Synchrotron radiation applications to macromolecular crystallography

Keith Moffat* and Zhong Ren†

Progress has been rapid in the development and application of four different types of macromolecular crystallographic experiment at synchrotron hard X-ray sources: multiwavelength anomalous diffraction; studies of crystals with very large unit cell dimensions; structure determination at atomic or near-atomic resolution; and time-resolved studies. The results illustrate the interplay between the advanced technical capabilities available at new beamlines and more challenging scientific issues.

Addresses
Department of Biochemistry and Molecular Biology, and Consortium for Advanced Radiation Sources, The University of Chicago, 920 East 58th Street, Chicago, IL 60637, USA
*e-mail: moffat@cars.uchicago.edu
†e-mail: renz@cars.uchicago.edu

Current Opinion in Structural Biology 1997, **7**:689–696

http://biomednet.com/elecref/0959440X00700689

Abbreviations

APS	Advanced Photon Source
CHESS	Cornell High Energy Synchrotron Source
DHFR	dihydrofolate reductase
ESRF	European Synchrotron Radiation Facility
HRV	human rhinovirus
MAD	multiwavelength anomalous diffraction
MIR	multiple isomorphous replacement
NSLS	National Synchrotron Light Source
PF	Photon Factory
PYP	photoactive yellow protein
TBP	TATA box binding protein
TF	transcription factor

Introduction

The past five years since the publication of Helliwell's *magnum opus* [1] on the applications of synchrotron radiation to macromolecular crystallography have seen a maturation of certain applications, the successful development of new applications, and proposals for revolutionary new applications [2•,3]. These advances have been made largely possible by the commissioning of purpose-built insertion device beamlines at third generation sources such as the European Synchrotron Radiation Facility (ESRF), in Grenoble, France, the Advanced Photon Source (APS) at Argonne National Laboratory, USA, and (shortly) by SPring-8 outside Osaka, Japan. New beamlines have been complemented by the continuous upgrading and wider availability of beamlines at existing synchrotron facilities, such as the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory, USA, the Cornell High Energy Synchrotron Source (CHESS) at Ithaca, New York, USA, the Photon Factory (PF) at Tsukuba, Japan, and the Deutsche Elektronensynchrotron at Hamburg, Germany [4]. As the science drives the technology, the advances have been fuelled, first, by the evident success of the multiwavelength anomalous diffraction (MAD) phasing technique [5] and its consequent, wide adoption; second, by increasing numbers of experiments and refinement at atomic resolution (< 1.2 Å [6,7]); third, by continued structure determination of virus and multiprotein complexes that crystallize with very large unit cells — in excess of 500 Å and even 1000 Å (e.g. [8,9]); and fourth, by the development and successful application of time-resolved experiments on the timescales of kiloseconds, tens of seconds, milliseconds and nanoseconds [10,11••,12•,13••].

A characteristic of the field is the interplay between the science to be explored and the technology (in the broadest sense) with which to conduct it. The X-ray source, the beamline optics, the crystal manipulation devices, the detector and the software [14] are integral parts of the experiment and, for the best results, their properties have to be considered from the outset. The ability to conduct first-rate synchrotron experiments is often at least as much due to effective background reduction as to enhancement of the diffraction signal. Measurement of the highest resolution shells of monochromatic oscillation data, or of the intrinsically weak Laue diffraction patterns obtained with exceedingly brief, single X-ray pulses [15••], depends critically on the reduction of the background noise. Thus, seemingly mundane experimental details, such as the beamline slit system, the air path around the crystal, the method of mounting the crystal, and the intrinsic noise and mode of operation of the detector, become important. It is ironic that, while huge sums of money are available for enhancing the signal (via source brilliance), much smaller sums are typically available for reducing the noise.

The overall result has been a rapid and very substantial increase in the percentage of papers in macromolecular crystallography that utilize synchrotron radiation. Five or seven years ago, only the more adventurous crystallographers used synchrotron radiation on their most challenging problems; in 1996, Hendrickson and Brändén [16] noted that more than 60% of the X-ray studies reported in the journal *Structure* used synchrotron radiation, a percentage that is matched or even slightly higher in *Science*, *Nature* and *Cell*. The use of synchrotron radiation is fast becoming routine for a wide variety of structural projects, while challenging new applications that are currently by no means routine continue to be developed.

One point of our brief review is to illustrate why and how advances have occurred. We describe a variety

of experimental types, provide current examples and note pertinent technical advances. We do not intend (or pretend) that our literature citations are comprehensive and refer the reader to other, more comprehensive reviews [2•,4]. In particular, we pass over studies of microcrystals [17] and of capillary and other forms of microfocusing optics [18]. Note also the invaluable structural reports that appear at the back of each issue of *Current Opinion in Structural Biology* and of *Structure*. It is a mark of the wide acceptance of synchrotron radiation that these reports now seldom explicitly mention its use in a project.

MAD and related energy-dispersive synchrotron diffraction methods

MAD [5,19••] entered its production phase around 1994 and the trend of its use continues. All tricks to incorporate anomalous scatterers [20•] have been aptly used to their maximum extent. Two Cu-containing proteins or cupredoxins have been solved by measuring the robust diffraction at the K absorption edge of the intrinsic Cu [21,22]. The structures of a fragment of the iron-sulfur protein of the bovine heart mitochondrial cytochrome bc_1 complex and two binuclear zinc cluster proteins have been determined using MAD at the K absorption edges of the 2Fe-2S cluster and the binuclear Zn center, respectively [23–25]. A number of selenomethionine-substituted proteins [26–33] have been engineered (for a review, see Doublie [34]) and their structures solved successfully. Geiger *et al.* [35] demonstrated a MAD experiment that took advantage of both the Se and Br absorption edges in the TFIIA/TBP/DNA complex. Both subunits of TFIIA and TBP were substituted with selenomethionine and the oligonucleotide was substituted with five bromine atoms. Peat *et al.* [30,36] showed that a threonine or valine substitution for methionine avoided the oxidized selenium atoms on the intermolecular contact surface, and thereby improved the chance of crystallization.

