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X-ray diffraction experiment – the last experiment in the structure elucidation process

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Abstract

Recent years have brought not only an avalanche of new macromolecular structures, but also significant advances in the protein structure determination methodology only now making its way into structure-based drug discovery. In this paper, we review recent methodology developments in X-ray diffraction experiments that lead to fast and very accurate elucidation of three-dimensional structures of macromolecules. We will discuss the role of data collection as the last experiment performed in the crystal structure determination process. A statistical analysis of diffraction experiments that are reported in the Protein Data Bank is also presented.

Keywords

X-ray diffraction experiment; structure determination; Structural Genomics; synchrotron

Introduction

There are several experimental techniques that can be used to elucidate the three-dimensional structures of protein and viruses: among others X-ray crystallography, electron microscopy (EM), small angle X-ray scattering (SAXS), and nuclear magnetic resonance (NMR) spectroscopy. The application of the two most frequently used - NMR spectroscopy and X-ray crystallography - has resulted in the deposition of almost 60,000 structural models of biological macromolecules (proteins, DNA, RNA or viruses) into the Protein Data Bank (PDB) (Berman *et al.*, 2002). The ratio of X-ray and NMR structures is about 6 to 1. Thus X-ray crystallography is still the predominant technique used to determine the structures of biological macromolecules.

High-throughput structural biology initiatives —and in particular, Structural Genomics (SG) —efforts have solved the thousands of protein structures and amassed a vast amount of experience (Slabinski *et al.*, 2007, Burley *et al.*, 2008). This experience has resulted in many advances that have improved the success rate of all experimental stages of the structure determination process, namely cloning, expression, crystallization, data collection, data

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reduction, phasing, model building, and structure deposition and analysis. In particular, the experience of SG groups has resulted in many efficient new techniques and technologies for crystallographic data collection and structure solution.

In principle, X-ray diffraction experiments are very simple, and have not changed much from the time almost one hundred years ago when Max von Laue (with the assistance of Walter Friedrich and Paul Kipping) obtained photographs showing the X-ray diffraction pattern produced from zinc sulfide. In modern-day macromolecular X-ray diffraction experiments, the two-dimensional diffraction images are collected with the use of an experimental setup that usually has four components: an X-ray source, a goniostat that can drive the crystal along the spindle axis, an area detector and equipment for the cryocooling of macromolecular crystals. The difficulty of the experiment arises from the fact that the crystals used in macromolecular crystallography vary enormously. For example, crystal mosaicity may vary between 0.05 and 3.5°, unit cell volumes may vary between 10000 and 720,000,000 Å³, and every crystal may have additional problems like anisotropic mosaicity, twinning, etc. Selecting the best crystal for diffraction is critical as in many cutting-edge experiments the number of available crystals can be very limited. In extreme cases there may be only one crystal available. In addition, it is very difficult to identify experimental setup pathologies, as the correlations between crystal and equipment malfunctions are very unintuitive. For example, goniostats which use servomotors to drive the spindle axis produce poor quality data when almost perfect crystals with unusually low mosaicity are in the X-ray beam. For typical macromolecular crystals, with mosaicity of 1° or larger, the same experimental setup may yield ideal data, as the (effectively) larger measurement time averages the nonuniformity of the spindle axis movement. This example shows that knowledge of experimental setup limitations can be critical for the success of the experiment. Experimenters that are aware of the limitations of nonuniform spindle axis rotation may address the problem in one of two equivalent ways: either use another synchrotron station with a spindle axis driven by a stepper motor, or select a more mosaic (and unfortunately, lower quality) crystal or slower rotation with attenuated beam.

Experimental protocols

In current practice, macromolecular diffraction data are typically collected for three different purposes: phase determination by molecular replacement (MR), phase determination by multiple anomalous diffraction (MAD)/single anomalous diffraction (SAD), and data for the final refinement of a model. Other techniques are also sometimes used, such as phasing by Multiple Isomorphous Replacement (MIR) or *ab initio* methods, albeit much less frequently than the ones described above. In each case, the diffraction experimental protocol should be designed to produce data best suited for the purpose intended. In the first and third cases, the diffraction experiment is used to measure amplitudes and phases are derived from the known model. In the second case (SAD/MAD), the diffraction experiment measures a phase-sensitive signal that is typically produced by small differences between observed amplitudes (Hendrickson, 1991). Often data collected for MR or SAD/MAD phasing are later used for refinement of the structure. In that case, the experimental collection protocol is a compromise between a protocol optimized for MR phasing (complete data to a lower resolution optimal for phase determination by MR) and a protocol yielding a complete data set collected to the highest resolution possible (the diffraction limit).

