

A Master of Science course on macromolecular X-ray crystallography resembling a realistic research project

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Abstract

Macromolecular X-ray crystallography is a robust method to understand protein functions at the molecular level, deciphering their various roles in biochemical processes. This makes teaching the method relevant and important for all biochemistry majors. To promote student engagement and hands-on training, we have developed a “Macromolecular X-ray crystallography” course where students determine a protein structure while learning all the steps of the technique. The course is implemented through Moodle, a web-based Open Source learning platform. The interactive online tool facilitates teaching by providing the students easy access to the course material and by enabling efficient tracking of learning outcomes for teachers.

Introduction

Macromolecular X-ray crystallography is a fundamental method in structural biology, where crystal structures widely contribute to our understanding of biochemical processes at the molecular level. As of today, it is a method that consistently provides atomic and sub-atomic resolution molecular details, making it an invaluable tool for all biochemists. However, teaching of crystallography to undergraduate students is generally not an easy task, and understanding the technique is usually achieved by specialized PhD level courses and hands-on practice.

Educational literature contains a number of publications aimed at practical teaching of protein crystallography to undergraduate students. The majority of these only covers parts of the method, for example crystallization or model building [1–3], although more comprehensive teaching modules have also been published [4,5]. Student feedback on

these have indicated good learning outcomes [4,5]. In particular, research-inspired teaching has been highly appreciated by students [4].

Here we aim to promote the teaching of protein crystallography, efficiently covering all the theoretical and practical steps of the method by simulating a realistic research project. We have therefore developed a project-based MSc course called “Macromolecular X-ray crystallography”, and we implemented it at the University of Oulu (Finland). Our philosophy was to engage students in solving a realistic research problem. That includes working with the same protein target starting with its crystallization, through diffraction data collection and processing, to structure solution, model building, refinement and finally model validation. The course has been enhanced by introducing specific challenges, which mimic real-case scenarios. For example, the use of a search model which significantly differs from the target during the molecular replacement step. The challenges train the student problem-solving skills, at the same time encouraging them to learn the method comprehensively. The research-based approach of the course has been acknowledged as a positive feature by students in their feedback.

General description of the course

The course is built around the determination of the three-dimensional structure of *Tritirachium album* proteinase K (ProtK) that forms a project for students. ProtK is commercially available (Merck) and works well as an educational tool, thanks to its relatively small size (28.9 kDa) and its easy crystallizability, which consistently yields well-diffracting crystals [6,7]. Students learn the theory behind each step of the protein crystallography method from lectures and implement their learning in the project. Regarding *in silico* work, specific practicals are used to introduce the needed softwares and provide test cases before project work.

The lectures and practicals are stored on a local installation of the Moodle online learning platform (<https://moodle oulu fi>). It provides an easy access for students to the course material including lecture recordings and slides, instructions for the exercises and project notebooks. The system also helps teachers to follow student progress in every section of the course. Although the choice fell on Moodle, other learning platforms fit the purpose equally well. For example OneNote (Microsoft Office 365) or Open Source tools such as Canvas (<https://www.instructure.com/en-gb/canvas>), OpenOlat (<https://www.openolat.com>) and Sakai (<https://www.sakailms.org>).

The subject has been divided into five sections: crystallization, data collection and processing, structure determination, model building and refinement and model validation. Each section is introduced by a theoretical lecture, followed by either lab work on the project or a practical regarding *in silico* work to facilitate the project work. The detailed timetable of the course is presented in Table I. In summary, students perform the crystallization of ProtK, track and score the crystallization outcome using the IceBear software [8] and choose the best looking crystals to be used in data collection, which is

then carried out on the X-ray home source diffractometer (Bruker MicrostarX8 Proteum). They proceed to data processing by using Proteum3 (Bruker) and Mosfilm [9], solve the structure through Phaser, and carry out the model building and refinement tasks using Coot [10], and phenix.refine, a component of the Phenix software suite [11] Finally, students validate their models through Coot [10], MolProbity [12] and the Protein Data Bank [13] validation server (<https://validate-rcsb-1.wwpdb.org/>). As they progress through the course, students are asked to log their activity and experimental observations in an online notebook, guided by section specific questions in order to help students to make a laboratory notebook of the project with relevant notes. This notebook is stored on Moodle.

Table I. Timetable of the sections of the macromolecular X-ray crystallography course

| Section* | Lecture time [h] | Practice mode and time [h]** | Project mode and time [h]*** |
|---|------------------|------------------------------|--|
| Crystallization | 2 | 0 | Laboratory, 11 <i>In silico</i> , 1 |
| Basic concepts of X-ray crystallography | 12 | 0 | 0 |
| Data collection and processing | 2 | <i>In silico</i> , 2 | Laboratory, 2 <i>In silico</i> , 4 |
| Experimental phasing | 2 | 0 | 0 |
| Molecular replacement | 2 | <i>In silico</i> , 2 | <i>In silico</i> , 2 |
| Model building and refinement | 6 | <i>In silico</i> , 2 | <i>In silico</i> , 18 |
| Model validation | 2 | <i>In silico</i> , 2 | <i>In silico</i> , 2 |

*Listed from up to down in accordance with procedure. Each section always starts with a lecture followed by practice and/or project work

**Each practice aims to facilitate the corresponding individual project work through training with examples cases provided by a teacher

***Project work on ProtK

General learning aims for students after a completion of the course are abilities to i) discuss the key aspects of protein crystallization methods and interpret the results, ii) describe the diffraction of X-rays and the importance of crystal symmetry, iii) describe the importance of the Fourier transform method in the structure determination, iv) describe the phase problem and tell the methods to solve it, v) apply knowledge on protein chemistry to refinement of a crystal structure and vi) judge the quality of a protein structure.

