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Energy optimization of a regular macromolecular crystallography beamline for ultra-high-resolution crystallography

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A practical method for operating existing undulator synchrotron beamlines at photon energies considerably higher than their standard operating range is described and applied at beamline 19-ID of the Structural Biology Center at the Advanced Photon Source enabling operation at 30 keV. Adjustments to the undulator spectrum were critical to enhance the 30 keV flux while reducing the lower- and higher-energy harmonic contamination. A Pd-coated mirror and Al attenuators acted as effective low- and high-bandpass filters. The resulting flux at 30 keV, although significantly lower than with X-ray optics designed and optimized for this energy, allowed for accurate data collection on crystals of the small protein crambin to 0.38 Å resolution.

Keywords: undulator; high energy; monochromator; harmonic.

1. Introduction

Collection of diffraction data at high photon energies (>25 keV) has numerous useful applications, among them obtaining ultra-high-resolution data on macromolecules, polycrystalline and amorphous materials, and structural investigations on small molecules. In particular, ultra-high-resolution data on macromolecular crystals can yield more accurate structures, reveal limited hydrogen atom positions, extract information about motions of atoms and macromolecules, and enable charge density analysis of structures. To date, nearly 600 structures in the Protein Data Bank have been solved at higher than 1.0 Å, with 47 structures resolved beyond 0.8 Å. These structures contain valuable information about bond lengths and angles, hydrogen atom positions, and therefore reveal great detail about the chemistry within protein structures and especially enzyme active sites. However, beamline operation at high energies and collection of ultra-high-resolution diffraction data remain challenging, and can involve major hardware adjustments. Beamline 19-ID at the Advanced Photon Source (APS) is an undulator beamline equipped with a Si(111) monochromator, a vertically focusing mirror with three lanes (Pd-coated, uncoated and Pt-coated), an array of Al and Ag filters for attenuation, and an ADSC Quantum 315r CCD detector (Rosenbaum *et al.*, 2006). The standard operational energy range is 6.5–13.5 keV, extendable with some special effort to 19.5 keV. This report describes a method of utilizing undulator, monochromator, mirror and attenuator settings to operate 19-ID at 30 keV (0.413 Å) that may be applicable to similar stations at other synchrotrons.

2. Results and discussion

There are two general options for collecting ultra-high-resolution data. One option involves moving the detector into a position suitable for collecting high-angle reflections. At 19-ID, available degrees of freedom of the detector include vertical offset, 2θ adjustment and crystal-to-detector distance. Adjustment of offset offers little gain in maximum resolution, and the 2θ adjustment leads to loss of reciprocal-space sampling, a variety of spot shapes, high background and problems with data-processing program suites. A close crystal-to-detector distance results in significant shadowing from the goniostat and extreme elongation of spots at the edge of the detector. Another option to address many of the aforementioned problems is to operate the beamline at a higher energy setting resulting in the compression of the diffraction pattern on the detector. The majority of macromolecular crystallography (MX) beamlines use Si(111) as a monochromator crystal and have an operational short wavelength limit of ≥ 0.75 Å (16 keV). With some effort it is mostly possible to extend the energy range up to $\sim$20 keV. A photon energy of 21 keV was used to collect 0.55 Å data on Z-DNA crystals (Brzezinski *et al.*, 2011), and similar high-energy settings (25 keV) were used to collect 0.48 Å data on crystals of crambin (Schmidt *et al.*, 2011). To achieve a target resolution of 0.4 Å at 16 keV would require $2\theta = 140^\circ$. Even at 19.5 keV the scattering angle would be 105°. As MX beamline end-stations are not set up for these scattering angles, it is necessary to go to higher energy, *i.e.* shorter-wavelength settings to collect ultra-high-resolution reflections in an efficient and accurate manner. To efficiently use the detector area and avoid excessive spot elongation the maximum scattering angle should be $2\theta \leq 60^\circ$, leading to a general metric where the wavelength of radiation used is equal to the desired highest resolution. In order to obtain 0.4 Å resolution data at $2\theta = 60^\circ$ one would need $\lambda = d_{\min} = 0.4$ Å (31 keV). The detector should also be placed in a position to avoid shadowing from the goniometer head and other beamline components, and to avoid unusual effects at high energy, such as beam stop fluorescence.

Table 1
Calculated flux [photons s^{-1} (0.1% bandwidth)$^{-1}$] at 10, 30, 40, 50 and 70 keV for different harmonics of the undulator tuned to 30 keV. Beamline 19-ID at the APS, undulator A, flux through primary aperture, calculated using program *SPECTRA* (version 9.0.2) (Tanaka, undated).