Heavy atoms incorporated via chemical modification [33,37–41,42••] are still commonly used. The use of Yb^{3+} ion [33,40,41,42••] suggests that lanthanide elements are also well suited for MAD experiments of large proteins. Lanthanides can be incorporated into macromolecules by substitution for intrinsic Ca^{2+} or Mg^{2+} ions. The suitability of multimetal clusters for phasing very large macromolecular assemblies by multiple isomorphous replacement (MIR) has been investigated [43•]. Such clusters, for example Ta_6Br_{14}, may also be used as the anomalous scatterers in a MAD experiment.

A set of experimental phases of exceptional quality (both accurate and complete) has been obtained by Burling *et al.* [42••] from a 230-residue Yb^{3+}-substituted subtilisin fragment of mannose-binding protein A. Perfect isomorphism, long claimed to be an advantage of MAD, has now been fully demonstrated. Because of the accuracy of the purely experimental MAD phases, the electron density map is not influenced by any refined models and shows such features as partially reduced disulfide bonds, local disorder, mobility of chemically equivalent molecules and a diffuse, partially disordered solvent layer around exposed hydrophobic groups (for comments, see [44•]).

If the heavy-atom sites can be located using such other means as a direct methods approach, $|F_A|$ and ϕ_A are no longer unknowns in the phasing process and only $|F_T|$ and ϕ_T remain to be determined. The parameter-to-observation ratio is therefore reduced, and more reflections can be phased. A MAD data set from the biotinyl domain of acetyl-coenzyme A carboxylase [45] did not result in an interpretable density map using the conventional MAD phasing protocol but generated an interpretable map using the modified phasing process. The success of the modification is due more to the increase in completeness of the phased data set (from 85% to 99.7% in the resolution range from 20 to 2.5 Å) than to the improvement of phase accuracy (50° mean phase error for both cases). Direct methods have also been used to help locate the selenium sites in the MAD phasing of the UmuD′ protein [36] and the DNA-binding domain of OmpR [31].

The algorithms underlying MAD phasing are generally classified as algebraic or probabilistic. The former is a straightforward least-squares approach, whereas the latter is based on Bayesian statistics, which theoretically should result in less biased phases and may be more popular in the future [38]. (For an 'under-the-hood' look, see [19••,46,47] and literature cited therein.) It has been shown that MAD phasing can be treated as a special case of MIR [47–49]. This approach seems to be gaining in popularity [25,29–31,35,37,38,50].

MAD data collection can be thought of as a set of monochromatic oscillation experiments at three or more wavelengths, except that the looping over spindle angle, wavelength, and inverse beam can be altered for the purpose of minimizing systematic errors arising from, for example, decay due to radiation damage. A diamond trichromator has been designed to select three desired wavelengths [51] such that diffraction data at these three wavelengths can be measured simultaneously. Another polychromator combining a curved crystal and a grid screen has been proposed to select several wavelengths and focus them at the sample from slightly different incident angles [52]. Results from these new instruments are still awaited.

Studies of crystals with large unit cell dimensions

It has long been known that brilliant synchrotron X-ray sources offer major advantages over laboratory sources for the study of crystals with large (e.g. exceeding 300 Å) cell dimensions, and this experimental type is now relatively mature (e.g. see [53–59]). Almost all experiments continue to employ the standard monochromatic oscillation tech-

nique using wiggler sources and record the data on film or, increasingly, on imaging plates. The Laue technique, however, has also proved effective at revealing small structural changes [60].

Interesting wrinkles emerge. For example, Zhao *et al.* [54] finally discovered that, after data collection at a nominal X-ray wavelength of 0.91 Å, post refinement of cell dimensions and other diffraction parameters, structure determination and crystallographic refinement, their model of human rhinovirus (HRV) 3 was systematically expanded with respect to the known structure of HRV14. The bond lengths of HRV3 were apparently systematically larger than those of HRV14; the length scale was somehow wrong. This effect was ultimately traced to the magnitude of the wavelength used in HRV3 data collection, and, when corrected, necessitated a decrease in the unit cell (and hence of all structural dimensions, expressed in fractional cell coordinates) derived from post refinement by only 7 parts in 1000. That such a small effect was clearly detectable emphasizes the necessity for wavelength stability of the beamline during such experiments, and the desirability of explicitly measuring the wavelength during the course of macromolecular data collection, for example, by examining a standard small molecule crystal of unimpeachable cell dimensions.

Because of the high implicit redundancy in the diffraction data that arises from crystals with high noncrystallographic symmetry, even data sets of low completeness may prove to be adequate for structure determination, always provided they are of sufficient accuracy. For example, Llamas-Saiz, Rossmann and coworkers [61] successfully solved the structure of a mutant canine parvovirus, although the data were only 32.5% complete, to 3.25 Å resolution, with an excellent R_{merge} of 9.0% (after rejection of weak reflections with $I < 2\sigma[I]$).

One of the frontiers now lies in successful data collection on crystals with even larger cells in the 750–1000 Å range [9,58,62], for which the anticipated structure of bluetongue virus by the Oxford group headed by David Stuart is likely to set the pace. High brilliance, undulator sources are essential to resolve such densely packed diffraction patterns. A second frontier lies in systems with much less or no noncrystallographic symmetry, including multiprotein and nucleoprotein complexes such as the 20S proteasome [63], cytochrome *c* oxidase [64,65••], photosystem I [66] and light-harvesting complex II [67]. The 'Holy Grail' is the successful structure determination of the intact ribosome and ribosomal subunits; but here, curiously, data collection itself is no longer the problem but rather, the development of suitable methods of phase determination that involve metal clusters (see [43•] and references therein).

