

A method to stabilize the incident X-ray energy for anomalous diffraction measurements

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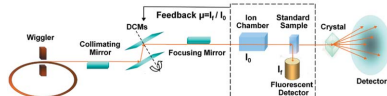
A method to calibrate and stabilize the incident X-ray energy for anomalous diffraction data collection is provided and has been successfully used at the single-crystal diffraction beamline 1W2B at the Beijing Synchrotron Radiation Facilities. Employing a feedback loop to control the movement of the double-crystal monochromator, this new method enables the incident X-ray energy to be kept within a 0.2 eV range at the inflection point of the absorption edge.

1. Introduction

Anomalous diffraction data, collected using an X-ray energy in the vicinity of an absorption edge, are routinely utilized to determine the positions of heavy atoms, and thereafter to solve the phase problem in the protein structure determination procedure (Hendrickson *et al.*, 1990). In order to maximize the anomalous signal, the peak point, which maximizes the imaginary component of the anomalous scattering factor f'' , and the inflection point, where the minimum f' is reached, are often selected for data collection (González, 2003; De la Fortelle & Bricogne, 1997). However, anomalous diffraction data collected at these two points are highly sensitive to energy instability. A small drift of energy can cause a significant change in the atomic scattering factor of heavy atoms. For example, at the K -edge of selenium a 5.6 eV increase in the incident X-ray energy results in a $5.4\times$ rise in f'' (varies from 1.2 to 6.3).

It is known that after anomalous scattering the intensities of a reflection hkl and its Bijvoet mate $\bar{h}\bar{k}\bar{l}$ are no longer equal, and the square of their deviation $[|F(+)-F(-)|^2]$ is used as the Patterson function for heavy-atom position calculations. $F(+)$ and $F(-)$ always appear in different diffraction patterns, so they are affected differently by the energy fluctuation. Thus, energy instability can cause errors in the Patterson function, and may lead to a failure in heavy-atom position calculation and structural determination.

There are many methods to reduce the error in $|F(+)-F(-)|^2$. Hendrickson *et al.* (1990) showed that symmetry-equivalent measurements of $F+$ and $F-$ can be made simultaneously on the same image if a crystal is aligned in a certain way. It is also feasible to collect data of $F+$ and $F-$ in adjacent images. That is to say, the orientation of the crystal is rotated in a $0^\circ-180^\circ-1^\circ-181^\circ-2^\circ-182^\circ$ way. The two methods above allow systematic errors of $|F(+)-F(-)|^2$ to be balanced to some extent during data collection.



Another way is to keep the energy of the incident X-rays stabilized during data collection. Evans & Pettifer (1996) used an apparatus to stabilize the incident X-ray energy that employed a back diffraction comparison method of a silicon crystal. In this paper an alternative technique exploiting the steep absorption edge of the heavy element is provided; it was successfully used at the single-crystal diffraction beamline 1W2B at the Beijing Synchrotron Radiation Facility (BSRF).

2. Description of the method

The basic design concept is based on the feedback amplification principle. As shown in Fig. 1, the absorption factor (μ) is highly sensitive to energy stability at the edge of the absorption spectrum. Therefore, the absorption edge can be used to monitor the incident X-ray energy. At a reference point

(E_0, μ_0), an increase or decrease of energy by ΔE can be detected as a rise or fall of the absorption factor by $\Delta\mu_1$ or $\Delta\mu_2$, respectively. Thus, a slight variation in energy can be significantly amplified as a detectable change of the absorption factor in the spectrum.

The arrangement of the optical elements of beamline 1W2B is shown in Fig. 2. Synchrotron beam emitted from the wiggler is reflected by the collimating mirror, the double-crystal monochromator (DCM), the focusing mirror, and incident onto the energy stabilization system. The system is composed of an ion chamber (Oxford; to record the incident X-ray intensity I_0), a heavy-element sample (abbreviated as ‘standard sample’ hereon) and a fluorescence detector (Lytle, to collect the fluorescence signal I_f). During anomalous diffraction experiments, a fraction of the incident beam is absorbed by the standard sample, transferred into fluorescence signal, and recorded by the fluorescence

detector. Apart from this small amount, most of the energy passes through the standard sample and proceeds to the normal diffraction experiment. If the absorption factor (calculated using the function $\mu = I_f/I_0$) is out of a certain range, the DCM will make a corresponding adjustment to revert to the initial energy.