In principle, the data collection protocols should be very simple as there are typically only four parameters that are under the user's control: crystal-to-detector distance, exposure time, oscillation angle, and wavelength of X-ray radiation (Bourenkov & Popov, 2006, Popov & Bourenkov, 2003). The difficulty of the experiment arises from the fact that not only is the quality of the crystal beyond the experimenter's control, but also destruction of the sample

with the X-ray dose due to crystal decay caused by the radiation. The difficulty of choosing user-controlled parameters (protocol) that minimize the detriments of poor crystal quality and radiation decay and maximize the signal of interest is to decide what trade-offs are most beneficial for a particular crystal, application and experimental setup. For example, using a longer exposure time for each image produces better counting statistics but may lead to incomplete high resolution data due to crystal decay. In addition, longer exposure time may lead to incomplete low resolution data due to the limited dynamic range of most detectors that are currently used at synchrotron facilities (Figure 1). For the latter case, it may be appropriate to collect data in multiple passes, measuring the low resolution data first (Dauter, 2005). A small oscillation range helps avoid overlaps of diffraction spots and for other reason is in principle beneficial to data quality (Pflugrath, 1999). However, smaller oscillations may be detrimental with experimental systems with poor shutter/goniostat synchronization and/or detectors with relatively large read-out noise. Another parameter is detector distance; moving the detector farther away from the sample decreases X-ray noise and helps to avoid overlaps for large unit cell crystals, but limit the resolution of the recorded diffraction data. A list of possible tradeoffs could continue for many pages but the best illustration of all of these difficulties is one fact: even though there are many programs that calculate the best collection strategy for diffraction experiments. It has been reported the need to collect 57 full data sets on average (Holton, 2005) to make one PDB deposit.

Choice of diffraction protocol

Data collection is the last experiment that is performed during the crystal structure determination process. As diffraction experiments measure only intensities (which are related to amplitudes of structure factors), the intended method for generating phases corresponding to those intensities must be decided before the diffraction experiment begins. This is necessary to optimize the data collection protocol to generate data most suited for the intended goal. In the simplest case, data for structure refinement, it is enough to concentrate on collection of an accurate and complete data set. The major goal of such an experiment is to obtain the highest possible resolution of the data.

The data collection protocol for MR phasing (Rossmann, 1962, 1964) is usually very similar to the protocol for experiment described above. However, in the case of MR, more care has to be taken to collect the low resolution data, as high resolution data are not critical to generate a MR solution. Specifically, complete low resolution data are very important for all methods that use Patterson functions (such as MR). That having been said, the success of MR depends more on the availability of an appropriate search model (Lebedev *et al.*, 2008) than on the accuracy of the data. It was shown recently that search models do not have to have very high sequence identity to the structures of interest (Figure 2), and in some cases MR methods have been successful for search structures with less than 20% sequence identity to the experimental structure. Nevertheless, even having a highly homologous structure does not guarantee the success of MR. Having many copies of the macromolecule in the asymmetric unit has been a serious obstacle for MR, though recent developments of software and methodology have produced greatly improved multi-copy translation and rotation searches (Vagin & Teplyakov, 2000, McCoy, 2007). In other situations, non-crystallographic symmetry operators are close to the crystallographic symmetry operators. This results in “pseudosymmetry,” which may additionally complicate or even prevent phase solution by MR (Dauter *et al.*, 2005, Jaskolski *et al.*, 2006). This problem is not rare; it has been estimated that around 6% of all structures deposited in the PDB exhibit pseudosymmetry (Zwart *et al.*, 2008).