Atomic resolution

Crystallography at atomic resolution demands crystals of low mosaic spread, high order, low sensitivity to radiation damage, and the recording of their diffraction patterns with extremely low background [6,7]. Although the third is now routinely achieved by cryocrystallographic techniques [68•], flash freezing almost invariably introduces a substantial increase in mosaic spread and hence is inconsistent with the first and second requirements. The dilemma is illustrated in Figure 6 of Chayen *et al.* [2•]. If the highest resolution is required, should ambient temperatures, which retain the lowest mosaic spread and the highest peak intensity to background intensity ratio for each reflection but run the near-certain risk of increased radiation damage, be employed? Or should cryogenic temperatures be employed? The decision is further clouded by the properties of the X-ray source and beamline to be employed. Ideally, the emittance of the source radiation (the product of its spatial extent and angular extent — a key property which is conserved as the beam propagates down a perfect beamline) should be matched to the acceptance of the crystal (the product of its linear dimensions and its mosaic spread). The emittance for third generation synchrotron sources is generally well matched to the acceptance of excellent protein crystals, but this match is lost when the acceptance of the protein crystal is increased by orders of magnitude upon freezing. As numerous samples are generally available for crystals that diffract to atomic resolution, it may well be preferable to preserve a low mosaic spread at all costs and to replace samples frequently as radiation damage appears. An excellent example is provided by Longhi *et al.* [69•].

The existence of thermal damage must also not be ignored. Although the crystal heating rate arising from X-ray absorption in focused, bending magnet, Laue beams can be several hundred degrees per second [70], it has not been sufficiently appreciated that focused, monochromatic beams from undulator sources at the ESRF and the APS can impose a similar heating rate and thus buckle the crystal and reduce (often reversibly!) its order. If the heating rate exceeds the cooling rate generated by heat loss to the cryogen from the crystal surface [71], the temperature will rise monotonically until the crystal is destroyed.

Accurate diffraction data at atomic or near-atomic resolution is essential to the application of direct methods for phase determination. The 'Shake-and-Bake' method of Hauptman and his coworkers (for a review, see Ealick [72]) has now proved applicable to structures with as many as 64 amino acids. The prospects for its extension to even larger structures, via the incorporation of anomalous scattering information and more efficient computational procedures, appear excellent, but the technique will continue to depend on diffraction data of the highest quality to the highest resolution, and hence on synchrotron data acquisition.

Time-resolved studies

Strategies for time-resolved crystallography depend on the lifetime of the intermediate(s) whose structure is to be

determined [73•,74]. The traditional approach is to prepare indefinitely stable chemical variants of the intermediates, and it continues to prove powerful as in the recent example of dihydrofolate reductase (DHFR). The reaction catalyzed by DHFR passes through five kinetically distinguishable intermediates. Sawaya and Kraut [75] have prepared isomorphous, stable crystal structures believed to be analogous to each intermediate and to a presumptive transition state. From these six stable structures, they pieced together a 'movie' at 2.1 Å resolution which reveals, among other features, loop and subdomain movements related to the overall mechanism.

The traditional approach has been denoted 'chemical trapping' [70,76], in which the lifetime of intermediates is prolonged by chemical manipulation. With the very brief X-ray exposures available at intense synchrotron sources, the lifetime need not be indefinitely long, and the extent of chemical perturbation necessary to stabilize the normally transient intermediates can be reduced. An excellent example is provided by the comprehensive time-resolved Laue crystallographic studies of Stoddard and coworkers [77,78,79••,80•], in combination with site specific mutagenesis, molecular dynamics calculations and optical studies, on isocitrate dehydrogenase with a time resolution of tens of milliseconds. A further example is provided by a study of two ferryl intermediates of catalase, denoted compound I and compound II, by Gouet, Hajdu and coworkers ([11••]; see also [76]). These intermediates have lifetimes in the tens of minutes range, and hence the restraints on crystallographic data collection are quite relaxed. They used monochromatic, Weissenberg crystallography [81,82] at the PF and recorded diffraction data on very large, $40 \times 40\,cm^2$, image plates at a long crystal-to-detector distance of 43 cm and thus reduced the background markedly — but at the expense of a long scan time. Data were collected with an exposure time of 20–40 seconds per degree of crystal rotation, for a total data acquisition time of 600–1200 seconds over a 30° rotation.

A second approach employs 'physical trapping' [70,76] and stabilizes intermediates by cryogenic techniques. The desired reaction is either initiated in the crystal at room temperature, allowed to proceed for a known time, then quenched by flash freezing to trap the intermediate; or the crystal is flash frozen, and the entire reaction initiation and evolution is conducted at low temperature. Scott *et al.* [83••] used the former strategy to study a catalytic RNA intermediate in the self-cleavage reaction of the hammerhead ribozyme. The time resolution is set by the time courses of both the reaction initiation process and the freezing process; the latter is typically tens of milliseconds or longer [70] and depends on the crystal dimensions and the freezing protocol. Hartmann *et al.* [84] and Teng *et al.* [10] both used the latter strategy to study the photolysis and rebinding of carbon monoxide from myoglobin at temperatures of 36–40K. Teng *et al.* [85] further addressed the issue of why the results of Hartmann *et al.* [84] and of two earlier studies [10,86] did not agree in detail, even though all were studying the same reaction via ostensibly the same experimental approach. In time-resolved studies extending over several hours, Teng *et al.* [85] followed the excruciatingly slow drift (< 1 Å per hour) of the photolyzed carbon monoxide away from the heme, under conditions of continuous illumination at 40K. Its trajectory mapped well on to a time-independent ligand probability distribution derived from computational studies [87]. This result, as with the parallel crystallographic and molecular dynamics studies of isocitrate dehydrogenase [79••], gives further confidence in the interpretation of the crystallographic results. The structural differences among the earlier studies are attributed largely to differences in the photolysis protocols, although inadvertent temperature jumps and differences among space groups and crystals may also contribute.