The standard samples which were used to produce fluorescence signals should be prepared with different valences so as to match the absorption spectrum of the heavy element inside the crystal to be measured. For example, if we would like to stabilize the X-ray energy at the inflection point of a crystal, it should be located on the steep rising part of the absorption edge of the standard sample (unnecessarily located exactly at the inflection point of the standard sample). In this case, we can say that there is a match between the crystal and the standard sample. The

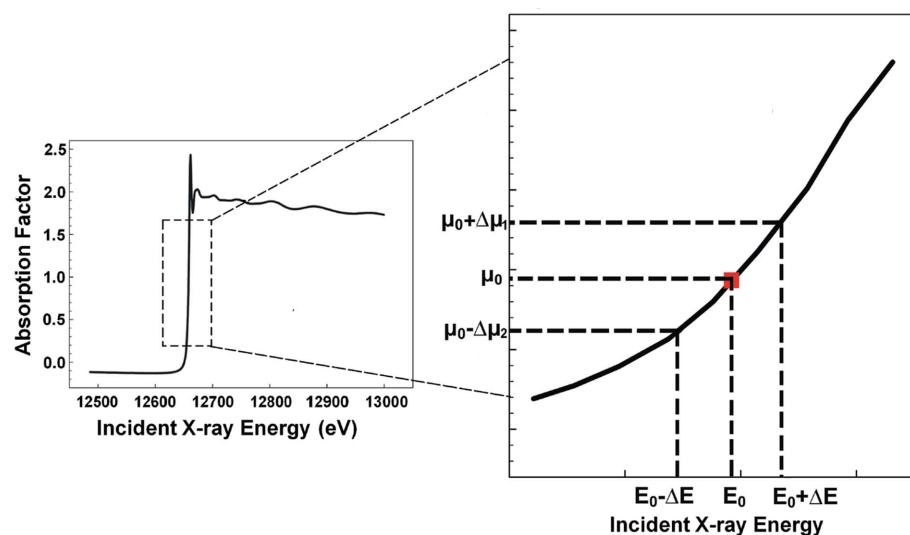


Figure 1

Schematic diagram illustrating the principle of the system for monitoring the incident X-ray energy using the inflection point of the absorption edge. At a reference energy E_0 , the absorption factor is μ_0 . A positive shift of energy, for example, to $E_0 + \Delta E$, causes the absorption factor to change to $\mu_0 + \Delta\mu_1$, while a negative shift of energy to $E_0 - \Delta E$ induces an absorption factor move to $\mu_0 - \Delta\mu_1$. Thus, the fluctuations of the incident X-ray energy can be observed and recorded as movements of the absorption factor.