If there is no suitable model for molecular replacement or the MR method fails for any reason, the only possibility is *de novo* phase determination. SAD/MAD methods (Dauter *et*

et al., 2002) are the most popular experimental phase determination techniques (Grabowski *et al.*, 2009), and in the last 10 years have effectively eliminated application of Multiple/Single Isomorphous Replacement (MIR/SIR) methods. In particular, SAD has gained significant popularity (Chruszcz *et al.*, 2008), due to its relative experimental simplicity and development of improved SAD phasing programs (Wang *et al.*, 2006). As a result, SAD is becoming most often used technique to phase structures of 'novel' macromolecules (Chruszcz *et al.*, 2008). Despite its "simplicity," SAD techniques require the most accurate data and is the most demanding in the sense of diffraction protocols, hardware and software. Sometimes a useful anomalous signal can be obtained for proteins that have only one ordered selenium atom per 200 residues (Figure 3). In principle, almost all proteins (with sufficient methionine and/or cysteine residues) could be phased using the anomalous signal from sulfur (Hendrickson & Teeter, 1981, Wang, 1985, Dauter *et al.*, 1999). Unfortunately, sulfur SAD is typically only successful for well-diffracting crystals and the highest-quality hardware and software combinations. In all other cases, the macromolecule sample must be derivatized prior to data collection, as only a fraction of macromolecules naturally contain anomalously scattering elements other than sulfur in quantities sufficient for successful phasing. For example, metal-ion-containing proteins may not require any special treatment (Shi *et al.*, 2005, Ascone & Strange, 2009), but measuring the X-ray absorption spectrum of the protein crystal to confirm presence of particular element prior to data collection is recommended (Walsh *et al.*, 1999).

Experimental setup

Cryocooling is the only major addition to the current experimental procedure in comparison with the procedure used by Max von Laue one hundred years ago (he also did not use rotational method). Low temperatures (~100K) are used in almost all recent macromolecular diffraction experiments (Chruszcz *et al.*, 2008). In 2009, 90.5% of all PDB deposits reported data collection temperatures below 130K. Cryocooling (Hope, 1988, Garman & Owen, 2006, Garman & McSweeney, 2007), in connection with the use of cryo-loops (Teng, 1990), has enormously increased the success rate of data collection process, by decreasing—but not eliminating—the rate of radiation decay of protein samples. However, the choice of cryosolution is still a challenge and many diffraction patterns are obtained from suboptimal cryocooling conditions. New software under development (Otwinowski *et al.*, 2003, Borek *et al.* 2009) can correct the values of intensities to account for radiation decay, but they are not able to restore high resolution reflections that cannot be measured due to crystal damage.

The fact that macromolecular crystals are typically much smaller and diffract orders of magnitude more weakly than the crystals used for diffraction 100 years ago (small molecule compounds) prompted the development of high brilliance X-ray sources. Currently almost 80% of all PDB deposits report the use of synchrotron radiation. This fraction is even higher when non-redundant structures (defined as the set of protein structures with less than 30% sequence identity to structures previously reported to PDB) are taken into account (Grabowski *et al.*, 2009). Synchrotrons not only provide high brilliance radiation source but most importantly allow to change energy (or wavelength) of the incoming radiation, which is critical for SAD/MAD experiments. The majority of currently existing over 120 macromolecular crystallography synchrotron stations permit the use of a wide range of energies. However, an analysis of the wavelengths used for structure determination of PDB deposits shows that only a small fraction were solved with collection wavelengths outside the 0.9 – 1.0 Å range (Figure 4). It seems that it would be beneficial for the structural biology community to build cheaper and simpler high-intensity fixed wavelength stations with energy set at the selenium absorption edge.

The biggest technological change affecting macromolecular diffraction experiments is in the development of devices used to record diffraction patterns. Ordinary film was still in use at most synchrotron stations as of the mid 1980s but soon was replaced by the TV detector constructed by Ulli Arndt for Enraf-Nonius (Schierbeek & Parlevliet, 1991), and Image Plate (IP) detectors developed by MAR, Rigaku and Mac Science (Sakabe *et al.*, 1997, Sakabe, 1991). At the same time, Nicolet and San Diego Multiwire (currently Area Detector System Corporations or ADSC) independently developed very successful multiwire detectors for use with rotating anode X-ray generators (Hamlin *et al.*, 1975, Hamlin *et al.*, 1981). At the beginning of the 1990s, a new charge-coupled device (CCD) detector (Gruner, 1994, Gruner *et al.*, 2002) constructed by Sol Gruner was extremely successful at CHESS synchrotron. Now, CCD detectors are responsible for almost 100% of data collected at synchrotron stations and are frequently used on “home” X-ray diffraction stations as well (Figure 1). In 2008, a new Pixel Array Detector became operational at the Swiss Light Source (Bech *et al.*, 2008). The very fast readout time of that detector allows for shutter-less data collection and full characterization of a crystal within a few seconds.