The course workload corresponds to five credits in the European Credit Transfer and Accumulation System and it is planned to run over a 3.5 week period (Table I). The lectures regarding the theory are mostly based on the books written by Bernhard Rupp [14], David Blow [15] and Jan Drenth [16]. In addition, many research articles are cited throughout the notes, to give the course a more research-oriented flavour. The contents of lectures and practicals are reported in the supplementary materials (sections 1 – 10) whereas the workflow of the project is described here.

Crystallization

Students are divided in groups of 1-2 persons and they are first guided to evaluate the physico-chemical properties of ProtK, which include its molecular weight, pI, amino acid sequence and biochemical function. They are also taught the importance of factors affecting crystallization, such as inhibitors, cofactors, pH, temperature, oxidizing and reducing agents. The crystallization practical involves initial screening of proteinase K against commercial cocktail formulations, followed by the optimization of results using custom-made crystallization solutions. The crystallization experiments are carried out at 20 °C, and set up in iQ 96-well plates (SPT Labtech) by the vapour diffusion sitting-drop geometry, using a Mosquito liquid handler (SPT Labtech). The crystallization plates are then imaged in bright-field and UV spectrum with a RI54 imager (Formulatrix). Students are introduced to the different methods used to unambiguously detect protein crystals and scoring them. The principles behind the use of UV light, bright-field microscopy, polarized light, SONICC (second order nonlinear imaging of chiral crystals) and X-rays to analyze crystallization results are briefly summarized. Scoring relies on the IceBear framework [8], which is used to teach the students how to monitor, score and annotate the outcome of crystallization trials (Figure 1a). Based on the results, all students are asked to design one optimization experiment, which can be carried out using a systematic screening of sodium formate, ammonium sulfate and PEG. Alternatively, students may choose a grid-based screening strategy using Bis-Tris, ammonium acetate and PEG. The initial crystallization trials are set up with 20 and 40 mg/ml-concentrated ProtK, to help the students explore the effect of initial protein concentration in a crystallization experiment. Students also test the effect of a stabilizing factor on crystallization by supplementing the protein solution with 10 mM benzamidine, a ProtK inhibitor. They are also asked to justify their choice of an optimization strategy. Based on their results, students choose which crystals they will fish for data collection.

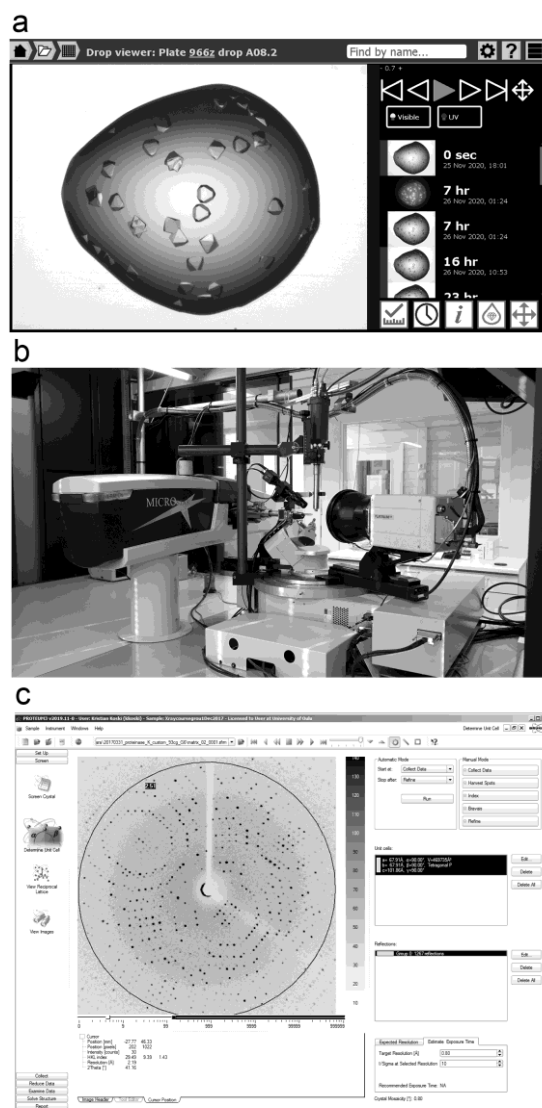


Figure 1. Crystallization and data collection of ProtK (a) Observation of ProtK crystals through IceBear[8] (b) The X-ray diffractometer (Bruker MicrostarX8 Proteum) used for data collection (c) A diffraction pattern of ProtK visualized with the Proteum3 software (Bruker).