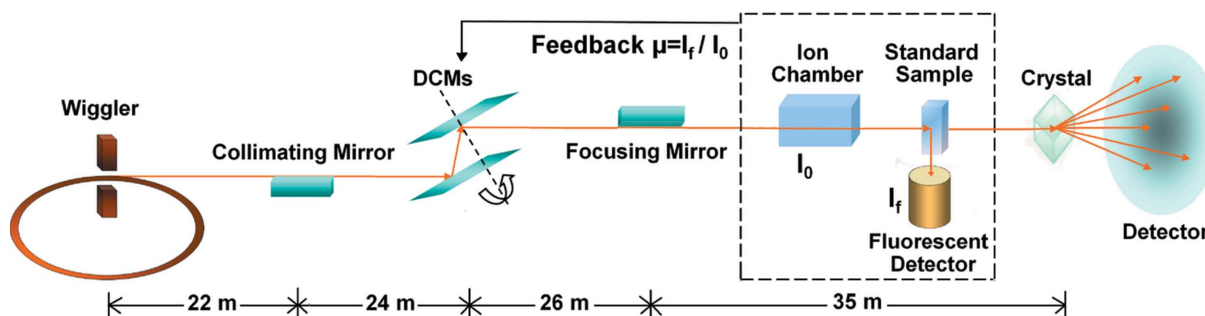


Figure 2

Schematic diagram of the system to stabilize the incident X-ray energy when performing anomalous diffraction experiments. X-rays from the synchrotron pass through the optical element of the beamline and to the energy stabilization setup, which is composed of an ion chamber, a standard sample and a fluorescence detector (shown inside the dashed box). The standard samples can be made into either films or cuvettes of solution depending on their physical state. A small part of the beam is absorbed by the standard sample, and the emitted fluorescence light is recorded by the fluorescence detector. Most of the beam passes through the energy stabilization system and proceeds to the crystal under study.

standard samples can be either in solid or liquid form. The metal samples were covered with Kapton film of thickness 100 nm. This value was optimized by balancing between yielding a sufficient fluorescence signal and maintaining a more direct light (about 3% of the incident light is lost through the film). The liquid and powder samples were encapsulated in cuvettes with mica windows. The concentration of heavy element in the solution or powder sample was 50 mM ml^{-1} , and the thickness of the standard sample was 1 mm. The standard samples were located at 45° from the incident light and the fluorescence detector in order to receive the maximum fluorescence energy. The prepared standard samples covered most of the heavy elements that are frequently used in the isomorphous replacement method (such as selenium, gold, platinum, mercury, iodine, bromine, ferrum, cuprum, *etc.*). For each element, most of its common valences were prepared (typical standard samples are listed in Table 1).

Table 1

Standard samples used for monitoring the incident X-ray energy.

Se	SeO ₂	SeO ₃	Selenocysteine
Pt	PtO	PtO ₂	
Hg	HgS	Hg ₂ Cl ₂	
Br	BrK	HBrO ₃	
I ₂	KI	NaIO ₃	NaIO ₄
Au	Au ₂ O ₃	AuCl	
Fe	FeO	Fe ₂ O ₃	Fe ₃ O ₄
Cu	CuSO ₄	Cu ₂ O	

A schematic flowchart illustrating the procedure for stabilizing the incident X-ray energy during anomalous data collection at the inflection point is shown in Fig. 3. Before data collection, the absorption spectrum of the standard samples and the crystal were collected and compared. The standard sample whose absorption spectrum matches best with that of the crystal was selected for energy monitoring. Once a certain energy E_0 at the inflection point of the absorption edge was chosen for anomalous data collection, the initial absorption factor μ_0 and the permitted range of μ ($\mu_0 - \Delta\mu_1, \mu_0 + \Delta\mu_2$) of the standard sample were calculated based on the absorption spectrum. The absorption factor μ of the standard sample is monitored continuously during the anomalous data collection procedure. If $(\mu_0 - \mu_2) < \mu < (\mu_0 + \mu_1)$, the data collection procedure would proceed, otherwise a software feedback loop to the DCM would be triggered to recover to the permitted range of μ ($\mu_0 - \Delta\mu_1, \mu_0 + \Delta\mu_2$). The programs in charge of data acquisition and DCM movements were written using *LabVIEW* (Johnson, 1997).

