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Quick Fluorescence–EXAFS: an Improved Method for Collection of Conventional XAFS Data and for Studying Reaction Intermediates in Dilute Systems

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The quick EXAFS (QuEXAFS) technique provides an alternative way of recording X-ray absorption fine-structure (XAFS) data where the scan time is reduced by moving the monochromator at a constant angular speed and recording the data 'on the fly'. Results are presented to show that the use of fluorescence detection with QuEXAFS is eminently suitable for studying reactions in dilute systems such as metalloproteins at a sub-minute time scale. In addition, we show that the fluorescence–QuEXAFS technique can reduce the overall time for normal data collection by some 50% compared with conventional step-by-step scanning EXAFS using the same optical system, thus reducing the total X-ray exposures of the samples. The use of X-rays for studying *in situ* redox reactions is demonstrated.

Keywords: QuEXAFS; XAFS; radiation damage; metalloproteins.

1. Introduction

Extended X-ray absorption fine structure (EXAFS) is one of the very few spectroscopic techniques which inherently contain structural information about an atomic centre such as a metal centre in metalloproteins, quite often the site of enzymatic reactions. Thus, the technique has major potential for providing structural interpretation of kinetic data on a variety of chemical and biochemical systems.

Measurements of EXAFS put stringent demands on instrument stability, as the EXAFS signal is small and the amplitude reduces as a function of k^2. For biological systems, the predominance of weak backscattering atoms further increases the demands on instrument perfo- nance. In order to collect a spectrum with a large enough spectral range to allow quantitative analysis, it is generally necessary to collect data to more than 10 $Å^{-1}$ with a signal-to-noise ratio (S/N) > 2.

One method of collecting time-resolved data is to adopt the dispersive EXAFS geometry (EDE), in which a wide band of X-ray energies, dispersed in angle, are focused on the sample (Matshushita & Phizackerley, 1981; Lee *et al.*, 1994). The beam, which is transmitted through the sample, subsequently disperses and is measured by a linear position-sensitive detector. This currently allows complete EXAFS spectra on concentrated systems (50–100 mM) to be collected in as short a time as a few ms and is expected to become shorter with third-generation sources and faster electronics. Many samples, however, cannot be prepared to these high concentrations, necessary for achieving sufficient absorption contrast at the edge to allow data of high enough quality to be recorded, so methods based on X-ray fluorescence (Jaklevic *et al.*, 1977; Hasnain, Quinn, Diakun, Wardell & Garner, 1984) must be used. In conventional EXAFS, step-by-step measurement of dilute systems with fluorescence detection is adopted. Multi-element solid-state detectors have recently become available which are capable of high count rates (*ca* 3–5 × 10^4 counts s^{-1}) and have an energy resolution of *ca* 3% in the X-ray region (4–25 keV). This resolution is sufficient to select only the X-ray fluorescence signal of interest and reject scattered X-rays and fluorescence from the neighbouring elements in the Periodic Table. Such detection techniques, combined with focusing optics (van der Hoek *et al.*, 1986) on a second-generation source such as SRS, make it possible to measure fluorescence EXAFS on samples with concentrations as low as 0.5 mM (Corrie, Evans, Hubbard, Strange & Hasnain, 1991).

The speeding up of the conventional scanning method has been achieved by eliminating the overheads associated with moving the monochromator on a point-by-point basis, *i.e.* having to wait whilst the monochromator moves and whilst it mechanically settles from the acceleration/deceleration of the move. If the monochromator is scanned at constant speed, these overheads disappear. It is then important to synchronize the data collection to the monochromator movement by recording the fluorescence at regular time intervals. The application of transmission QuEXAFS (Frahm, 1989, 1991) and a preliminary study on fluorescence–QuEXAFS have been reported elsewhere (Dobson, Hasnain, Neu, Ramsdale & Murphy, 1993). Here, we demonstrate the potential of the fluo-

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rescence–QuEXAFS technique with two metalloproteins, transferrin and plastocyanin, where the metal ion concentration is as low as 2.5 m*M*.