The goniostat used for macromolecular diffraction experiments can be very simple – just a single rotation axis can be used to collect a complete data set. Few or no observations are lost in the so-called blind region of missing reflections given the relatively low resolution limits typical for macromolecular experiments. A three-axis goniostat seems to add some benefits compared to a single axis system, but requires additional precautions due to the possibility of physical collisions between the goniostat and the detector, cryo-cooling device, or other equipment. Experience has shown that goniostats are the most neglected components of the X-ray diffraction apparatus in terms of technical advancement. Most experimental errors in macromolecular X-ray diffraction are caused by uneven goniostat movement and/or poor coordination between the goniostat and X-ray shutter.

Data processing

Software development and software/hardware integration plays one of the major roles in the transformation of structural biology into an analytical tool that can be more easily used by non-crystallographers. Traditionally, the result of a synchrotron experiment was a set of diffraction images, or at best, scaled diffraction intensities with associated errors. During the last three years the trend is toward integrated systems that combine data collection, data reduction, phasing and model building. Such systems significantly accelerate the process of structure determination and on average minimize the number of data sets and amount of synchrotron time required for structure solution. The typical end result (while the crystal is still on the goniostat) is an interpretable electron density map with a partially built structure and, in some cases, an almost complete refined model. The current development of systems like HKL-3000 (Minor *et al.*, 2006) or PHENIX (Adams *et al.*, 2002) is oriented towards very fast structure solution, in order to provide feedback during the diffraction experiment. HKL-3000 has proved its usefulness as over 900 PDB deposits list it as the program used for structure determination.

Data and structure quality

In an ideal world, the only parameter that should describe structure quality is the resolution of the data used for structure refinement. In practice however, there is no agreement how to define the resolution of the data. The analysis of mean $I/\sigma I$ presented on Figure 5 shows that if we disregard obvious mistakes in deposits (e.g. if the mean $I/\sigma I$ for the highest resolution shell is the same or larger than the mean for the whole data set), a substantial fraction of the remaining structures had mean $I/\sigma I$ values much larger than 5 in the highest resolution shell, which suggests that refinement of many structures are not performed to the true crystal

diffraction limit. Moreover, several deposits have very incomplete data in the highest resolution shell (Grabowski *et al.*, 2009). Similar conclusions may be derived by analyzing R_{merge} values (Figure 6). The quality of the data does not directly implicate the quality of structures. It is striking, however, that the quality of structures as represented on an R vs. resolution plot (Figure 7) differs substantially for all four large PSI2 centers (JCSG, MCSG, NESGC, NYSGXC), even when only structures deposited during the last two years are taken into consideration.

Conclusions

Inexperienced experimenters can be assured that they are close to performing the best experiments possible by preparing experiments based on analysis of successful experiments and protocols used on the most productive synchrotron stations (Rosenbaum *et al.*, 2006, Grabowski *et al.*, 2009). Unfortunately, it is much easier to analyze single experiments than to find out in general what experimental practices are the most successful (in the sense of successful structure solution). The currently used format of the PDB header (Berman *et al.*, 2002) does not allow for a more detailed description of the diffraction experiment. In many cases, data are collected at different wavelengths for phasing calculations and structure refinement. For example, the data collected with a chromium anode X-ray generator are generally not useful for refinement due to resolution restrictions for long wavelengths, and the use of longer wavelength may be underrepresented in PDB deposits. As of September 2009, there are only 10 deposits collected at synchrotrons and 9 at home sources that reported the use of wavelengths longer than 2.0 Å. The new practices applied at the Center for Structural Genomics of Infectious Diseases (Anderson, 2009), Seattle Structural Genomics Center for Infectious Diseases (Myler *et al.*, 2009), and the Joint Center for Structural Genomics (Lesley & Wilson, 2005) to provide raw diffraction images together with a rudimentary description about how the structure was solved. It has to be stressed that data collection is the last experiment in the structure determination process, which usually cannot be repeated as crystals are typically destroyed during synchrotron experiment. Furthermore, repetition of data collection is impossible if there are no more crystals of similar or better quality.