The most challenging form of time-resolved crystallography is provided by 'no trapping', in which the chemical and physical manipulation of intermediates and associated artefacts are avoided, but the necessity for ultrarapid crystallographic measurement is introduced. In one such study, Moffat, Getzoff and coworkers [12•,88,89] used time-resolved Laue diffraction at beamline X26C, NSLS, with a 10 ms time resolution, to study the decay of a small bacterial photosensor, photoactive yellow protein (PYP), from a saturated, photostationary state obtained by brief laser illumination of small crystals of typical dimensions $50 \times 50 \times 350\,\mu m^3$. They were able to observe the structural change that is associated with late stages in the PYP photocycle and to infer those associated with earlier stages. The novel, 4-hydroxycinnamic acid chromophore of PYP is *trans* in the dark, resting state and is inferred to rapidly isomerize to *cis* on absorption of a blue photon. Isomerization conveys structural changes to the surrounding protein over the microseconds to hundreds of milliseconds time range, and thus generates the structural signaling state directly observed in these experiments. The chromophore finally re-isomerizes to the resting state over a few seconds. As the PYP photocycle is fully reversible and the crystals are resistant to X-ray- and laser-induced damage, highly redundant accurate Laue data could be accumulated over numerous reaction cycles on the same crystal. Successful structural analysis was aided by using novel algorithms for the reduction of static [90–92] and time-resolved [89] Laue data.

Spectroscopic studies of both carbonmonoxy myoglobin photolysis and PYP reveal very fast spectral changes down to the 100 fs timescale that are presumably accompanied by structural changes in the chromophore and the protein. The ultimate goal of the 'no trapping' approach is to conduct ultrarapid X-ray studies and thus provide a structural foundation for these spectroscopic studies and for computational approaches that presently extend only to the few nanoseconds timescale. The X-ray pulse duration emitted by a single-particle bunch circulating in the ESRF,

APS or CHESS is around 150 ps and repeats every few microseconds – the circulation time in the storage ring. Some time ago, a 150 picosecond exposure from an undulator temporarily installed at CHESS was shown to be sufficient to yield an X-ray diffraction pattern of very modest quality from a large, well-ordered protein crystal [93]. However, it required the careful design, development and commissioning of the focused, white beam BL3/ID9 beamline at the ESRF by Wulff and coworkers [15••] to demonstrate that entire Laue data sets of high accuracy could be obtained on smaller crystals. To obtain the desired time resolution of nanoseconds and below, the fast shutter train had to be slaved to the accelerator ring clock, and triggering signals had to be developed to fire a pulsed laser for reaction initiation and to arm the sensitive, low-noise, image-intensified charge-couple device (CCD) detector. With these elaborate experimental developments in hand [15••], it proved possible to conduct the first time-resolved experiments with nanosecond time resolution and achieve a six orders of magnitude increase in time resolution on carbonmonoxy myoglobin photolysis at physiological temperature ([13••]; see also [94]). This reaction, as that of PYP, is fully reversible in the crystal, and indeed carbon monoxide rebinding is fully complete in ~100 ms, as shown both by the X-ray results and by parallel optical studies. A putative location for the outgoing carbon monoxide could be visualized and falls neatly beside the ligand trajectory visualized at cryogenic temperature [85], thus tying together the results at cryogenic and physiological temperatures. In these initial studies, structural changes at the heme could readily be detected on the nanosecond to tens of microseconds timescale, but those in the globin were barely distinguishable from noise.

In all such time-resolved experiments, a particular time point may reveal a superposition of structures, and it is the individual components of this superposition whose structures are required. The final piece of the time-resolved puzzle involves techniques for unscrambling this superposition ([95]; W Krebs, Z Ren, K Moffat, unpublished data), which continues to remain a challenge.

Conclusions and frontiers

Synchrotron-based macromolecular crystallography is firmly established, and structural biologists will increasingly exploit the fruits of the numerous genome projects. Others will pursue a more physics-directed path. For example, the time resolution may be extended to the 100 fs range by completely new and admittedly challenging strategies [96•], and certain experiments may seek to exploit laser-based or linear accelerator based hard X-ray sources [97]. But, travel time to and from our X-ray sources will still be orders of magnitude longer than the total exposure time we use when we are there!

Acknowledgements

Supported by grants from the National Institutes of Health to Keith Moffat.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Helliwell JR: **Macromolecular crystallography with synchrotron radiation.** Cambridge, UK: Cambridge University Press; 1992.

2. • Chayen NE, Boggon TJ, Cassetta A, Deacon A, Gleichmann T, Habash J, Harrop SJ, Helliwell JR, Nieh YP, Peterson MR *et al.*: **Trends and challenges in experimental macromolecular crystallography.** *Quart Rev Biophys* 1996, **29**:227-278.
A thorough review with a substantial section on synchrotron-related developments.

3. Helliwell JR: **Overview of synchrotron radiation and macromolecular crystallography.** *Methods Enzymol* 1997, **276A**: 203-217.

4. Ealick S, Walter R: **Synchrotron beamlines for macromolecular crystallography.** *Curr Opin Struct Biol* 1993, **3**:725-736.

5. Hendrickson WA: **Determination of macromolecular structures from anomalous diffraction of synchrotron radiation.** *Science* 1991, **254**:51-58.

6. Dauter Z, Lamzin VS, Wilson KS: **Proteins at atomic resolution.** *Curr Opin Struct Biol* 1995, **5**:784-790.

7. Dauter Z: **Atomic resolution protein crystal structures.** *Synchrotron Rad News* 1997, **10**:22-25.

8. Zhou L, Luo M: **Crystallization and preliminary X-ray analysis of two density populations of feline calcivirus particles.** *Acta Crystallogr D* 1995, **51**:850-852.