XAFS studies on a number of different metalloproteins have been previously undertaken using the step-by-step method with fluorescence detection [see reviews by Cramer & Hodgson (1979) and Hasnain (1987)]. These experiments have provided valuable information on the nature of metal binding sites in metalloproteins. In the case of transferrins, XAFS has been used to probe the stereochemistry of the iron binding sites (Garratt, Evans, Hasnain & Lindley, 1991; Neu, Strange, Hasnain, Lindley & Evans, 1991). However, it is still not known how the metal reaches the binding site which lies buried approximately 15 Å deep in the interdomain cleft. To investigate a possible mechanism for metal absorption we have previously studied the non-physiological reaction intermediate of diferric human serum transferrin (Fe_2HST) with cyanide (Swope, Chasteen, Weber & Harris, 1988) in a pseudo-time-resolved mode. The reaction of cyanide with Fe_2HST is quite slow, taking 12 h to remove iron from the C-lobe at room temperature. The reaction intermediate, formed *ca* 40 min after mixing, has a very distinct XAFS and electron paramagnetic resonance spectrum and is expected to represent one of the initial iron binding sites upon its uptake. This intermediate can be stabilized by freezing, and studies have been performed on intermediates frozen at different times after mixing. Here, we study the *in situ* reaction of cyanide with transferrin using the fluorescence–QuEXAFS method of data collection.

The copper-containing metalloprotein plastocyanin has also been the subject of a number of XAFS studies (Scott, Hahn, Doniach, Freeman & Hodgson, 1982; Penner-Hahn, Murata, Hodgson & Freeman, 1989; Murphy, Hasnain, Strange, Harvey & Ingeldew, 1991). As a redox protein, plastocyanin readily cycles between the Cu^{I} and Cu^{II} oxidation states (Adman, 1985). Here we have examined the use of X-rays for studying this reaction at room temperature during QuEXAFS data collection. We show that the autoreduction of these redox proteins requires less than one X-ray photon per molecule at room temperature.

We have also collected data on plastocyanin at *ca* 80 K using both QuEXAFS and normal step-by-step EXAFS scanning modes in order to quantify the benefit of the fluorescence–QuEXAFS method for obtaining normal XAFS data on dilute systems. We show that for high-quality XAFS data for a dilute metalloprotein, there is a net saving of *ca* 50% in data-collection time using the QuEXAFS approach.

2. Experimental

2.1. *Instrumentation*

Data were collected using the EXAFS station 9.3 on the 5 T wiggler beamline at the Daresbury SRS. The station is equipped with a Canberra 13-element Ge solid-state detector with electronics developed in-house to optimize data throughput rate, allowing the detection system to be linear to 3×10^4 counts s^{-1} (Derbyshire *et al.*, 1992). The station has a 1 m long fused silica plane mirror that is bent to provide vertical focusing. The mirror is positioned at the 1:1.3 position in the beamline giving rise to a vertical focus of 400 μm FWHM. A flux of *ca* 10^{10} photons s^{-1}/200 mA impinges on a 10×0.4 mm^2 sample (as there is no horizontal focusing, higher flux is available for larger samples). The angle of incidence onto the mirror can be varied to allow the cut-off due to the critical angle of the mirror to be used to suppress higher harmonics from the monochromator. The silicon (220) monochromator, positioned 2 m before the sample position, is a two-crystal design with the first crystal directly water-cooled [similar to the monochromator on SRS station 8.1 (van der Hoek *et al.*, 1986)]. The second crystal is a long flat crystal positioned in the 'channel-cut' position, so the monochromator can be moved through its complete working range with the diffracted beam from the first crystal 'walking' up and down the second crystal. The second crystal is, however, mounted on three piezoelectric Inchworm (Burleigh Instruments) drives which have a travel of 25 mm with a smallest step increment of 2 nm. These are used to align the two crystals so that the crystal planes are parallel. The second crystal is then slightly offset in angle to improve harmonic rejection. The perfection of the second crystal, the possibility of exactly aligning the two crystals and the stability of the Inchworm drives and the remaining monochromator assembly allow the monochromator to be operated without active servoing of the second crystal position. This is important for QuEXAFS applications as servo systems generally introduce a settling time to the system which would introduce noise into a QuEXAFS scan. Finally, the monochromator is driven by a d.c. servo motor controlled by the output from a Heidehain ROD800 encoder, capable of encoding the Bragg angle position to 0.1 mdeg. The servo control system not only controls the static stability of the system, but can be optimized to perform scans at constant angular speed. The advantage of this system over previously reported systems using stepper motors for QuEXAFS experiments is that the d.c. motor can slew at much higher speeds (1° s^{-1}), whilst still achieving a positional accuracy of ± 0.1 mdeg. Stepper motor systems have a further disadvantage in that they move in a series of small sharp steps. When the monochromator moves, a series of small high-frequency pulses are created which can disturb a monochromator and introduce intensity fluctuations in the monochromator output. The system was further improved, prior to acquisition of the plastocyanin data but after the transferrin data were collected, by the addition of a further encoder on the actual motor drive shaft. This creates a velocity signal which the modified d.c. servo control system uses for more accurate control of the angular speed, whilst still retaining the high accuracy of the ROD800 Bragg-angle encoder.