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References

- Adams PD, Grosse-Kunstleve RW, Hung LW, Ioerger TR, McCoy AJ, Moriarty NW, Read RJ, Sacchettini JC, Sauter NK, Terwilliger TC. *Acta Crystallogr.* 2002; D58:1948–1954.
- Anderson WF. *Infect Disord Drug Targets.* 2009; 9:507–517. [PubMed: 19860716]
- Ascone I, Strange R. *J Synchrotron Radiat.* 2009; 16:413–421. [PubMed: 19395808]
- Bech M, Bunk O, David C, Kraft P, Bronnimann C, Eikenberry EF, Pfeiffer F. *Appl Radiat Isot.* 2008; 66:474–478. [PubMed: 18035547]
- Berman HM, Battistuz T, Bhat TN, Bluhm WF, Bourne PE, Burkhardt K, Feng Z, Gilliland GL, Iype L, Jain S, Fagan P, Marvin J, Padilla D, Ravichandran V, Schneider B, Thanki N, Weissig H, Westbrook JD, Zardecki C. *Acta Crystallogr.* 2002; D58:899–907.
- Borek D, Cymborowski M, Machius M, Minor W, Otwinowski Z. *Acta Crystallogr D.* in print.
- Bourenkov GP, Popov AN. *Acta Crystallogr.* 2006; D62:58–64.
- Burley SK, Joachimiak A, Montelione GT, Wilson IA. *Structure.* 2008; 16:5–11. [PubMed: 18184575]