Crystal testing and data collection

Crystal handling and diffraction data collection are combined in a single practical. Here students learn how to harvest protein crystals and set up the data collection experiment using a X-ray diffractometer (Figure 1b). The crystal testing and handling practical consists of three sessions over the course of three days, each session divided in four 45-minutes slots and running in parallel with the data processing practical. During the exercise the students are divided into two groups, group 1 and group 2. While students in group 1 are occupied with crystal handling and data collection, group 2 takes part in the

data processing practical in the computer classroom (Supplementary material, section 4). The two groups swap roles at the end of the session.

Students participating in the crystal handling practical are further divided into group A and B. Helped by a teacher, group A starts with crystal harvesting using a dedicated stereo microscope (Molecular Dimensions). Students harvest crystals from their own crystallization plates, or those from another group if their own trials did not yield any usable sample. Before collecting the crystals, they are asked to compare the underlying crystallization conditions to those tested by Garman & Owen [17], in order to select the best cryoprotectant. Typically, students prepare cryo-solutions containing either PEG 400, glycerol or ethylene glycol as a cryoprotectant supplemented in a mother liquor. After that, students fish the crystals using pre-mounted nylon loops (Hampton Research) attached to a magnetic wand tool (Molecular Dimensions). Crystals are then transferred into cryocooled SPINE pucks, kept in a foam dewar (Molecular Dimensions) filled with liquid nitrogen, and subsequently stored within a dry shipper (Wharton Taylor). Meanwhile, students from group B are introduced to operating the diffractometer (Bruker MicrostarX8 Proteum), followed by a demonstration on how to set up a data collection experiment. The practical is timed so that group A and B can swap task at the half-time of the three-hour session. Group B harvests crystals, while group A undergoes data collection training. On the second day, group 2 will have the Crystal handling and Data collection practical and group 1 will have the Data processing practical.

During the course of the third day, students individually mount their own crystals onto a goniometer using cryo tongs (Hampton Research). Under the supervision of a teacher, they center a crystal in the X-ray beam and take few exposures to determine its diffraction properties (Figure 1c). Students evaluate the crystals and their choice of a cryoprotectant by assessing the quality of the diffraction pattern. After each session, students answer questions about the practical in their project notebooks. The questions aim to draw the student's attention to the principal aspects of setting up a data collection experiment. The student's ability to justify their cryoprotection strategy, list the relevant data collection parameters, and describe the outcome of the testing of their own crystal is evaluated. H5P interactive applets embedded into the notebook are used to assess the student's ability to name the different parts of the diffractometer.

Data processing

Students are provided with a ProtK data set collected at 1.5 Å resolution on a synchrotron beamline (ESRF ID23-1, France), which they process taking advantage of what they have learnt during the data processing practical (Supplementary material, section 4). They independently index and integrate the diffraction data through mosflm, and scale the integrated intensities with AIMLESS, both accessed through the iMosflm GUI [9]. The enantiomeric space groups ($P4_32_12$ and $P4_12_12$) reflect a realistic situation, where the right solution is only known after a successful molecular replacement. The students are encouraged to process the data in both space group options, and later choose the correct

solution. In the computer classroom, a teacher follows the process and provides guidance to the students when needed. Students are also allowed to decide the resolution cutoff by themselves, based on the information given to them during the data collection practical. Students are expected to report in a table the data processing statistics from both the in-house data set (used in the training practical) and the synchrotron data set (project work), explaining the meaning of the key parameters (completeness, I/σ , R_{merge} , $CC_{1/2}$) and justifying the resolution cutoff criteria. The statistics of then compared at the end.

Solving the structure

Before trying molecular replacement on ProtK, students practice the task on test data sets (Supplementary material, section 6), using the tools available within the Phenix program suite [11]. The input information for each step is available in the data processing notes. Calculation of the Matthews coefficient [18], carried out in the Xtriage analysis, should indicate a presence of one molecule in the asymmetric unit; students are asked to report the value of the Matthews coefficient and explain what the value means. The students are provided with a sequence alignment between ProtK and the search model, *Bacillus lentus subtilisin* serine proteinase (PDB id 1GCI) [19]. The sequence comparison shows 36% identity; its relevance for manual model building is discussed during the model building and refinement section. Students utilize the information obtained from the sequence comparison and from the Xtriage analysis and perform a molecular replacement using Phaser-MR, available in Phenix (simple, one-component interface) [11]. The students are asked to report the molecular replacement results, such as how many molecules the solution contains and what is the space group for the solution (one molecule in the asymmetric unit, $P4_32_12$). The key educational question for the students is to evaluate the correctness of the solution. For this purpose, students are taught to visualize it using the Coot program [10]. Students are expected to answer by referring to the RFZ and TFZ values and by providing a figure from Coot [10] that shows the absence of clashes with neighboring protein molecules. After a successful molecular replacement, students learn which one of the reflection files (saved in mtz format) they need to use for the model building and refinement.