	Flux through primary aperture				
Undulator harmonic	10 keV Si(111)	30 keV Si(333)	40 keV Si(444)	50 keV Si(555)	70 keV Si(777)
3rd	3.77×10^{14}	5.08×10^{13}	4.78×10^{12}	6.35×10^{12}	6.96×10^{11}
4th	5.61×10^{11}	7.07×10^{13}	7.32×10^{12}	4.11×10^{12}	1.17×10^{12}
5th	3.10×10^{13}	1.03×10^{14}	3.24×10^{13}	9.67×10^{12}	8.79×10^{12}
6th	1.04×10^{14}	1.18×10^{14}	5.69×10^{13}	2.60×10^{13}	1.35×10^{13}

Table 2
Calculated flux at output of monochromator at 10, 40, 50 and 70 keV relative to the flux at 30 keV for different harmonics of the undulator tuned to 30 keV (based on incident fluxes listed in Table 1).

	Flux at monochromator output relative to 30 keV				
Undulator harmonic	10 keV Si(111)	30 keV Si(333)	40 keV Si(444)	50 keV Si(555)	70 keV Si(777)
3rd	115	1	0.060	0.028	0.0009
4th	0.123	1	0.067	0.013	0.0011
5th	4.67	1	0.203	0.021	0.0058
6th	13.7	1	0.311	0.049	0.0078

Out of the ~150 biological beamlines at synchrotrons worldwide (http://biosync.sbkb.org), only four MX end-stations are rated for standard operation at 30 keV or higher energy, with none currently operational in North America. Since the design of the monochromator at 19-ID does not allow operation at 30 keV with Si(111), one possibility is to replace the monochromator crystal with a higher-index cut, *e.g.* Si(311) or Si(400). This is impractical as it involves a multi-day procedure of warming up the liquid-nitrogen-cooled monochromator, replacing the crystals, cooling the monochromator and re-calibration of the system. A more practical option is to use a higher-index reflection of the existing monochromator crystal, allowing for operation of the beamline in the normal Bragg-angle range. For example, using the Si(333) reflection provides a 30 keV output beam at the same Bragg-angle setting as for 10 keV from the Si(111) reflection. The major disadvantage of this is lower intensity: the bandwidth of Si(333) is 0.065 relative to Si(111), 0.32 relative to Si(311) and 0.36 relative to Si(400). Another disadvantage of using Si(333) is the presence of contaminating energies at 10 keV Si(111), 40 keV Si(444) and 50 keV Si(555), whereas Si(311) or Si(400) would have a single contaminating energy at 90 keV or 60 keV, respectively, both well separated from 30 keV and, thus, more effectively rejected by a mirror.

The strongest and potentially most serious contamination is the 10 keV sub-harmonic (Tables 1 and 2). If the diffraction pattern is recorded with a photon-counting pixel array detector, 10 keV photons are not counted. However, for integrating detectors, like CCD detectors, strong low-order *h*,*k*,*l*-peaks from 10 keV photons are integrated by the same pixels as the weaker 3*h*,3*k*,3*l*-peaks from 30 keV photons. The relative contribution of the 10 keV signal on the CCD is magnified by two factors. The integrated intensity of the diffraction peaks is proportional to λ^2 and the primary quantum efficiency of the scintillator in front of the CCD is only ~15% at 30 keV compared with 94% at 10 keV. Thus, even with the three-times-higher light output per absorbed 30 keV photon the average signal per incident photon is higher for 10 keV photons than for 30 keV photons. The resulting strength, recorded by a CCD detector, of diffraction peaks arising from the contamination relative to the 30 keV component is listed in Table 3. Considering the $\sim\lambda^3$ dependence of the absorption coefficient, the 10 keV photon flux on the sample should be <1/90 of the 30 keV flux in order to keep the 10 keV dose at <1/10 of the 30 keV dose.

Table 3
Integrated intensities recorded by a CCD detector (ADSC Q315r) for the same diffraction peak generated by the 10, 40, 50 and 70 keV contaminations relative to the integrated intensity by the 30 keV component for different harmonics of the undulator tuned to 30 keV (without filters and assuming the same mirror reflectivity for all photon energies).