Similarly, there are two ways to stabilize energy at the peak point. One way is to use the peak point of the standard sample to monitor the X-ray energy [abbreviated as peak–peak monitoring, Fig. 4(a); a schematic flowchart is shown in Fig. S1A of the supporting information]. In this situation, the absorption spectrum of the standard sample should accurately match that of the heavy element inside the crystal at the peak point. The other way is to monitor the incident X-ray energy by the region around the inflection point of the standard

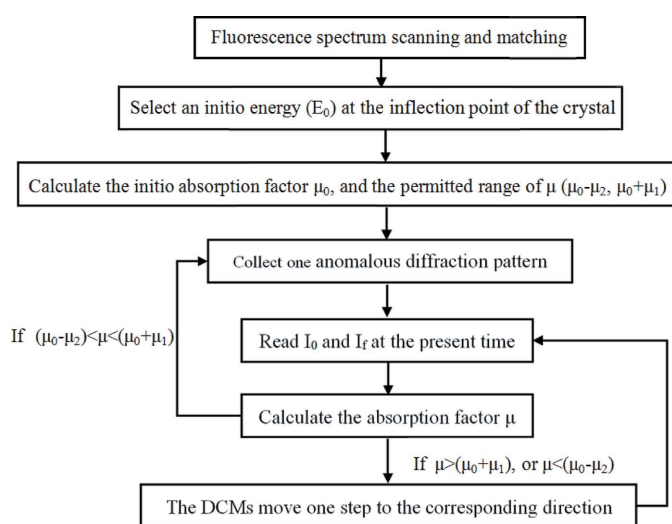


Figure 3
Flowchart of the energy stabilization system for inflection–inflection monitoring.

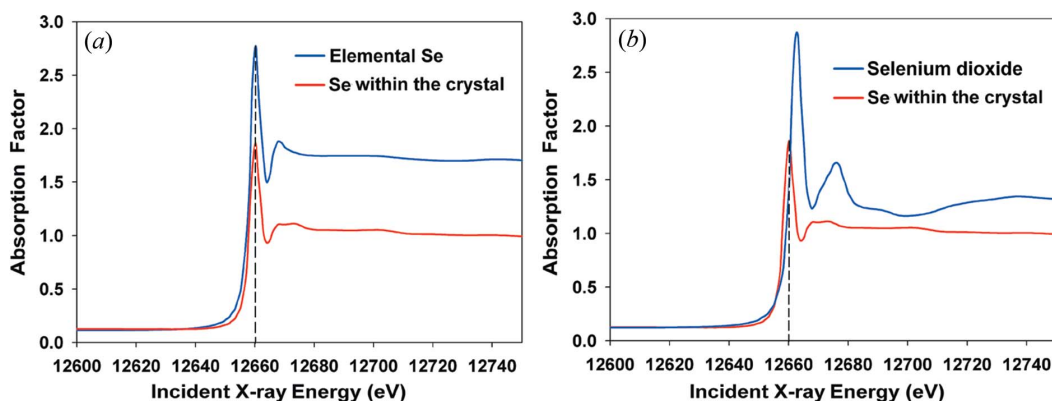


Figure 4
Schematic diagram of peak–peak monitoring and inflection–peak monitoring of the incident X-ray energy. (a) The peak point of Se within the crystal located at the peak point of elemental Se. Thus, the peak point of the standard sample (elemental Se) was used to monitor the energy at the peak point of the crystal, abbreviated as peak–peak monitoring. (b) When the peak point of Se inside the crystal is located on the steep rising part of the absorption edge of the standard sample (selenium dioxide), it is able to use the region near the inflection point of the standard sample to monitor the energy at the peak point of the crystal. This is called inflection–peak monitoring.

sample when the inflection point of the crystal is located on the steep rising part of the absorption edge [abbreviated as inflection–peak monitoring, Fig. 4(b); a schematic flowchart is shown in Fig. S1B of the supporting information]. The inflection–peak monitoring method can reach a higher energy resolution than the peak–peak monitoring method (0.2 eV *versus* 0.5 eV). However, it can work only when it is possible to find one standard sample whose inflection region coincides well with the peak region of the crystal. Sometimes this occurs for the same element with different valences. As a result, if possible, it is better to monitor the energy at the peak point with the inflection point of the standard sample.