Model building and refinement

The low sequence identity between the search model and the actual target (36%), simulates a real-world situation, where the MR solution carries a high model bias. This is reflected by the R -factors high value (R -work and R -free over 0.4) for the MR solution, and creates an optimal target for manual model building.

Before the students start to individually work on the ProtK model, the key principles of model building are introduced to them during the ‘model building’ practical (Supplementary material, section 8). After that, a brief set of instructions are provided to

help them start the refinement process. Students are instructed to first perform a rigid body refinement followed by a restrained refinement of the MR solution. Next, they perform a density fit analysis of the model in Coot [10] and delete all the residues, which do not fit into the electron density map. After that a restrained refinement is carried out. When all the students have reached this step, a teacher explains the importance of the sequence comparison and model building. The teacher starts from the sequence comparison and shows how to proceed further by mutating mismatching search model residues into the corresponding ones of ProtK. After the introduction, students are allowed to proceed individually with model building. At the same time, the teacher continues working on the model, pointing out to the students any aspect worth considering during the model-building process. Students are encouraged to ask help from the teacher, as well as discuss issues among themselves. It is understandable that the initial poor quality of the electron density maps may cause challenges on interpreting and fixing some regions of the model (Figures 2a and 2b); individual help for model building and refinement is provided throughout the session. The improvement of the electron density maps is clearly visible as the model is iteratively corrected throughout refinement cycles. Typically, students can complete the task approximately within 10 cycles (Figure 2c), over the course of 4-5 days. As a learning outcome, students get very familiar with the structure of amino acids and the various features of Coot [10]. Students are asked to provide a short description of changes implemented into the model and also report the *R*-factors obtained from each building and refinement cycle. In addition, students need to answer some questions related to model building and refinement. During this stage of the course, the notebooks help to monitor each student's performance and the teacher can provide guidance to those who need it.

Model validation

In the final steps, hydrogens are added to the model and the final refinement is carried out with phenix.refine. At this time, the validation tools of Coot [10] are also introduced to students during a practical (Supplementary material, section 10). After that, they use the same validation tools for their ProtK models. They also perform a sequence comparison to confirm that the model contains the correct amino acid sequence. Finally, the students perform a validation of their models using the PDB [13] validation server. Students are asked to attach the PDB validation report to their notebooks, and fill a table showing model building and refinement statistics.

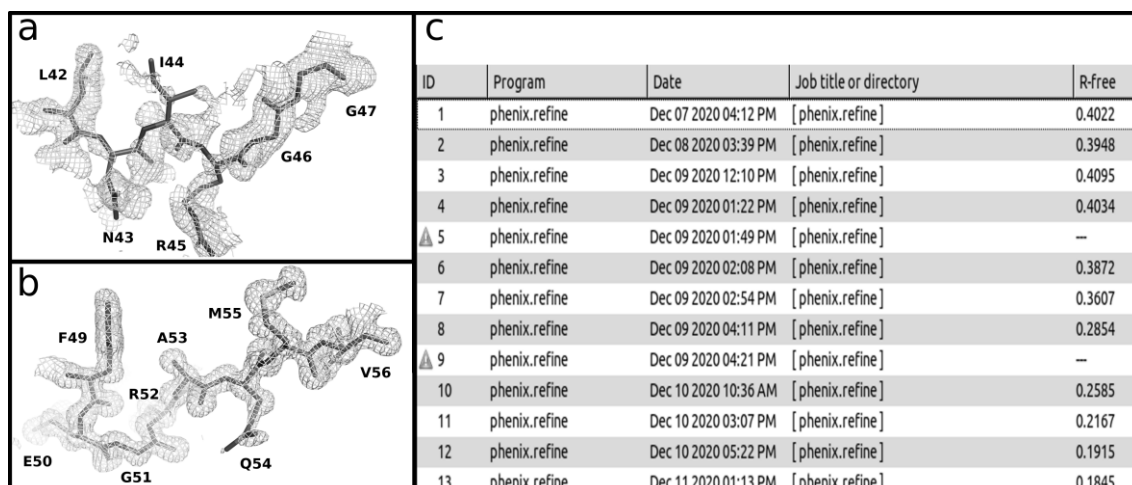


Figure 2. Refinement and model building of the ProtK model (a) An example of a region with a poor post-MR electron density map, (b). Map of the same region shown in (a) after being successfully built and refined. Sigma A weighted $2Fo-Fc$ electron density maps are contoured at 1.0σ in (a) and (b). Amino acid residues are presented as stick models. (c) An example of a refinement history from Phenix [11] shows student's progress with R -free values after each cycle (ID). The R -free increase after cycle 3 indicates a mistake in rebuilding the model. The errors messages obtained from cycles 5 and 9 (exclamation mark next to the cycle number) indicate technical problems with the refinement.

