystallography. Recently, these questions have been turned into an Internet-accessible quiz resembling the popular Swiss-Quiz offered through the ExPASy Molecular Biology Server (http://www.expasy.ch/swiss-quiz/). Our PXQuiz (http://www.man.poznan.pl/CBB/PXQuiz.html) can be used for mid-term tests as well as for final examinations. In the examination mode, it can e-mail the results to the examiner. It can also be used in an educational mode, revealing the correct answers to the student. PXQuiz is a multiple-choice test, always with one correct answer to be found among four possibilities. The questions are drawn at random from the full database, which at the present moment holds about 250 entries. This question database grows as the discipline and our experience develop. Originally in Polish, it is being converted into English to promote studying in this language among our students and to make it of more general interest. The program driving it through the Web page is an adaptation of the Perl script *Quirex*1.3 written by Thomas Tsoi (http://www.cgi.com.hk). The welcome page of PXQuiz and a sample run are shown in Fig. 1.

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