9. Burroughs JN, Grimes JM, Mertens PP, Stuart DI: **Crystallization and preliminary X-ray analysis of the core particle of bluetongue virus.** *Virology* 1995, **210**:217-220.

10. Teng T-Y, Srajer V, Moffat K: **Photolysis-induced structural changes in single crystals of carbonmonoxy myoglobin at 40 K.** *Nat Struct Biol* 1994, **1**:701-705.

11. •• Gouet P, Jouve H-M, Williams PA, Andersson I, Andreoletti P, Nussaume L, Hajdu J: **Ferryl intermediates of catalase captured by time-resolved Weissenberg crystallography and UV-VIS spectroscopy.** *Nat Struct Biol* 1996, **3**:951-956.
The state of the art in slower time-resolved crystallography, carefully linked to optical studies.

12. • Genick UK, Borgstahl GE, Ng K, Ren Z, Pradervand C, Burke PM, Srajer V, Teng T-Y, Schildkamp W, McRee DE *et al.*: **Structure of a protein photocycle intermediate by millisecond time-resolved crystallography.** *Science* 1997, **275**:1471-1475.
Quite fast time-resolved studies can be conducted efficiently at second-generation sources, here, the National Synchrotron Light Source. The intrinsic light-sensitivity of photoactive yellow protein is exploited to generate a light-driven reaction in the crystal, which reveals later stages in the rather complex photocycle of this protein with 10 ms time resolution. The interpretation of results is complicated by unexpected structural heterogeneity in the photostationary state.

13. •• Srajer V, Teng T-Y, Ursby T, Pradervand C, Ren Z, Adachi S-I, Schildkamp W, Bourgeois D, Wulff M, Moffat K: **Photolysis of the carbon monoxide complex of myoglobin: nanosecond time-resolved crystallography.** *Science* 1996, **274**:1726-1729.
The elaborate experimental techniques developed in [15••] finally yield real structure results.

14. Szebenyi DME, Arvai A, Ealick S, Laiuppa JM, Nielsen C: **A system for integrated collection and analysis of crystallographic diffraction data.** *J Synchrotron Rad* 1997, **4**:128-135.

15. •• Bourgeois D, Ursby T, Wulff M, Pradervand C, LeGrand A, Schildkamp W, Laboure S, Srajer V, Teng T-Y, Roth M, Moffat K: **Feasibility and realization of single pulse Laue diffraction on**

macromolecular crystals at ESRF. *J Synchrotron Rad* 1996, **3**:65-74.
Ultrarapid time-resolved crystallography poses severe experimental demands; this paper explains how they have been met.

16. Hendrickson WA, Brändén CI: **Editorial.** *Structure* 1997, **5(1)**.

17. Harding MM: **Recording diffraction data for structure determination for very small crystals.** *J Synchrotron Rad* 1996, **3**:250-259.

18. Balaic DX, Barnea Z, Nugent KA, Garrett RF, Varghese JN, Wilkins SW: **Protein crystal diffraction patterns using a capillary-focused synchrotron X-ray beam.** *J Synchrotron Rad* 1996, **3**:289-295.

19. •• Hendrickson WA, Ogata CM: **Phase determination from multiwavelength anomalous diffraction measurements.** *Methods Enzymol* 1997, **276A**:494-523.
A recent overview and survey of the MAD phasing technique. It contains an explicit deduction of MAD phasing theory and lists of commonly accessible absorption edges, MAD beamline parameters, and structures determined by MAD phasing.

20. • Pappa HS, Stewart AE, McDonald NQ: **Incorporating anomalous scattering centers into macromolecules.** *Curr Opin Struct Biol* 1996, **6**:611-616.
A review on incorporation of heavy atoms into macromolecules using chemical modification, protein engineering and *in vivo* labeling.

21. Guss JM, Merritt EA, Phizackerley, RP, Freeman HC: **The structure of a phytocyanin, the basic blue protein from cucumber, refined at 1.8 Å resolution.** *J Mol Biol* 1996, **262**:686-705.

22. Walter RL, Ealick SE, Friedman AM, Blake RC II, Proctor P, Shoham M: **Multiple wavelength anomalous diffraction (MAD) crystal structure of rusticyanin: a highly oxidizing cupredoxin with extreme acid stability.** *J Mol Biol* 1996, **263**:730-751.

23. Iwata S, Saynovits M, Link TA, Michel H: **Structure of a water soluble fragment of the 'Rieske' iron-sulfur protein of the bovine heart mitochondrial cytochrome bc_1 complex determined by MAD phasing at 1.5 Å resolution.** *Structure* 1996, **4**:567-579.

24. Concha NO, Rasmussen BA, Bush K, Herzberg O: **Crystal structure of the wide-spectrum binuclear zinc β-lactamase from *Bacteroides fragilis*.** *Structure* 1996, **4**:823-836.

25. Bellon SF, Rodgers KK, Schatz DG, Coleman JE, Steitz TA: **Crystal structure of the RAG1 dimerization domain reveals multiple zinc-binding motifs including a novel zinc binuclear cluster.** *Nat Struct Biol* 1997, **4**:586-591.

26. Davies C, White SW, Ramakrishnan V: **The structure of ribosomal protein L14 reveals an important organizational component of the translational apparatus.** *Structure* 1996, **4**:55-66.

27. Lee Y-H, Ogata C, Pflugrath JW, Levitt DG, Sarma R, Banaszak LJ, Pilkis SJ: **Crystal structure of the rat liver fructose-2,6-bisphosphatase based on selenomethionine multiwavelength anomalous dispersion phases.** *Biochemistry* 1996, **35**:6010-6019.

28. Reinemer P, Prade L, Hof P, Neuefeind T, Huber R, Zettl R, Palme K, Schell J, Koelln I, Bartunik HD, Bieseler B: **Three-dimensional structure of glutathione S-transferase from *Arabidopsis thaliana* at 2.2 Å resolution: structural characterization of herbicide-conjugating plant glutathione S-transferases and a novel active site architecture.** *J Mol Biol* 1996, **255**:289-309.