- Chruszcz M, Wlodawer A, Minor W. *Biophys J*. 2008; 95:1–9. [PubMed: 18441029]
- Dauter Z. *Prog Biophys Mol Biol*. 2005; 89:153–172. [PubMed: 15910916]
- Dauter Z, Botos I, LaRonde-LeBlanc N, Wlodawer A. *Acta Crystallogr*. 2005; D61:967–975.
- Dauter Z, Dauter M, de La Fortelle E, Bricogne G, Sheldrick GM. *J Mol Biol*. 1999; 289:83–92. [PubMed: 10339407]
- Dauter Z, Dauter M, Dodson E. *Acta Crystallogr*. 2002; D58:494–506.
- Garman E, Owen RL. *Methods Mol Biol*. 2006; 364:1–18. [PubMed: 17172757]
- Garman EF, McSweeney SM. *J Synchrotron Radiat*. 2007; 14:1–3. [PubMed: 17211066]
- Grabowski M, Chruszcz M, Zimmerman MD, Kirillova O, Minor W. *Infect Disord Drug Targets*. 2009; 9:459–474. [PubMed: 19594422]
- Gruner SM. *Current Opinion in Structural Biology*. 1994; 4:765–769.
- Gruner SM, Tate MW, Eikenberry EF. *Review of Scientific Instruments*. 2002; 73:2815–2842.
- Hamlin R, Cork C, Howard A, Nielsen C, Vernon W, Matthews D, Xuong NH. *J Appl Crystallogr*. 1981; 14:85–93.
- Hamlin R, Cork C, Nielsen C, Vernon W, Xuong NH. *Acta Crystallogr*. 1975; A31:S234–S234.
- Hendrickson WA. *Science*. 1991; 254:51–58. [PubMed: 1925561]
- Hendrickson WA, Teeter MM. *Nature (London)*. 1981; 290:107–113.
- Holton, J. Teaching Elves to Collect Data: An Analysis of the Last Million Diffraction Images from ALS 8.3.1; Annual Meeting of the American Crystallographic Association; Orlando, FL, USA. 2005.
- Hope H. *Acta Crystallogr*. 1988; B44:22–26.
- Jaskolski M, Li M, Laco G, Gustchina A, Wlodawer A. *Acta Crystallogr*. 2006; D62:208–215.
- Lebedev AA, Vagin AA, Murshudov GN. *Acta Crystallogr*. 2008; D64:33–39.
- Lesley SA, Wilson IA. *J Struct Funct Genomics*. 2005; 6:71–79. [PubMed: 16211502]
- McCoy AJ. *Acta Crystallogr*. 2007; D63:32–41.
- Minor W, Cymborowski M, Otwinowski Z, Chruszcz M. *Acta Crystallogr*. 2006; D62:859–866.
- Myler PJ, Stacy R, Stewart L, Staker B, Van Voorhis WC, Varani G, Buchko GW. *Infect Disord Drug Targets*. 2009; 9:493–506. [PubMed: 19594426]
- Otwinowski Z, Borek D, Majewski W, Minor W. *Acta Crystallogr*. 2003; A59:228–234.
- Pflugrath JW. *Acta Crystallogr*. 1999; D55:1718–1725.
- Popov AN, Bourenkov GP. *Acta Crystallogr*. 2003; D59:1145–1153.
- Rosenbaum G, Alkire RW, Evans G, Rotella FJ, Lazarski K, Zhang RG, Ginell SL, Duke N, Naday I, Lazarz J, Molitsky MJ, Keefe L, Gonczy J, Rock L, Sanishvili R, Walsh MA, Westbrook E, Joachimiak A. *J Synchrotron Radiat*. 2006; 13:30–45. [PubMed: 16371706]
- Rossmann MG, Blow DM. *Acta Crystallogr*. 1964; 17:1474–1475.
- Rossmann MG, Blow DM. *Acta Crystallogr*. 1962; 15:24–31.
- Sakabe K, Sasaki K, Watanabe N, Suzuki M, Wang ZG, Miyahara J, Sakabe N. *J Synchrotron Radiat*. 1997; 4:136–146. [PubMed: 16699220]
- Sakabe N. *Nuclear Instruments & Methods in Physics Research Section a-Accelerators Spectrometers Detectors and Associated Equipment*. 1991; 303:448–463.
- Schierbeek AJ, Parlevliet D. *Nuclear Instruments & Methods in Physics Research Section a-Accelerators Spectrometers Detectors and Associated Equipment*. 1991; 310:571–575.
- Shi W, Zhan C, Ignatov A, Manjasetty BA, Marinkovic N, Sullivan M, Huang R, Chance MR. *Structure*. 2005; 13:1473–1486. [PubMed: 16216579]
- Slabinski L, Jaroszewski L, Rodrigues AP, Rychlewski L, Wilson IA, Lesley SA, Godzik A. *Protein Sci*. 2007; 16:2472–2482. [PubMed: 17962404]
- Teng TY. *J Appl Crystallogr*. 1990; 23:387–391.
- Vagin A, Teplyakov A. *Acta Crystallogr*. 2000; D56:1622–1624.
- Walsh MA, Evans G, Sanishvili R, Dementieva I, Joachimiak A. *Acta Crystallogr*. 1999; D55:1726–1732.
- Wang BC. *Methods Enzymol*. 1985; 115:90–112. [PubMed: 4079800]

Wang J, Dauter M, Dauter Z. Acta Crystallogr. 2006; D62:1475–1483.

Zwart PH, Grosse-Kunstleve RW, Lebedev AA, Murshudov GN, Adams PD. Acta Crystallogr. 2008; D64:99–107.

Figure 1. Fraction of deposits determined with the use of CCD detectors by data collection year. The blue bars represent the fraction of PDB deposits solved using synchrotron radiation, while the red bars represent the fraction of deposits solved using home systems.

Figure 2.

The resolution of structures solved by MR as a function of the sequence identity of the most similar homolog in the PDB at the time of deposition. The figure shows only cases where the sequence identity of the most similar structure smaller or equal to 90%. Red circles represent structures with one macromolecular chain in the asymmetric unit.

Figure 3. Ratio of total amino acid residues to number of ordered Se-Met residues, plotted as a function of the structure resolution. Structures solved by SAD are shown as red circles and those solved by MAD as green circles.

Figure 4. PDB deposits grouped by the wavelength used during the diffraction experiment. Only deposits obtained with synchrotron radiation are shown.

Figure 5. Distribution of mean $I/\sigma I$ vs. $I/\sigma I$ for the highest resolution shell for structures reported in the PDB.

Figure 6.
Distribution of R_{merge} vs. R_{merge} for the highest resolution shell for structures reported in the PDB.

Figure 7. R factor *vs.* resolution for four PSI centers. Each center is represented by different color of data points and trend lines.