The monochromator scans are controlled by standard data-acquisition software which has been modified to incorporate QuEXAFS scans. Switching between

QuEXAFS and EXAFS data-collection modes is entirely software-controlled as the monochromator is constructed so that the same drive system can operate either at constant angular speed or step by step. Thus, the same optical system is used for both operational modes. The total time for the scan and the angular step per data point can be selected, otherwise the scan uses the usual parameters for a scan such as angular range *etc*. The software ensures that the monochromator is positioned before the beginning of the scan to allow it time to accelerate to full angular speed. Data are recorded at constant time intervals (corresponding to angular range intervals) as the monochromator scans through the required range. The software then slows the monochromator in a controlled deceleration before resetting it.

For the transferrin and $FeCl_3$ samples, the fluorescence detector was used in the reflectance geometry due to insufficient time available for the changeover. For the later studies on plastocyanin, however, the fluorescence detector was mounted horizontally at 90° to the incident beam, a geometry suitable for such fluorescence measurements, as scatter from the sample is minimized in this geometry due to the polarization of the X-ray beam. For both samples the detector count rates per single element did not exceed more than 12 000 counts s^{-1}, with typical fluorescence ratios F_{edge}:$F_{pre\text{-}edge}$ of *ca* 13:1.

2.2. *Sample preparation*

Human serum transferrin (HST) was purchased from SERVA (Heidelberg) and used without further purification. Iron-free HST in 50 m*M* [*N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid)] (HEPES) buffer, pH 6.3, 20 m*M* $NaHCO_3$ was loaded to 45% iron saturation through the addition of bis(nitrilotriacetato)ferrate(III) solution (Fe-NTA), then dialysed against 0.1 *M* $NaClO_4$, pH 6.8, a further four changes of H_2O, and then a further three changes of 50 m*M* HEPES, pH 6.8. This method selectively loads only the C-lobe of HST with iron to give the species Fe-cHST. A 2 *M* NaCN solution was adjusted slowly to pH 10.4 with 1 *M* HCl and then equal volumes of Fe-cHST and 2 *M* NaCN were mixed *in situ* by means of a stopped flow cell at the beginning of the QuEXAFS scan to give CN-Fe-cHST. The final iron concentration was *ca* 2.5 m*M*. Spinach plastocyanin (Pc_s) was isolated using the acetone extraction method (Boulter, Haslet, Peacock, Ramshaw & Scawen, 1977). Lyophilized Pc_s was dissolved in 20 m*M* HEPES buffer, pH 8.0. One half of this sample was placed in an EXAFS cell and frozen in liquid nitrogen for data collection at *ca* 80 K using a liquid-nitrogen cryostat. The other half was placed in an EXAFS cell for data collection at room temperature. The copper concentration in all the Pc_s samples was 5 m*M*.

The transferrin and $FeCl_3$ data were recorded at the Fe *K*-edge and plastocyanin at the Cu *K*-edge. The counting time per step for equivalent energies for QuEXAFS and EXAFS scans on plastocyanin were identical. For each sample individual XAFS data sets were calibrated, weighted and summed using the Daresbury Laboratory program *EXCALIB* (Morrell *et al.*, 1989). Background subtraction and normalization was performed using the Daresbury Laboratory program *EXBACK* (Morrell *et al.*, 1989). The *S/N* analysis was performed using the Daresbury program *NOISE* (Dent, Stephenson & Greaves, 1992).