29. Thorn KS, Christensen HEM, Shigeta Jr R, Huddler D Jr, Shalaby L, Lindberg U, Chua N-H, Schutt CE: **The crystal structure of a major allergen from plants.** *Structure* 1997, **5**:19-32.

30. Peat TS, Frank EG, McDonald JP, Levine AS, Woodgate R, Hendrickson WA: **Structure of the UmuD′ protein and its regulation in response to DNA damage.** *Nature* 1996, **380**:727-734.

31. Martinez-Hackert E, Stock AM: **The DNA-binding domain of OmpR: crystal structure of a winged helix transcription factor.** *Structure* 1997, **5**:109-124.

32. Tong L, Pav S, White DM, Rogers S, Crane KM, Cywin CL, Brown ML, Pargellis CA: **A highly specific inhibitor of human p38 MAP kinase binds in the ATP pocket.** *Nat Struct Biol* 1997, **4**:311-316.

33. Blanchard H, Grochulski P, Li Y, Arthur JSC, Davies PL, Elce JS, Cygler M: **Structure of a calpain Ca^{2+}-binding domain reveals a novel EF-hand and Ca^{2+}-induced conformational changes.** *Nat Struct Biol* 1997, **4**:532-538.

34. Doublie S: **Preparation of selenomethionyl proteins for phase determination.** *Methods Enzymol* 1997, **276A**:523-530.

35. Geiger JH, Hahn S, Lee S, Sigler PB: **Crystal structure of the yeast TFIIA/TBP/DNA complex.** *Science* 1996, **272**:830-836.

36. Peat TS, Frank EG, Woodgate R, Hendrickson WA: **Production and crystallization of a selenomethionyl variant of UmuD′, an *Escherichia coli* SOS response protein.** *Proteins* 1996, **25**:506-509.

37. Sondek J, Bohm A, Lambright, DG, Hamm HE, Sigler PB: **Crystal structure of a GA protein βγ dimer at 2.1 Å resolution.** *Nature* 1996, **379**:369-374.

38. Boissy G, de La Fortelle E, Kahn R, Huet J-C, Bricogne G, Pernollet J-C, Brunie S: **Crystal structure of a fungal elicitor secreted by *Phytophthora cryptogea*, a member of a novel class of plant necrotic proteins.** *Structure* 1996, **4**:1429-1439.

39. Yamaguchi H, Hendrickson WA: **Structure basis for activation of human lymphocyte kinase Lck upon tyrosine phosphorylation.** *Nature* 1996, **384**:484-489.

40. Shapiro L, Fannon AM, Kwong PD, Thompson A, Lehmann MS, Grubel G, Legrand J-F, Als-Nielsen J, Colman DR, Hendrickson WA: **Structure basis of cell–cell adhesion by cadherins.** *Nature* 1995, **374**:327-337.

41. McDonald NQ, Panayotatos N, Hendrickson WA: **Crystal structure of dimeric human ciliary neurotrophic factor determined by MAD phasing.** *EMBO J* 1995, **14**:2689-2699.

42. •• Burling FT, Weis WI, Flaherty KM, Brunger AT: **Direct observation of protein solvation and discrete disorder with experimental crystallographic phases.** *Science* 1996, **271**:72-77.
The most accurate and complete experimental phase set obtained so far.

43. • Thygesen J, Weinstein S, Franceschi F, Yonath A: **The suitability of multi-metal clusters for phasing in crystallography of large macromolecular assemblies.** *Structure* 1996, **4**:513-518.
This paper describes procedures for soaking, cocrystallizing, and specific covalent binding of heavy atom clusters to very large macromolecular assemblies, with the aim of introducing sufficient phasing power for structure determination.

44. • Read RJ: **As MAD as can be.** *Structure* 1996, **4**:11-14.
Comments on the observed phase set in [42••].

45. Athappilly FK, Hendrickson WA: **Structure of the biotinyl domain of acetyl-coenzyme A carboxylase determined by MAD phasing.** *Structure* 1995, **3**:1407-1419.

46. Terwilliger TC: **Multiwavelength anomalous diffraction phasing of macromolecular structures: analysis of MAD data as single isomorphous replacement with anomalous scattering data using the MADMRG program.** *Methods Enzymol* 1997, **276A**:530-537.

47. Ramakrishnan V, Biou V: **Treatment of multiwavelength anomalous diffraction data as a special case of multiple isomorphous replacement.** *Methods Enzymol* 1997, **276A**:538-557.

48. Otwinowski Z: **Isomorphous replacement and anomalous scattering.** In *Proceedings of the CCP4 Study Weekend*. Edited by Wolf W, Evans PR, Leslie AGW. Warrington: SERC Daresbury Laboratory; 1991:80-86.

49. Furey W, Swaminathan S: **PHASES: a program package for the processing and analysis of diffraction data from macromolecules.** *Methods Enzymol* 1997, **276B**, in press.

50. Xu R-M, Koch C, Liu Y, Horton JR, Knapp D, Nasmyth K, Cheng X: **Crystal structure of the DNA-binding domain of Mbp1, a transcription factor important in cell-cycle control of DNA synthesis.** *Structure* 1997, **5**:349-358.

51. Yamamoto M, Fujisawa T, Nakasako M, Tanaka T, Uruga T, Kimura H, Yamaoka H, Inoue Y, Iwasaki H, Ishikawa T *et al.*: **Conceptual design of SPring-8 contract beamline for structural biology.** *Rev Sci Instrum* 1995, **66**:1833-1835.

52. Lee PL, Ogata CM: **Simultaneous multiwavelength anomalous diffraction (SMAD): an energy-dispersive synchrotron method for macromolecules.** *J Appl Crystallogr* 1995, **28**:661-665.