3. Results

The apparatus described above has been used at the biological macromolecular crystallography beamline 1W2B at BSRF. Typically, the intensities recorded by the fluorescence detector and the ion chamber are both about 10^6 counts s^{-1} with a background of about 10^4 counts s^{-1} . At these count rates, a 5% change of μ can be detected precisely. Corresponding to the resolution of μ , the energy of the incident X-rays can be measured with an accuracy of 0.1 eV and 0.5 eV at the inflection and the peak point of the absorption spectrum, respectively. When working at an energy of 10 keV, the step size of the Si(111) DCM is about 0.2 eV. Taking together the energy resolution of the equipment and the step size of the DCM, the incident X-ray energy can be kept within the scope of 0.2 eV and 0.5 eV, monitored by the inflection point and the peak point of the standard sample, respectively.

Using this equipment, the energy stability of beamline 1W2B at BSRF was measured by continuous recording of the absorption factor at the inflection point of a standard elemental Se sample over the lifetime of the synchrotron. It is observed that the absorption factor fluctuates in a wave-like manner, with a maximum value of about 1.4 and a minimum value of about 0.8 (Fig. 5a, black triangles). Due to the middle section of the absorption edge (the part near the inflection point, within the range $0.8 < \mu < 1.4$) being approximately

linear (Fig. 5b), the absorption factors were further transformed into the corresponding energy of the incident beam accordingly. A total energy shift of 1.4 eV was observed over the lifetime of the synchrotron (Fig. 5c, black triangles). Beam attenuation or injection of the synchrotron induces an increase or decrease of heat loading at the monochromator, and leads to a deformation of the monochromator. Thus, the same monochromator position can select different energies at different times. This illustrates that the continuous drift in energy is mainly caused by fluctuations in the X-ray intensity. Besides the continuous drift, an abrupt change in energy can also be observed (Fig. 5c, black triangles, from 3.7 h to 4.2 h). Considering that the deformation period of the DCM should be much larger, this kind of sudden change of energy was most probably caused by unpredictable beam position movements induced by electron shaking inside the storage ring.

Further, we recorded the absorption factors of the same standard sample during the equivalent synchrotron life-time period while the DCM feedback loop is in operation. The absorption factor was confined within the range 1.1–1.2 (Fig. 5a, red squares) during measurement, corresponding to an incident X-ray energy in the range 12657.7–12657.9 eV (Fig. 5c, red squares). Thus, after using the energy stabilization apparatus, the fluctuation of the X-ray energy was ensured to a limited range of 0.2 eV, significantly superior to the energy shift of 1.4 eV observed earlier.

So far, this method has been successfully used to stabilize the energy of the incident beam when collecting anomalous diffraction data at the crystallography beamline 1W2B. For example, published single-wavelength anomalous diffraction (SAD) data of Se-Met YafQ-DinJ [Protein Data Bank (PDB) id: 4ml0] and Se-Met YafQ mutant H87Q (PDB id: 4mmg) (Liang *et al.*, 2014) was obtained with the aid of this equipment. The absorption spectrum of Se within the protein matches well with that of the elemental Se (Fig. 6a). Thus, elemental Se was chosen as a standard sample to monitor the energy when collecting anomalous data at the peak point. The absorption factor of the standard sample was controlled within the range $2.75 < \mu < 2.80$ by the equipment, and the stability of