3. Results

3.1. *Quality of fluorescence–QuEXAFS data*

For oxidized Pc_s, pH 8.0, at *ca* 80 K, data were collected both in the QuEXAFS and conventional step-by-step EXAFS modes under the same experimental conditions. Fig. 1(*a*) shows EXAFS and QuEXAFS data collected over essentially identical total scan times (*ca* 250 min scan time). The QuEXAFS data set is the sum of 25 scans each taking 10 min (*i.e.* 250 min total summed scan time). The

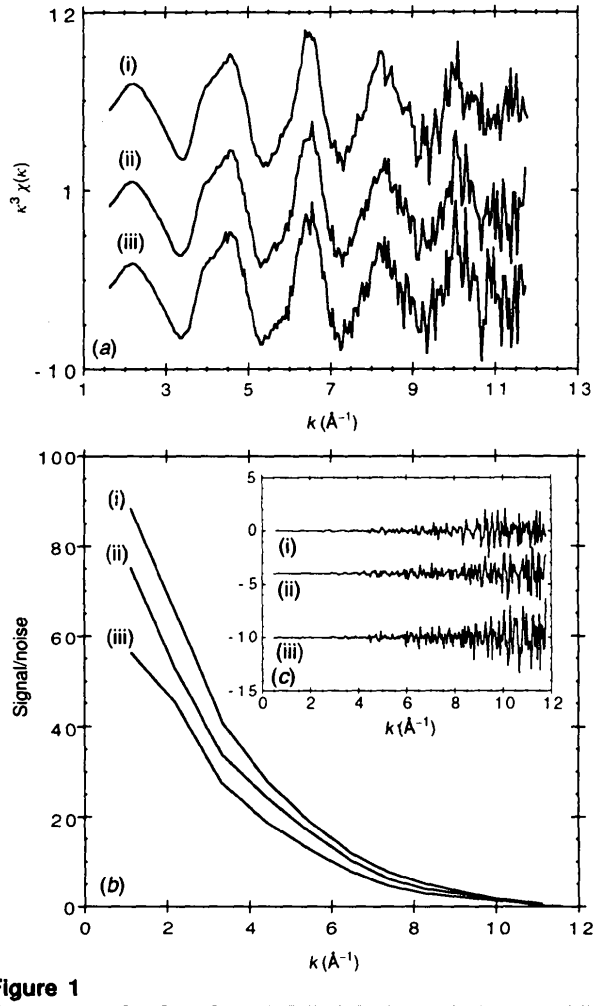

Figure 1
Cu *K*-edge QuEXAFS and EXAFS data of 5 m*M* oxidized plastocyanin, pH 8.0, at *ca* 80 K. (*a*) The k^3-weighted raw data, (*b*) the variation of *S/N* with k and (c) noise spectra extracted from the raw data (see text). In each case, (i) is the sum of 25 scans of QuEXAFS data collected over a total summed scan time of 250 min, (ii) is the sum of 25 scans of EXAFS data collected over a total summed scan time of 425 min, and (iii) is the sum of 15 scans of EXAFS data collected over a total summed scan time of 255 min.

EXAFS data set is the sum of 15 scans each taking 17 min (*i.e.* 255 min total summed scan time). The total summed scan time is defined as the real time for which the sample is in the X-ray beam and data being collected. The variations of *S/N* as a function of *k* for the two spectra are shown in Fig. 1(*b*), and the noise spectrum extracted from the Fourier filtering (0–6 Å) of the EXAFS data sets is shown in Fig. 1(*c*). The quality of the QuEXAFS data is demonstrably superior to that of the EXAFS data for 'identical' total summed scan times.

In Fig. 1(*a*) we also present EXAFS data for Pc_s, pH 8.0, which result from the same number of summed individual scans as recorded for the QuEXAFS data; the actual data-collection time per point is the same but the overheads differ. The EXAFS data set is the sum of 25 scans each taking 17 min (*i.e.* 425 min total summed scan time). In Fig. 1(*b*) the variation of *S/N* as a function of *k* for this data is also shown together with the noise spectrum extracted from Fourier filtering (0–6 Å); the EXAFS data set is presented in Fig. 1(*c*)(ii). The QuEXAFS data set has better *S/N* at low and medium *k*. The *S/N* ratios for these data sets converge at *ca* 10.1 $Å^{-1}$. The EXAFS data set has $S/N \geq 2$ up to 9.9 $Å^{-1}$. The quality of the QuEXAFS data set is still better than that of the EXAFS data set at most *k* values, even for an identical data-collection time per energy point. The reason for this improvement is unclear but may be due to a better averaging of the non-statistical variations in instrumental noise in the QuEXAFS method compared with the step-by-step data collection.