Evaluation of students

Student evaluation is based on their continuous assessment. For that purpose the electronic project notebook is a fundamental tool. After each section, students are asked to report their activity to the notebook and answer a set of specific questions. The activity log and answers to the questions are checked and accepted by the teacher in charge of each section. This helps assessing each student progress and comprehension of the subject, and allows teachers to provide corrections and asking for revisions until the quality of the answers is considered satisfactory. Finally, a failed/passed grade is awarded to the student after all the teachers have approved their sections in the online notebook.

Alternatively, a numerical grade (from 0 -fail to 5 -the best possible grade) could also be provided for students. In that case, the final evaluation should not be exclusively based on the project notebooks, due to a possible bias introduced by peer support. Due to the same reason, it may be difficult to evaluate individual answers given to the questions. In order to enforce uniform assessment criteria, an evaluation matrix can be employed. It is important that the evaluation criteria are agreed upon by all the teachers. An example matrix is shown in the Table SI.

Student feedback

Students' feedback is voluntary and it is collected anonymously using the online feedback system provided by the University of Oulu. The feedback is provided with numerical values to specific questions and also with some open questions regarding i) good practices, ii) things to develop and iii) other feedback. The questions with the average numerical results from the years 2016-2020 are presented in Table II, gathered from 25 students representing 49% of the total number of the students taking the course over the same time period.

Table II. Average feedback given by 25 students of 53 students participated in the course in the years 2016 – 2020.

| Question | Average value* |
|--|----------------|
| I achieved the course learning outcomes and course objectives | 4.5 |
| The course content helped me to achieve learning outcomes | 4.7 |
| The course content supported my progression towards expertise in my field | 4.7 |
| Teaching methods supported learning and helped me to achieve learning outcomes | 4.3 |
| Course material supported my learning | 4.3 |
| Instruction to the course tasks were clear | 4.3 |
| There was enough support and guidance in the course | 4.6 |
| Assessment methods and criteria supported my learning | 4.4 |
| There was enough time to complete the tasks in the course | 4.1 |

*The assessment criteria is as follows: 1 = totally disagree, 2 = somewhat disagree, 3 = don't disagree or agree, 4 = somewhat agree, 5 = totally agree.

Discussion

The course introduces the students to the entire workflow of protein crystallography, theory and practice. The project-based learning is supported by lectures efficiently linked to the laboratory or computational activities. The model protein ProtK project offers a real research target scenario and provides essential hands-on experience of all the steps of protein crystallography. In overall, students' feedback (Table II) has been very positive over the years with a minor critic addressed to the available time to complete the course tasks. This critic given by clear minority of the students is understandable as the course handles a broad content in a relatively short period of time that may affect individual learning abilities to adapt. The most time-consuming part of the course is the manual

model building and refinement and naturally, this could be speeded up by using the automated model building tools available in Phenix [11]. However, we provide support and encourage the students to employ manual model building because practical experience with residue-by-residue model modification really helps to develop the necessary skills to interpret electron density maps and to assess the model quality. Furthermore, it allows the students to become aware of many important practical aspects of model building, as well as features of the software used for the task. The manual model building is really appreciated by the students, who get eager to build the protein as they would solve a puzzle. This may indicate that efficient learning in this case occurs without being aware of it and finally by being able to solve the puzzle provides a successful feeling.

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Conflict of interest

The authors declare no conflict of interest.

Content of Supplementary material

1. Crystallization lecture
2. Lectures on basic concepts of crystallography
3. Data collection lecture
4. Data processing practical
5. Solving structure lecture
6. Solving structure practical
7. Model building and refinement lecture
8. Model building and refinement practical
9. Model validation lecture
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11. Table SI - An example evaluation matrix
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Supplementary materials

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1. Crystallization lecture

The course starts with a lecture on protein crystallization, where the aim of protein crystallography is first explained. Learning objectives of the lecture are understanding the concept of protein crystallization, common crystallization techniques, such as vapour-diffusion and also common features affecting crystallization. The theory of crystallization is introduced using the phase diagram for the vapour diffusion method as an example. That provides the basis for explaining nucleation and crystal formation. This is followed by individual introductions of the main factors affecting crystallization, such as the physico-chemical properties of the protein, its storage buffer and crystallization solution components. It is pointed out that crystallization is a multi-dimensional problem containing countless variables (protein and precipitant concentrations, pH, etc.). The concept is illustrated by a set of cartesian coordinates (x, y, z, etc.), each representing one of the main variables. This provides a rationale for using commercial crystallization cocktail formulations for the initial screening of crystallization conditions. Next, optimization strategies are presented. The explanation of the hydrophobic effect and the classification of solute molecules has a key role to guide the students towards understanding the need to maximize protein-protein interactions rather than protein-solute interactions. Finally, common crystallization methods and techniques are presented. Summarizing questions are asked to the students via e.g. Kahoot or Socrative to ensure the learning outcomes.