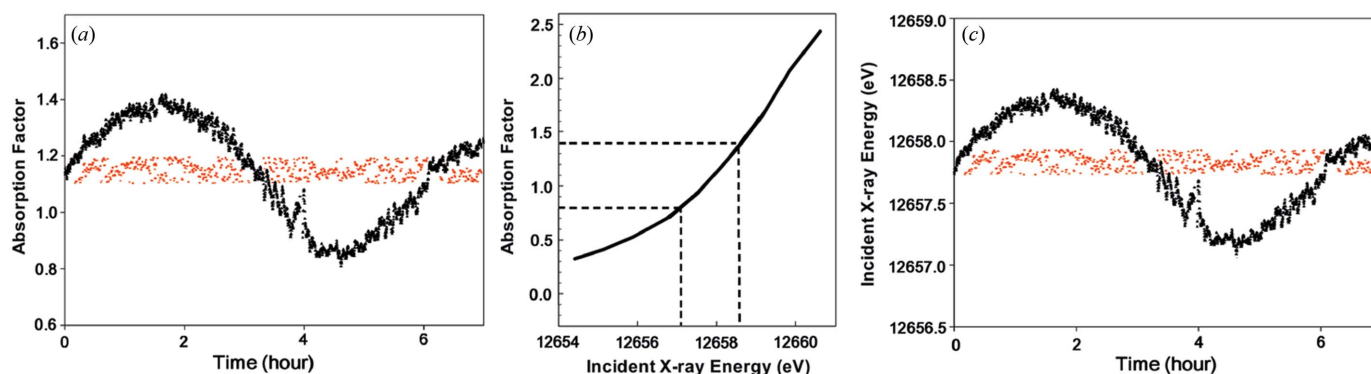


Figure 5

Comparison of the incident X-ray energy with (red squares) and without (black triangles) the feedback loop to the monochromator. (a) The absorption factor of the standard Se sample at the inflection point was measured at ~ 1 min intervals over the lifetime of the beam. (b) The absorption edge of elemental Se, by which the recorded absorption factor was converted to the energy of the incident beam. (c) Energy fluctuation of the incident beam calculated based on (a) and (b).

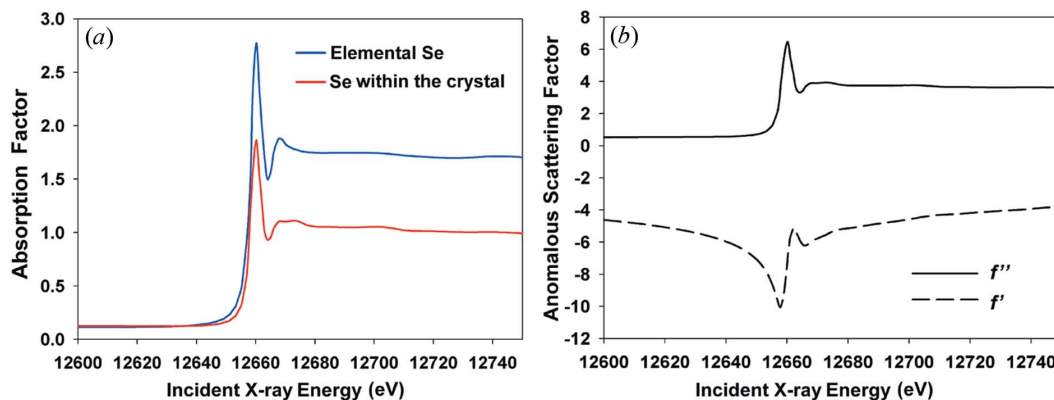


Figure 6

(a) Comparison of the absorption spectrum of Se within the protein crystal and elemental Se from the standard sample. (b) Anomalous scattering factor calculated based on the absorption spectrum of Se within the crystal.

the incident X-ray energy was kept to 0.5 eV for the duration of data collection. Thereafter, the sample's anomalous scattering factors f' and f'' , which were calculated based on the X-ray fluorescence spectra and by the program *CHOOCH* (Evans & Pettifer, 2001), were guaranteed within the ranges $-6.7 < f' < -7.3$ and $6.35 < f'' < 6.5$, respectively (Fig. 5b). If the energy has not been stabilized by the system, the maximum energy change can reach 1.4 eV, and the anomalous scattering factors f' and f'' would fluctuate in the ranges $-6.0 < f' < -8.5$ and $6.0 < f'' < 6.5$, respectively. This example illustrates the need of the system to keep the energy stable.