53. Munshi S, Liljas L, Cavarelli J, Bomu W, McKinney B, Reddy V, Johnson JE: **The 2.8 Å structure of a T=4 animal virus and its implications for membrane translocation of RNA.** *J Mol Biol* 1996, **261**:1-10.

54. Zhao R, Pevear DC, Kremer MJ, Giranda VL, Kofron JA, Kuhn RJ, Rossmann MG: **Human rhinovirus 3 at 3.0 Å resolution.** *Structure* 1996, **4**:1205-1210.

55. McKenna R, Bowman BR, Ilag LL, Rossmann MG, Fane BA: **Atomic structure of the degraded procapsid particle of the bacteriophage G_4: induced structural changes in the presence of calcium ions and functional implications.** *J Mol Biol* 1996, **256**:736-750.

56. Hadfield AT, Lee W-M, Zhao R, Oliveira MA, Minor I, Rueckert RR, Rossmann MG: **The refined structure of human rhinovirus 16 at 2.15 Å resolution: implications for the viral life cycle.** *Structure* 1997, **5**:427-441.

57. Golmohammadi R, Fridborg K, Bundule M, Valegard K, Liljas L: **The crystal structure of bacteriophage Qβ at 3.5 Å resolution.** *Structure* 1996, **4**:543-554.

58. Luo M, Toth KS, Zhou L, Pritchard A, Lipton HL: **The structure of a highly virulent Theiler's murine encephalomyelitis virus (GDVII) and implications for determinants of viral persistence.** *Virology* 1996, **220**:246-250.

59. Stehle T, Gamblin SJ, Yan Y, Harrison SC: **The structure of simian virus 40 refined at 3.1 Å resolution.** *Structure* 1996, **4**:165-182.

60. Hadfield A, Hajdu J, Chapman MS, Rossmann MG: **Laue diffraction studies of human rhinovirus 14 and canine parvovirus.** *Acta Crystallogr D* 1995, **51**:859-870.

61. Llamas-Saiz AL, Agbandje-McKenna M, Parker JSL, Wahid ATM, Parrish C, Rossmann MG: **Structure analysis of a mutation in canine parvovirus which controls antigenicity and host range.** *Virology* 1996, **225**:65-71.

62. Dokland T, McKenna R, Ilag LL, Bowman BR, Incardona NL, Fane BA, Rossmann MG: **Structure of a viral procapsid with molecular scaffolding.** *Nature* 1997, in press.

63. Lowe J, Stock D, Jap B, Zwickl P, Baumeister W, Huber R: **Crystal structure of the 20S proteasome from the archaeon *T. Acidophilum* at 3.4 Å resolution.** *Science* 1995, **268**:533-539.

64. Tsukihara T, Aoyama H, Yamashita E, Tomizaki T, Yamaguchi H, Shinzawa-Itoh K, Nakashima R, Yaono R, Yoshikawa S: **Structure of metal sites of oxidized bovine heart cytochrome *c* oxidase at 2.8 Å.** *Science* 1995, **269**:1069-1074.

65. •• Tsukihara T, Aoyama H, Yamashita E, Tomizaki T, Yamaguchi H, Shinzawa-Itoh K, Nakashima R, Yaono R, Yoshikawa S: **The whole structure of the 13-subunit oxidized cytochrome *c* oxidase at 2.8 Å.** *Science* 1996, **272**:1136-1144.

The cytochrome *c* oxidase structure [64,65••] has been eagerly anticipated; it will provide the basis for interpretation of decades of results by bioenergeticists. All cell dimensions are around 200 Å with two molecules per asymmetric unit. Structure determination at 2.6 Å resolution using isomorphous replacement, solvent flattening and phase extension benefited greatly from the high quality data collected with the Weissenberg camera [81,82] at the Photon Factory.

66. Krauss N, Schubert W-D, Klukas O, Fromme P, Witt HT, Saenger W: **Photosystem I at 4 resolution represents the first structural model of a joint photosynthetic reaction centre and core antenna system.** *Nat Struct Biol* 1996, **3**:965-973.

67. Koepke J, Hu X, Muenke C, Schulten K, Michel H: **The crystal structure of the light-harvesting complex II (B800-850) from *Rhodospirillum molischianum*.** *Structure* 1996, **4**:581-597.

68. • Garman EF, Schneider TR: **Macromolecular cryocrystallography.** *J Appl Crystallogr* 1997, **30**:211-237.

A comprehensive review of the principles and practice of cryocrystallography, essential to the effective use of beamlines that impose a high heating rate on the crystal.

69. • Longhi S, Czjzek M, Lamzin V, Nicholas A, Cambillau C: **Atomic resolution (1.0 Å) crystal structure of *Fusarium solani* cutinase: stereochemical analysis.** *J Mol Biol* 1997, **268**:779-799.

If your crystals are terrific, then careful structure analysis at atomic resolution can supply new data on, for example, the planarity of the peptide bond.

70. Moffat K, Henderson R: **Freeze trapping of reaction intermediates.** *Curr Opin Struct Biol* 1995, **5**:656-663.

71. Teng T-Y, Moffat K: **Cooling rates during flash cooling.** *J Appl Crystallogr* 1997, in press.

72. Ealick SE: **Now we're cooking: new successes for Shake-and-Bake.** *Structure* 1997, **5**:469-472.

73. • Mozzarelli A, Rossi GL: **Protein function in the crystal.** *Annu Rev Biophys Biomol Struct* 1996, **25**:343-365.

Remarkably few macromolecular crystals have had their activity quantitatively examined in the crystalline state, yet we routinely seek to correlate the crystal structure with functional proteins derived from studies in dilute solution in the absence of precipitants and of lattice forces. This paper reviews activity in the crystal from an enzymological and spectroscopic perspective.

