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Experimental phasing and radiation damage

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This issue contains articles based on talks presented at the 2009 CCP4 Study Weekend on Experimental Phasing and Radiation Damage. As is the remit of the CCP4 study weekend, speakers gave didactic overviews covering their areas of expertise, as well as reporting new developments and results. Experimental phasing and radiation damage were chosen as joint topics for the 2009 study weekend as, in a macromolecular crystallographic X-ray diffraction experiment, the two topics are intimately connected since the occurrence of the latter pivotally affects the success of the former. Moreover, radiation damage is an unavoidable aspect of X-ray diffraction experiments, accumulating from the moment X-rays first hit the sample. In view of this, after two introductory talks which reviewed the main concepts involved in experimental phasing (Garry Taylor) and radiation damage (Elspeth Garman), the following talks were divided into four sessions reflecting the flow through a crystallographic experiment and highlighting both the methods and advances in experimental phasing and the role played at each stage by radiation damage.

To this end, the presentations were split into groups covering: 'before the experiment', 'during the experiment', 'using the experiment', and 'after the experiment'. The first session, *Before the Experiment*, comprised talks discussing how choices made during sample generation can be optimized to ensure that the experiment has the maximum chance of success. In order to obtain crystals, protein must be obtained and purified, sometimes as a Seleno-met derivative so that *e.g.* Se-MAD phasing can be used to determine the structure. The first paper, by Helen Walden, summarizes current recombinant techniques for expression of protein suitable for crystallization trials including the generation of Seleno-Met derivatives in a variety of expression hosts (bacterial, yeast and insect cells). Historically, multiple-isomorphous replacement using heavy-atom soaking methods has been the main (and remains a common) method of phasing new structures, and Peter Sun presents a study describing rational approaches to conventional heavy-atom screening to define optimal derivatization methods that minimize non-isomorphism and maximize the efficiency of binding. The next vital stage of a 21st century macromolecular crystallographic experiment is to establish a successful cryocooling protocol, and Douglas Juers describes new results on the thermal contraction of different cryosolutions. These measurements should lead to an improvement in our understanding of the basic processes involved in flash-cooling, and in our ability to take more rational approaches to the choice of cryobuffers. A new phasing tool is presented by Tobias Beck: 'the magic triangle' consisting of a cluster of either three iodine or three bromine atoms in specially developed compounds which combine heavy atoms with functional groups that bind to proteins and can be soaked into crystals with a minimum of non-specific binding. As well as the issues involved in crystal preparation covered above, consideration of the lifetime of the crystal in the X-ray beam owing to radiation damage should be made even before the experiment starts. Knowledge of the dose that will be absorbed during the planned exposures is a prerequisite for planning an experimental strategy which takes into account the limitations imposed by a finite crystal life. The program *RADDOSE* is widely available at synchrotron beamlines to compute this dose and a guide to its everyday use, including some new developments, is outlined by Karthik Paithankar.

Having covered sample preparation, we turned our attention to the next stage of the diffraction experiment in the second session entitled *During the Experiment*. An optimized data collection strategy can significantly improve the data quality and thus the amount and accuracy of biological information that can be obtained from the final refined model. The important point made by the first paper in this section, by Zbyszek Dauter, is that the diffraction experiment is the last experimental stage of structure determination.