4. Discussion

The main properties of the X-ray beam that are essential for a successful SAD/MAD (single-wavelength anomalous diffraction/multi-wavelength anomalous diffraction) data collection include the beam size and energy resolution at the sample position, and stable beam intensity, beam position and beam energy (the beamline specifications of beamline 1W2B are shown in Table 2). The beam size and energy resolution contribute to the reflection width, and are immediately visible in the spot profile of low-resolution reflections. A large beam size and low energy resolution may introduce overlapping between reflections for crystals with very long unit cells. The energy resolution ($\leq 2 \times 10^{-4}$) and beam spot size (1.0 mm \times 0.6 mm) of 1W2B can satisfy the demand of high-precision data acquisition in MAD/SAD. Large fluctuations in the beam intensity produce variable background intensities, while large fluctuations in the beam position shifts the diffraction points positions in different diffraction patterns. These deviations, as long as they are not too large, can be corrected by routine scaling and refinement procedures using auto-indexing programs.

Unlike the items above, the energy of the incident beam was deemed to be stable by the auto-indexing programs by default, and there is no way of recognizing energy fluctuations by the scattering patterns directly, even though energy fluctuation is common during synchrotron exposures. It would be desirable, therefore, to design an instrument to stabilize the incident

Table 2

Specifications of beamline 1W2B, BSRF.

Photon energy range	5–18 keV
Energy resolution	$\leq 2 \times 10^{-4}$ [Si(111)]
Beam spot size (FWHM) at sample position	1.0 mm \times 0.6 mm (horizontal \times vertical)
Flux at sample position	10^{12} photons s^{-1}
Beam position fluctuation	≤ 10 μ m
Beam intensity fluctuation	$\leq 1\%$

X-ray energy during the exposures. In this investigation, a new method to calibrate and stabilize the incident X-ray energy has been reported and successfully utilized at the 1W2B X-ray crystallography beamline of BSRF. The method employs the inflection or peak point of the absorption spectrum to monitor the X-ray energy. It is of general use to anomalous diffraction or scattering experiments which are highly sensitive to energy stability.

In this energy stabilization system, the first priority is to select a suitable standard sample whose absorption edge coincides well with that of the crystal. Movement of the absorption spectrum depends greatly on the valence of the heavy element. The higher the valence, the further the spectra will move compared with its elemental form. Se-Met protein is mostly used to solve the phase problem in structural biology research. An excellent standard sample for Se-Met protein crystals is selenocysteine powder. It ensures that the adjacent anatomic structures of the Se element and the protein crystal are the same as that of the standard sample, and hence ensures the absolute coincidence of the absorption spectrum of the crystal and the standard sample. For anomalous diffraction experiments using a crystal containing other heavy elements, the valence of the heavy element inside the crystal is unknown. As a result, a series of standard samples with different valences should be prepared, and comparing the absorption spectrum of the crystal and that of the standard samples is essential for selecting the best match.

A significant feature of this apparatus is that it is not able to fix energies far away from the absorption edges of standard samples. Fortunately, for most synchrotron techniques including imaging, spectroscopy, normal scattering and diffraction, an energy fluctuation of 1–2 eV (meaning a 0.01–

0.02% change in energy when working at a typical synchrotron energy of 10 keV) is negligible. Adequate cooling matching the heat load of the incident X-ray beam (Kudo & Tanida, 2007; Proux *et al.*, 2006) and recalibration of the energy is sufficient to achieve the required stability in this case. On the contrary, anomalous data collected at the inflection and peak points of the absorption edge are more sensitive to energy instability. The 0.01–0.02% change of energy at the inflection point of the selenium *K*-edge can lead to an 80% change in the anomalous scattering coefficient f'' . As a result, the system is essential for stabilizing the incident X-ray energy and to obtain a more reliable anomalous scattering coefficient.

Using the instrument, the energy of the incident beam can be detected at a resolution of about 0.1 eV at the inflection point. If an accumulating counter is used to reduce the noise of the measured absorption factor, the energy resolution can be even higher. However, the smallest rotation step of the DCM at beamline 1W2B is only 0.2 eV. This indicates that the instrument has the potential to achieve even higher energy stability by using an accumulating counter and improving the DCM's rotation platform.

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