3.2. *Use of X-rays for studying redox reactions: autoreduction of plastocyanin*

The autoreduction at room temperature of oxidized Pc_s, pH 8.0, during repeated QuEXAFS scans was observed [see Fig. 2, where the XANES (X-ray absorption near-edge structure) of the first and last individual QuEXAFS scans are compared]. The individual scan time for these XANES scans was 68 s. The sample colour was bleached after 250 min of X-ray exposure, leaving a colourless band in the centre of the solution where the beam was hitting. The experimental set-up was not optimized for high-energy resolution, therefore the sharp feature often observed at *ca* 8983–8984 eV for samples in the copper(I) state (Kau, Spira-Solomon, Penner-Hahn, Hodgson & Solomon, 1987; Blackburn *et al.*, 1989) is observed here as a broad shoulder. However, the characteristic edge shift arising from the reduction of copper is clearly observed. The QuEXAFS scan recorded after a total of 220 min exposure to X-rays was identical to all subsequent scans, therefore indicating that the reduction was complete. The average flux actually hitting the sample was calculated as 2.8×10^{10} photons s^{-1} for a 10 × 2 mm beam. The total X-ray dosage required to reduce the 5 m*M* plastocyanin solution was therefore 1.85×10^{22} photons mol^{-1} Pc_s.

The course of autoreduction at room temperature has not been established quantitatively before. The high time resolution of our experimental set-up compared with the time course of the redox reaction has allowed us to define this accurately. The QuEXAFS set-up described here is capable of studying these reactions at much faster time scales and is primarily limited by the flux density on the sample. However, the full speed of the instrument and the minimum scan time (a few seconds) cannot be fully utilized without significant improvement in data throughput of the fluorescence detector.

3.3. *Rapid recording of fluorescence–QuEXAFS*

Fig. 3 shows the results of a 300 s scan of CN-Fe-cHST at an iron concentration of 2.5 m*M*. The protein was mixed with NaCN at the beginning of the scan, so this represents the first room-temperature recording

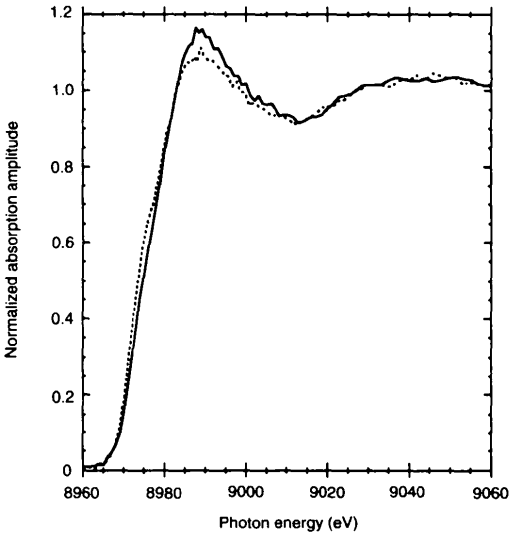

Figure 2
QuEXAFS scans of the Cu *K*-edge of 5 m*M* plastocyanin, pH 8.0, at room temperature showing X-ray induced reduction of the Cu atom. The oxidized data are shown by the solid line. After 220 min exposure to the X-ray beam the Cu atom is fully reduced, as shown by the dashed line. The scan time for the individual scans was 68 s.