If your data are *sub par*, then it does not matter how powerful the downstream processing is, your final model will be limited by the quality of the collected diffraction data. In his paper, he describes how the optimal diffraction data collection strategy can vary depending on the type of experiment; for example, the best approaches for molecular replacement and experimental phasing are quite different. At the study weekend, Gerard Bricogne spoke on interleaved data collection protocols for optimally exploiting anomalous scattering and its anisotropy for phase determination, but unfortunately he has not provided a written version of his talk. A treatise on the theoretical limits of macromolecular crystallography is contributed by James Holton, and includes a detailed exposition on the minimum crystal size required to obtain a complete data set before radiation damage compromises the sample order. James also comprehensively examines the contributions of the sample, source and detector characteristics to the diffraction pattern, providing a summary of over 100 years of literature. The program *BEST* is already becoming widely used to plan a data strategy once initial images are available and Sasha Popov reports the inclusion of radiation damage into the diffraction model used within the software, improving its estimates of crystal lifetime and hence optimal strategy. Two real-life case studies are described by Susan Lea in which it was only by combining experimental and molecular replacement phasing approaches that the crystal structures could be determined. If radiation damage has occurred, despite the best efforts of the crystallographer, then there are strategies by which it can be corrected during data processing. These are outlined by Zbyszek Otwinowski who discusses the various manifestations of radiation-induced changes in diffraction data and considers how best to handle these in data analysis and processing.

In the third session of the meeting, *Using the Experiment* the emphasis shifted to considering the positive aspects of the radiation damage process as, although it is detrimental to many aspects of the diffraction experiment, it can in fact be used to elucidate mechanism and biological function as well as to provide additional phase information. Martin Weik describes how to use specific radiation damage to probe structural protein dynamics *via* a combination of different data-collection temperatures and utilizing X-ray induced changes to initiate catalytic reactions. The symmetries in reflection intensities can be broken by site-specific radiation damage and the polarization anisotropy of anomalous scattering. Marc Schiltz probes the use of unmerged data in phasing for both these cases. A recent development on synchrotron beamlines is the availability of on-line microspectrophotometers for UV–vis and Raman scattering measurements, and Arwen Pearson reviewed the currently available instrumentation and discussed how single-crystal spectroscopy can guide data collection strategy. (A current review of the types of spectroscopy available at synchrotron beamlines can be found in Pearson & Owen, 2009, so has not been repeated in this issue).

In the final session, *After the Experiment*, issues relevant to data manipulation after the experiment were addressed, and Airlie McCoy's paper summarizes, in an accessible manner, recent developments in experimental phasing as well as discussing the potential pitfalls to be avoided during substructure determination. Following a successful structure solution, density modification to improve the initial set of phases is often the next step, and Kevin Cowtan presents a modified classical approach for improving phase estimates which gives improved results and is much faster than statistical methods. Model building into those electron-density maps follows, and George Sheldrick gives a general account of experimental phasing using the *SHELXC/D/E* suite of programs, the last of which now includes an automated protein main-chain tracing algorithm. Lastly in this issue, Paul Emsley gives a full description of the graphics program *Coot* for model manipulation and validation, and a detailed guide to its use.

To complete the weekend, Sean McSweeney led a discussion of the future and challenges for macromolecular crystallography at high brilliance synchrotron sources that are capable of delivering doses approaching the experimental dose limit within seconds of exposure. A particular challenge presented to the participants was the need to establish optimized protocols for the use of microbeams and microcrystals. Sean paraphrased a certain American politician when describing our current understanding of radiation damage and its effects on diffraction data collection. There are 'known knowns', 'known unknowns' and 'unknown unknowns', and any investigations undertaken to increase our understanding must be systematic and statistically significant.

In the future, it is expected that the tools already developed to monitor and deal with radiation damage (*e.g. BEST*, *eDNA*, on-line spectroscopy, routine flux and beam shape measurements) will be used to inform experimental strategies in order to maximize the information that can be obtained in a diffraction experiment from a given crystal. Even if radiation damage is unavoidable there are chances of turning something initially thought of as a curse into a useful tool for phasing as well as for biological interpretation. The presentations and discussions during the study weekend revealed an optimistic outlook, showing the potential for further deepening of our understanding of both experimental phasing and radiation damage (and their interaction). The speakers also made clear how the development of new phasing tools and protocols in combination with informed data-collection strategies will ensure that we make maximal use of the ongoing developments in X-ray light sources to deliver better structures that reveal in detail how macromolecular structure is linked to biological function.

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References

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