	Integrated intensities (in ADUs) of peaks *h*,*k*,*l* relative to 30 keV				
Undulator harmonic	10 keV Si(111)	30 keV Si(333)	40 keV Si(444)	50 keV Si(555)	70 keV Si(777)
3rd	2160	1	0.022	4.6×10^{-3}	4.5×10^{-5}
4th	2.3	1	0.024	2.1×10^{-3}	5.5×10^{-5}
5th	88	1	0.074	3.4×10^{-3}	2.8×10^{-4}
6th	257	1	0.113	8.0×10^{-3}	3.8×10^{-4}

The undulator X-ray source offers the unique ability to manipulate the emission spectrum by changing the gap between the magnets. Normally, a gap is set so that one of the peaks of the emission spectrum is at the desired photon energy. In this case, the aim is to find a gap setting that minimizes contamination at 10, 40 and 50 keV relative to the intensity at 30 keV. The standard approach would be to set the undulator gap for the third or fifth harmonic at 30 keV; however, both emission spectra show a significant contamination of 10, 40 and 50 keV harmonics (Fig. 1*a*, Table 1). A more favorable emission spectrum is achieved by moving the fourth harmonic to 30 keV, reducing the 10 keV sub-harmonic by three orders of magnitude relative to the 30 keV flux while the 40 and 50 keV harmonics are about the same or reduced (Fig. 1*b*, Table 2). The even undulator harmonics are labeled as weak, although this is only true

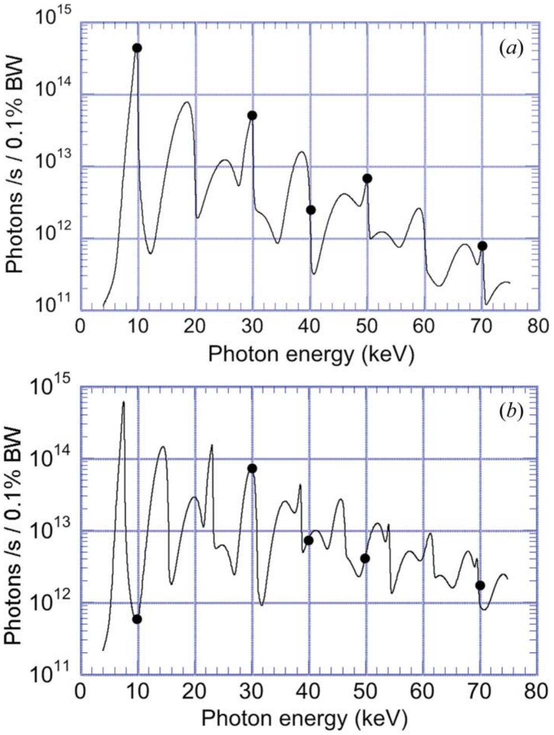

Figure 1
Emission spectra for undulator A with the gap set for (*a*) the third harmonic peak at 30 keV and (*b*) the fourth harmonic peak at 30 keV. Most noticeable is the reduction of the 10 keV emission by three orders of magnitude when selecting the fourth harmonic. Dots mark the intensities at photon energies reflected by the monochromator crystal.

J. Synchrotron Rad. (2015). **22**, 172–174 Gerd Rosenbaum *et al.* • Energy optimization of an MX beamline **173**

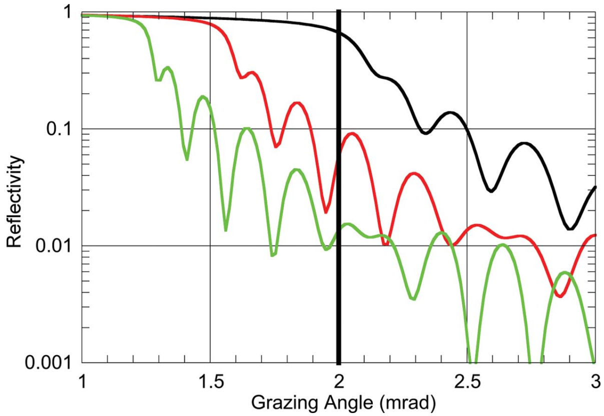

Figure 2
Calculated reflectivity of the vertically focusing mirror for 30 keV (black), 40 keV (red) and 50 keV (green) photons as a function of grazing angle of incidence (Stepanov, undated). Reflecting surface: Pd, 400 Å thick, density correction factor 0.9, vapor-deposited on a glass substrate. The rapid fluctuations of the reflectivity are not observed due to averaging over the grazing angle interval of ±0.1 mrad over the footprint of the beam. The vertical bar represents the optimal mirror angle at 2.0 mrad.

for the on-axis beam (see supporting information[1]). The intensity into the central cone is quite strong (Table 1). The even-harmonics maxima are also wider than those for the odd harmonics which gives more freedom to optimize the ratios of the contaminations to the 30 keV radiation without going too much off the maximum flux.