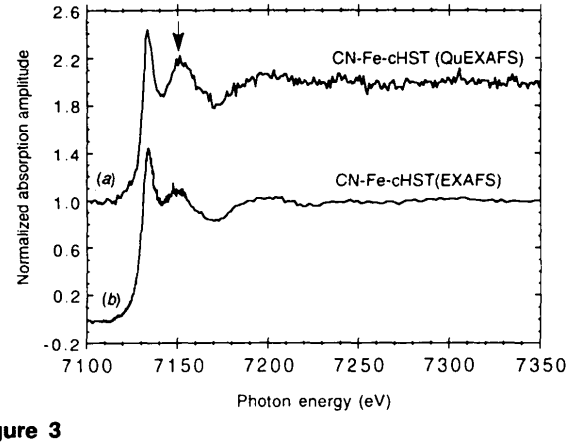

Figure 3
Fe *K*-edge XAFS of 5 m*M* CN-Fe-cHST (see text); (*a*) data recorded over 300 s using QuEXAFS scanning and (*b*) data recorded over 1 h using conventional EXAFS scanning.

of the transient state of chelation described above. The feature marked is characteristic of cyanide ligation to iron (Edwards, Garner & Stedman, 1989). Fig. 3 also shows the conventional step-by-step EXAFS scan of the CN-Fe-cHST complex, which took 1 h to collect. The two spectra of the CN-Fe-cHST complex are at slightly different stages of the reaction as assessed by the intensity of the CN^- resonance.

Fig. 4 shows the fluorescence EXAFS spectrum for 10 m*M* $FeCl_3$ solution at room temperature. The scan time was 30 s and the data were recorded every 30 mdeg. The spectrum clearly shows the EXAFS oscillations and represents the fastest scan that produced acceptable *S/N* in the data at these concentrations. With a fully optimized detector geometry, however, it would be expected that the scan time could be further reduced by a factor of two.

4. Conclusions

The results presented here demonstrate that the QuEXAFS technique, when combined with fluorescence detection, offers particular advantages to dilute chemical/biochemical systems. Not only is the technique applicable for the study of reaction intermediates, but it also has the advantage of reducing the X-ray exposure time over the conventional step-by-step method to collect data of equal quality. The prerequisites for such a technique are a suitable stable monochromator design, d.c. servo motor drive of the Bragg angle, sensitive fluorescence detectors and high photon flux.

Data collection on dilute biological systems is generally very time consuming; therefore, a method which significantly reduces the data-acquisition time is an important development. We have demonstrated here that fluorescence–QuEXAFS is capable of reducing the time by nearly half over conventional step-by-step EXAFS data collection. This is due to the increased number of QuEXAFS scans that can be performed in a given time period compared with EXAFS scans, where the actual data-collection time per point is the same but the overheads differ. Fluorescence–QuEXAFS offers a reduction in data-collection time and thus helps to reduce the X-ray exposures of the sample.

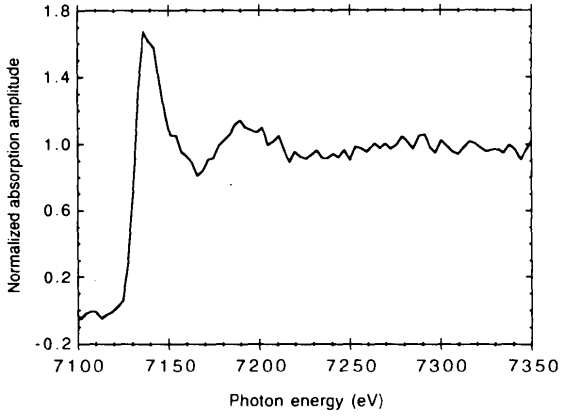

Figure 4
Fe *K*-edge QuEXAFS spectrum of 10 m*M* $FeCl_3$ solution. The scan took 30 s with the data collected at 30 mdeg intervals.

The instrumentation described here has been used to obtain acceptable XANES data on dilute systems in as short a time as 30 s. Many biological systems can only be studied as very dilute samples, hence energy-dispersive fluorescence detectors are generally working well within their linear range. Therefore, the main limitation to time resolution is the X-ray flux available to ensure good *S/N* ratios at high scanning speeds. A new experimental station, Ultra-dilute spectroscopy 16.5, is being commissioned at the SRS for EXAFS which will provide at least an order of magnitude more flux for elements with $Z \geq 25$, while proposed developments with focusing optics on the original EXAFS station at the SRS 7.1 would provide a factor of 60 increase for $Z \leq 25$ (Hasnain, Dobson, Harvey & Linford, unpublished results). Such facilities, when established on the much more powerful third-generation multi-GeV sources, *e.g.* ESRF, APS and SPring-8, will greatly advance the potential of this technique for studying the environment of metal centres in dilute chemical and biochemical systems, enabling reaction intermediates to be studied on a realistic time scale.