74. Moffat K: **Time-resolved macromolecular crystallography.** *Synchrotron Rad News* 1996, **9**:15-18.

75. Sawaya MR, Kraut J: **Loop and subdomain movement in the mechanism of *E. coli* dihydrofolate reductase: crystallographic evidence.** *Biochemistry* 1997, **36**:586-603.

76. Stoddard BL: **Caught in a chemical trap.** *Nat Struct Biol* 1996, **3**:907-909.

77. Stoddard BL, Koshland DE Jr: **Structure of isocitrate dehydrogenase with α-ketoglutarate at 2.7- Å resolution: conformational changes induced by decarboxylation of isocitrate.** *Biochemistry* 1993, **32**:9317-9322.

78. Bolduc JM, Dyer DH, Scott WG, Singer P, Sweet RM, Koshland DE Jr, Stoddard BL: **Mutagenesis and Laue structures of enzyme intermediates: isocitrate dehydrogenase.** *Science* 1995, **268**:1312-1318.

79. •• Stoddard BL, Dean A, Bash PA: **Combining Laue diffraction and molecular dynamics to study enzyme intermediates.** *Nat Struct Biol* 1996, **3**:590-595.

Transient structural states are often present at lower occupancy and may be characterized by higher temperature factors on key atoms, which provides an additional challenge to crystallographic methods. Computational molecular dynamics studies can complement the crystallography, particularly in mobile regions of the structure, and can provide more confident mechanistic insights. Conversely, the computational studies are heavily dependent on excellent stereochemical information from the crystallography.

80. Mesecar AD, Stoddard BL, Koshland DE Jr: **Orbital steering in
• the catalytic power of enzymes: small structure changes with large catalytic consequences.** *Science* 1997, **277**:202-206.
The relationship between structure, energetics, and the origins of rate acceleration in enzyme mechanisms is not always straightforward. Freeze trapping of active complexes of isocitrate dehydrogenase probes this relation and re-examines the role of orbital steering.

81. Sakabe K, Sasaki K, Watanabe N, Susuki M, Wang ZG, Miyahara J, Sakabe N: **Large-format imaging plate and Weissenberg camera for accurate protein crystallographic data collection using synchrotron radiation.** *J Synchrotron Rad* 1997, **4**:136-146.

82. Kamiya N, Sasaki K, Watanabe N, Sakabe N, Sakabe K: **Time-resolved protein crystallography with large-angle oscillations: an application of a protein data-collection system using the Weissenberg technique and a large-format imaging plate.** *J Synchrotron Rad* 1997, **4**:14-16.

83. Scott WG, Murray JB, Arnold JRP, Stoddard BL, Klug A:
•• **Capturing the structure of a catalytic RNA intermediate: the hammerhead ribozyme.** *Science* 1996, **274**:2065-2069.
Freeze trapping of an intermediate in the divalent cation catalyzed self-cleavage of a ribozyme reveals structure changes that are largely restricted to the active site. On the basis of these and more conventional, static studies, the structure of a transition state can be inferred and the reaction mechanism can be pieced together.

84. Hartmann H, Zinser S, Komninos P, Schneider RT, Nienhaus GU, Parak F: **X-ray structure determination of a metastable state of carbonmonoxy myoglobin after photodissociation.** *Proc Natl Acad Sci USA* 1996, **93**:7013-7016.

85. Teng T-Y, Srajer V, Moffat K: **The initial trajectory of carbon monoxide after photodissociation from myoglobin at cryogenic temperatures.** *Biochemistry* 1997, in press.

86. Schlichting I, Berendzen J, Phillips GN Jr, Sweet RM: **Crystal structure of photolysed carbonmonoxy-myoglobin.** *Nature* 1994, **371**:808-812.

87. Vitkup D, Petsko GA, Karplus M: **A comparison between molecular dynamics and X-ray results for dissociated CO in myoglobin.** *Nat Struct Biol* 1997, **4**:202-208.

88. Ng K, Getzoff ED, Moffat K: **Optical studies of a bacterial photoreceptor protein, photoactive yellow protein, in single crystals.** *Biochemistry* 1995, **34**:879-890.

89. Ren Z, Ng K, Borgstahl GEO, Getzoff ED, Moffat K: **Quantitative analysis of time-resolved Laue diffraction patterns.** *J Appl Crystallogr* 1996, **29**:246-260.

90. Ren Z, Moffat K: **Quantitative analysis of synchrotron Laue diffraction patterns in macromolecular crystallography.** *J Appl Crystallogr* 1995, **28**:461-481.

91. Ren Z, Moffat K: **Deconvolution of energy overlaps in Laue diffraction.** *J Appl Crystallogr* 1995, **28**:482-493.

92. Bourgeois D, Longhi S, Wulff M, Cambillau C: **Accuracy of structural information obtained at the European Synchrotron Radiation Facility from very rapid Laue data collection on macromolecules.** *J Appl Crystallogr* 1997, **30**:153-163.

93. Szebenyi DME, Bilderback DH, LeGrand A, Moffat K, Schildkamp W, Smith Temple B, Teng T-Y: **Quantitative analysis of Laue diffraction patterns recorded with a 120 ps exposure from an X-ray undulator.** *J Appl Crystallogr* 1992, **25**:414-423.

94. Eaton WA, Henry ER, Hofrichter J: **Nanosecond crystallographic snapshots of protein structural changes.** *Science* 1996, **274**:1631-1632.

95. Moffat K: **Time-resolved macromolecular crystallography.** *Annu Rev Biophys Biophys Chem* 1989, **18**:309-332.

96. Neutze R, Hajdu J: **Femtosecond time resolution in X-ray
• diffraction experiments.** *Proc Natl Acad Sci USA* 1997, **94**:5651-5655.
A thought-provoking new approach to structural kinetics on the subnanosecond timescale.

97. Doniach S: **Studies of the structure of matter with photons from a X-ray free-electron laser.** *J Synchrotron Rad* 1996, **3**:260-267.