2. Lectures on basic concepts of crystallography

After the crystallization part, lectures continue by introducing the basic concepts of crystallography. The learning objective is to provide basic knowledge by which the common principles of X-ray diffraction, electron diffraction (including cryoEM) and neutron diffraction can be appreciated and by which the importance of the Argand diagram and the Ewald sphere can be understood. The lectures are based on the books by Bernhard Rupp [1], David Blow [2], Jan Drenth [3] and Eaton Latman & Patrick Loll [4]. At first, the importance of the Fourier transform is introduced: the intensities of the diffraction pattern correspond to the amplitudes of the Fourier components defining the scattering object. This allows the introduction of the Argand diagram and the atomic scattering factor. Subsequently, the importance of crystals and their possible symmetry is introduced. The theory is further explained by discussing the discoveries of von Laue, Bragg, Ewald and Bijvoet.

Von Laue: (i) X-rays are waves and (ii) the diffraction pattern is an interference phenomenon, due to elastic scattering by the electrons, resulting in the Laue conditions because of destructive and constructive interference.

Bragg: The interference pattern can also be described as a reflection against sets of planes separated by a distance d , resulting in Bragg's law ($2 \cdot d \cdot \sin\theta = n \cdot \lambda$), which allowed solving structures.

Ewald: The diffraction pattern is a three-dimensional function, but at a particular orientation of the scattering object, the only visible part of the diffraction pattern is a spherical section of the total scattering figure, due to elastic scattering. This spherical intersection is called the Ewald sphere.

Bijvoet: If in a unit cell there are a few anomalous scatterers, this causes $F(hkl)$ to be different from $F(-h, -k, -l)$ (for acentric reflections), because of the anomalous component in the scattering factors of the anomalous scattering centers. This is beautifully explained in the book by David Blow, using the concept of atomic scattering factors (“Outline of Crystallography for Biologists”)[2].

Using this outline, over the course of six lectures the students are able to understand (i) the geometry of the punctuated diffraction pattern (using the Ewald sphere) and (ii) how to solve the phase problem (in the Argand diagram).

3. Data collection lecture

The main learning objective of this lecture is to make the students understand the various strategies used to collect a complete and best possible quality dataset from a given crystal. The lecture starts with a brief revisiting of diffraction basics, Bragg’s law and Ewald sphere concepts. Then the major part of the lecture is dedicated to various strategies used in the data collection. First, why a rotation/oscillation method is used for data collection is briefly explained and then the important aspects to consider during the oscillation data collection method, i.e. data collection strategies, are discussed. This includes, for example, how to decide the optimal crystal-to-detector distance, oscillation angle, exposure time etc. All these data collection parameters are dependent on the properties of crystals such as unit cell parameters, diffraction quality, mosaicity, anisotropy and twinning. Such details in the lecture enable students to make their own decisions while planning their data collection with their ProtK crystals. For example, a large unit cell results in the close spacing of diffraction spots. Therefore, the student should be able to decide the scan-angle such that there is no overlap of the diffraction spots recorded on the same image. They additionally need to take care not to lose the high resolution diffraction spots, which get weaker as the detector is moved farther away from the crystal, as well as possibly end up lying outside the detector area. The geometry of the data collection in a typical oscillation data collection experiment is described. The advantages of data collection at a home source vs. at a synchrotron, and the various parameters that affect each of these, such as radiation damage and thin slicing, is only briefly explained as the students will be limited to using a home source instrument in their data collection practical. Also, the frequently-faced problem of missing data from the ‘blind region’ is discussed and how to overcome it e.g. using a kappa goniometer. Furthermore, a brief historical perspective of transitioning from a room temperature data collection to cryo-freezing crystals (**related to project work**) is discussed, highlighting the more recently

developed ‘diffraction-before-destruction’ room temperature, single-frame data collection from multiple crystals.

The major defects observed in crystals, such as mosaicity, anisotropy, and twinning are introduced. Twinning, in particular, is discussed in more detail describing different types of twinning and how to detect and quantify the percentage of twinning from intensity statistics [5]. Then the concept of B-factors and Wilson B are described, as well as the need for Lorentz and polarization correction of the diffraction data.

Data processing is also briefly introduced during the same lecture. The actual practical steps in data processing are left for the practical. In the lecture we try to give a perspective on how data processing works. It is often challenging for the students to visualize how the orientation matrix is determined by the data processing software. Emphasis is given to assess the outcome of data processing using parameters such as I/σ , R_{merge} and $CC_{1/2}$.

4. Data processing practical

The data processing practical takes place in a computer classroom, where each student has a Linux computer. Students are shortly introduced to some basic Linux commands, which are needed to carry out the practical. Initially, students follow the teacher processing the ProtK dataset collected at 1.9 Å resolution with the home source. Processing is performed using the Proteum 2 program package, which is well suited for teaching thank to an intuitive graphical user interface (Figure 1C). During this session, the data indexing, integration and scaling are demonstrated step-by-step to the students. Also, the space group selection is described in detail. The same dataset is then processed with iMosflm [6], with the students individually processing the same data while following the teacher. Finally, the intensity statistics, Bravais lattice and unit cell dimensions from both processing sessions are collected to a table. Since the ProtK crystal belongs to the $P4_32_12$ space group, the data are processed in both enantiomeric space groups, $P4_12_12$ and $P4_32_12$.