Reduction of the higher harmonics intensities relative to the 30 keV flux is achieved by careful setting of the grazing angle and selection of the surface coated lane of the vertical focusing mirror. Calculations of the reflectivity for 30, 40 and 50 keV radiation (Stepanov, undated) show that, given the choice of Pd or Pt as the mirror coating, Pd gives the best ratio of 30 keV *versus* 40 keV reflectivity (Fig. 2). The calculations also suggest a grazing angle of about 2.0 mrad as the best balance between reasonable reflectivity at 30 keV and good rejection of 40 and 50 keV. The actual best mirror angle has to be determined experimentally since the actual density of the vapor-deposited surface coating is different from the bulk metal density and is not exactly known.

Reduction of the strong 10 keV contamination is easily achieved with absorption filters. Aluminium, pure or common alloys, are suitable choices since there are no absorption edges between 10 keV and 30 keV and the ratio of the absorption coefficients is high. Al filters with attenuations at 10 keV between 30 (0.5 mm) and 10^4 (1.5 mm) are readily available from the filter array at most beamlines. Absorption filters will enhance the flux of the higher harmonics relative to 30 keV. Therefore, the attenuation factor should be kept at the minimum necessary for 10 keV reduction. To estimate the 10 keV contamination of the beam incident on the sample, a series of Al filters of increasing thickness were inserted and the transmitted flux measured with an ion chamber. By analyzing the decay *versus* filter thickness and accounting for the absorption coefficients of the filter material and the ion chamber gas (N_2), an estimate of the ratio of 10 keV to 30 keV fluxes can be obtained. The higher energy contaminations are too small to be estimated by this method, but they are of less concern. Knowing the 10 keV contamination of the incident beam is only a first step. Most important is the detector signal of diffraction peaks attributable to 10 keV photons, relative to those attributable to 30 keV photons. An initial estimate of the detector signal of the 10 keV component can be obtained using the uncoated lane of the mirror surface, which does not reflect 30 keV photons, and recording diffraction images without and with filters. Then, after returning the mirror to the Pd lane that reflects the 30 keV flux, the filter thickness should be gradually increased until, estimating from the decrease of the diffraction signal on the detector with increasing filter thickness, the remaining 10 keV contribution is small enough that it can be estimated well enough for correction of the diffraction data before processing. Filter thicknesses that absorb more than 30% at 30 keV (*i.e.* 1.3 mm Al) should be avoided because they noticeably enhance the higher harmonics.

Table 4
Data collection and processing statistics. Highest-resolution shell in parentheses (0.39–0.38 Å). Data were integrated and merged in *HKL3000*. A 0.75 mm-thick Al filter was inserted to reduce the 10 keV contamination.

Energy/wavelength	30 keV/0.413 Å
Temperature	10 K
Data collection settings	75 mm crystal-to-detector distance; 0.5° oscillation; 360 frames; 30 s exposure per frame; crystal volume ∼0.2 mm^3
Resolution	0.38 Å
Space group	$P2_1$
Unit cell (Å)	a = 22.32, b = 18.49, c = 40.81, β = 90.7°
Completeness (%)	97.1 (84.2)
$I/\sigma(I)$	18.2 (1.4)
R_{sym}	0.056 (0.623)
Reflections	313234 (8777)

It should be noted that accessing higher photon energies in the manner proposed here results in a greatly decreased flux and detector signal. In addition to the factors discussed above, such as the smaller bandwidth of the monochromator, the decrease of the integrated intensity of diffraction peaks with λ^2, and the low quantum efficiency of the detector at 30 keV, the undulator emission also decreases with increasing photon energy. Fortunately, at high-energy third-generation storage rings like the APS there is enough flux at high photon energies to collect ultra-high-resolution data in a reasonable time despite the less-than-optimal way of accessing the very high photon energy regime.

The 30 keV beam was tested on crystals of the small protein crambin. Crambin forms the best-ordered macromolecular crystals known, diffracting to a published resolution of 0.48 Å (Schmidt *et al.*, 2011). This setting enabled collection of a complete high-quality dataset at 19-ID at 10 K on a large (∼0.2 mm^3) crystal, yielding data to a nominal resolution of 0.38 Å and an overall R_{sym} of 5.4% (Table 4). The low temperature of data collection has the advantage of decreased B-factors and background scattering, contributing to the extraordinary resolution obtained. A detailed structural refinement is currently underway, in addition to efforts to further extend the resolution through careful data collection protocols and management of radiation damage.

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[1] Supporting information for this paper is available from the IUCr electronic archives (Reference: CN5058).