5. Solving a structure lecture

This lecture is generally based on the books by Bernhard Rupp [1] and David Blow [2]. The main learning objective of these lectures is to provide the students an understanding of various phasing methods so that they learn when and how to use each of these methods to determine the protein structure. At first the importance of phases in determining the structure and the ‘phase problem’ is explained. The first lecture mainly covers the experimental phasing approaches such as multiple isomorphous replacement, anomalous dispersion and a combination of these two methods [7,8]. Due to the limited time available, experimental phasing is only covered during the theoretical lecture. Emphasis is given on how knowledge of the ‘heavy atom/anomalous atom sub-structure’, as obtained from the difference Patterson function, will help to estimate the phases for each reflection using the Argand diagram. The importance of the figure-of-merit to estimate the phase quality is mentioned. Then, briefly, how the heavy atom soaking or Se-Met

labelling is carried out and the advantage of using synchrotron radiation for these methods over a home source are explained. Examples of structures determined by these methods are discussed, to demonstrate the various challenges faced and how to overcome them. Examples provided include some of historical importance such as myoglobin [9] and crambin [10] and some of our own molecular targets [11,12].

The second lecture on phasing is dedicated to molecular replacement, introducing the method and when it can be successfully applied. The lecture is focused on the principle underlying the technique, and it will help the students to better understand the molecular replacement practical. The reason why in many programs the six-dimensional search problem is divided into two three-dimensional search problem of ‘rotation’ and ‘translation’ function is explained, mainly based on Patterson methods. This is visualized using a 3 or 5-atom molecule as the model due to its simplicity. The example gives a perspective to the students on the complexity of the problem when dealing with protein molecules containing thousands of atoms. After this, the advantage of using the maximum likelihood method is briefly explained without going much into the mathematical background. The different molecular replacement programs [13,14] available are briefly introduced with emphasis on Phaser, which will be used during the practical session. A few case-studies [15,16] are discussed to demonstrate the power as well as the challenges accompanying the use of this phasing method. Examples include the use of molecular replacement to phase multi-domain proteins, multi-protein complexes as well as multi-copy models.

6. Solving a structure practical

The aim of the practical is to help students to understand the importance of analyzing data quality and crystal content for successful molecular replacement. The practical provides the knowledge on the required information and familiarize the programs for students so that they are able to solve the phase problem for ProtK. Initially, the students perform crystal content analysis using the Xtriage program for β -galactosidases from *Trichoderma reesei* (PDB ID 3OG2) [17] and *Bacillus circulans* sp. *alkalophilus* (PDB ID 3TTS) [18], which are used as test cases. Based on the information obtained from Xtriage, combined with the information and search models provided in the practical, students carry out molecular replacement for the proteins using Phaser, through the ‘simple interface’ Phenix module. The solution for *T. reesei* β -galactosidase should contain one molecule in the asymmetric unit in the space group *P*1. Students are instructed to visualize the molecular replacement solution using the software Coot [19]. In particular, students are encouraged to check the crystal packing by showing neighbouring protein chains related to the solution by symmetry. In the case of *B. circulans* sp. *alkalophilus* β -galactosidase, students try to solve the structure by using several search models. The asymmetric unit of the protein contains six molecules arranged as a “dimer of trimers”. This particular structure provides an excellent example of non-crystallographic symmetry for the students and it can also be used to emphasize the

importance of choosing the right search model. Using a trimer of *Thermus thermophilus* A4 β -galactosidase [20] as the search model (sequence identity 33%) leads to the correct solution, whereas a monomer results in no solution.

7. Model building and refinement lectures

The first model building and refinement lecture starts with the definition of an electron density map and the description of the maps used for model building. The learning objectives from the lectures are understanding of the model building process and to get familiar with the refinement in real space and in reciprocal space. It is emphasized how the data and our knowledge of protein structures affect these strategies. Lectures are tightly connected to the practical work carried out after each lecture. The concept of resolution is addressed and the effects of B-factors and resolution on the electron density maps and on the precision of the atomic coordinates is discussed. Effects of data collection issues and how they affect the resulting electron density maps are addressed and further visualized with the help of movies prepared by James Holton (<https://bl831.als.lbl.gov/~jamesh/movies/>). Model bias and errors in the model that should be corrected are showcased through examples. The overall model building and refinement process is explained with a focus on local real space refinement. Reciprocal space refinement and restraints, knowledge of what amino-acids and proteins look like, is the topic of the following lecture. Cross validation and avoidance of over-parametrization due to a poor data-to-parameter ratio, and how we improve the situation through restraints, are introduced. Refinement strategies at different resolutions are discussed together with students to recapitulate the content. The subsequent lecture addresses the practical aspects of model building and refinement and it is designed to match the approximate status of the students in their project work. How to address bulk solvent, ordered solvent, the possible presence of buffer molecules, metal ions and ligands are topics discussed during the course of the lecture. Possible problems and advanced scenarios like multiple conformations and missing atoms in the model are explained through examples. “When is the model ready?” is the most recurring question that puzzles students and seasoned crystallographers alike, and it is discussed at the end of the lecture.

8. Model building and refinement practical

Students are introduced to practical model building through three examples simulating typical issues caused by MR search model bias. The learning is to get familiar with the tools used and applied in the own model building and refinement project. The practical work expands the knowledge gained from the lecture. In the first exercise, a region showing a mismatch between the electron density map and the model is pointed out. The region contains many negative and positive electron density peaks due to the presence of the wrong amino acids. Students are instructed to correct the protein sequence by replacing the wrong residues with the correct ones and perform a restrained refinement. After that, students will notice a change in the electron density map, which will then fit perfectly to the model. The second practical requires building residues into the electron

density map according to a given sequence. In addition, students are directed by specific questions to pay attention to secondary structure constraints while building the missing parts. This teaches them to build the residues by ensuring the correct formation of the hydrogen bond network. The final exercise deals with waters and other solvent molecules. Students are guided to build a few missing waters and DMSO molecule into the electron density. They are asked to pay attention to correctly fitting DMSO while preserving hydrogen bond formation. Finally, students save the coordinates containing their changes and perform a refinement using Phenix [21]. After the refinement is ready, students are instructed to check the *R*-work and *R*-free, which should have decreased significantly compared to the starting values. The latter are available in the PDB database together with the structure factor file needed for the refinement.

9. Model validation lecture

Validation is a crucial step in crystallography and it is a duty of the crystallographer to deliver a high quality final model. The main learning objective is that the students will be able to judge the quality of a protein structure. Biochemists should be able to identify possible errors and assess the quality of the structures produced by other scientists, as they form the basis for their own work. Typical errors observed in the electron density maps and in model geometry are discussed during the course of the lecture in the form of examples. The principles behind the validation tools used in the project are described. The tools include Ramachandran plots, the analysis of bad contacts between atoms and the correlation coefficients between the model and the electron density map. Overall quality of the model is assessed with MolProbity [22], accessed through Phenix [21]. Finally the refinement statistics, their meaning and expected values reported in publications are discussed, followed by the description of specific aspects of model validation and deposition into the Protein Data Bank [23].

10. Model validation practical

Before validating the ProtK model, students practice the use of the validation tools available in Coot by making corrections to the model deposited to PDB (PDB id. 1LEE) [24]. This will support and complete the learning objective regarding the assessment of the quality of a protein structure. Students are guided to first perform a difference map peak analysis. Specific questions are asked related to problems that arose during the analysis. Students then check the correct placement of water molecules included in the model and perform corrections when needed. Next, students analyze the Ramachandran plot. They are instructed to locate and describe the outliers, and they are asked to correct some of them. Finally, they need to save the corrected model and perform restrained refinement. Finally, students are asked to compare the pre- and post-refinement *R*-factors noting down their significant improvement.

| | Grade 1 | Grade 2 | Grade 3 | Grade 4 | Grade 5 |
|--|---|---|---|---|--|
| Project reporting | Some reporting is missing. Answers are very short and mostly irrelevant. Based on answers, a student has clear difficulties to understand the subject. Language is difficult to understand. | All sections are reported. Answers are short and partly irrelevant. Based on answers, a student has clear difficulties to understand the subject. Language is reasonably containing few mistakes. | All sections are reported. Answers cover the primary subject and are mostly relevant. Based on answers, a student demonstrates some understanding of the subject. Language is reasonable. | All sections are reported. Answers clearly cover the subject and are relevant. Based on answers, a student mostly understands the subject. Language is mostly fluent. | All sections are reported. Answers clearly cover the subject and are very relevant. Based on answers, a student clearly understands the subject. Language is fluent. |
| Development of the research skills. As a teacher, give your evaluation to this in the end of your teaching task. What kind of development a student has achieved during your teaching? | Student cannot carry out of the project step/s individually and needs heavy supervision everywhere. Student has no understanding of the subject/s. | Student is able to carry out some of the project step/s independently, but some steps require heavy supervision. Student has some understanding of the subject/s. | Reasonable primary understanding of the subject/s is achieved. Student is able to work independently in the project step/s and does not require heavy supervision. | Primary understanding of the subject/s is achieved. Student is able to carry out project steps independently, but some cases supervision is needed. | Understanding of the subject/s is achieved. Student is able to carry out all project steps independently, with little supervision. |
| Own evaluation of learning regarding to study goals given in the course description | I have not achieved any of the learning goals. | I have achieved some of the learning goals. | I have achieved majority of the learning goals. I have achieved a reasonable | I have achieved almost all the learning goals. I have a basic | I have achieved all of the learning goals. I have a good |

| | | | | | |
|--|--|--|-----------------------------------|---|---|
| Student evaluates himself/herself in the end of the course | | | understanding of the subjects. | understanding of all subjects of the course. | understanding of all subjects of the course. |
|--|--|--|-----------------------------------|---|---|

Table SI. An example evaluation matrix

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