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References

Adman, E. T. (1985). *Top. Mol. Struct. Biol.* **6**, 1–42.
Blackburn, N. J., Strange, R. W., Reedijk, J., Volbeda, A., Farooq, A., Karlin, K. D. & Zubieta, J. (1989). *Inorg. Chem.* **28**, 1349–1357.
Boulter, D., Haslet, B. G., Peacock, D., Ramshaw, J. A. & Scawen, M. D. (1977). *Int. Rev. Biochem. Plant Biochem.* II, **13**, 1–40.
Corrie, A. R., Evans, M. C. W., Hubbard, J. A. M., Strange, R. W. & Hasnain, S. S. (1991). *X-ray Absorption Fine Structure*, edited by S. S. Hasnain, pp. 178–180. Chichester: Ellis Horwood.
Cramer, S. & Hodgson, K. O. (1979). *Prog. Inorg. Chem.* **25**, 1–39.
Dent, A. J., Stephenson, P. C. & Greaves, G. N. (1992). *Rev. Sci. Instrum.* **63**, 856–858.
Derbyshire, G. E., Dent, A. J., Dobson, B. R., Farrow, R. C., Felton, A., Greaves, G. N., Morrell, C. & Wells, M. P. (1992). *Rev. Sci Instrum.* **63**, 814–815.
Dobson, B. R., Hasnain, S. S., Neu, M., Ramsdale, C. A. & Murphy, L. M. (1993). *Jpn. J. Phys.* **32**(2), 192–194.
Edwards, B., Garner, C. D. & Stedman, G. (1989). *Physica B*, **158**, 223–224.
Frahm, R. (1989). *Rev. Sci. Instrum.* **60**, 2515–2518.
Frahm, R. (1991). *X-ray Absorption and Fine Structure*, edited by S. S. Hasnain, pp. 731–737. Chichester: Ellis Horwood.
Garratt, R. C., Evans, R. W., Hasnain, S. S. & Lindley, P. F. (1991). *Biochem. J.* **280**, 151–155.
Hasnain, S. S. (1987). *Life Chem. Rep.* **4**, 273–331.
Hasnain, S. S., Quinn, P. D., Diakun, G. P., Wardell, E. M. & Garner, C. D. (1984). *J. Phys. E*, **17**, 40–43.
Hoek, M. J. van der, Werner, W., Vanzuylen, P., Dobson, B. R., Hasnain, S. S., Worgan, J. S. & Luijckx, G. (1986). *Nucl. Instrum. Methods*, **246**, 380–384.

Jaklevic, J., Kirby, J. A., Klein, M. P., Robertson, A. S., Brown, G. S. & Eisenburger, P. (1977). *Solid State Commun.* **23**, 679–682.

Kau, L., Spira-Solomon, D., Penner-Hahn, J. E., Hodgson, K. O. & Solomon, E. I. (1987). *J. Am. Chem. Soc.* **109**, 6433–6442.

Lee, P. L., Beno, M. B., Jennings, G., Ramanathan, M., Knapp, G. S., Huang, K., Bai, J. & Montano, P. A. (1994). *Rev. Sci. Instrum.* **65**, 1–6.

Matshushita, T. & Phizackerley, R. P. (1981). *Jpn. J. Appl. Phys.* **20**, 2223–2228.

Morrell, C., Baines, J. T. M., Campbell, J. W., Diakun, G. P., Dobson, B. R., Greaves, G. N. & Hasnain, S. S. (1989). *EXAFS Users' Manual.* Daresbury Laboratory, Warrington, UK.

Murphy, L. M., Hasnain, S. S., Strange, R. W., Harvey, I. & Ingeldew, W. J. (1991). *X-ray Absorption and Fine Structure*, edited by S. S. Hasnain, pp. 152–155. Chichester: Ellis Horwood.

Neu, M., Strange, R. W., Hasnain, S. S., Lindley, P. F. & Evans, R. W. (1991). *X-ray Absorption and Fine Structure*, edited by S. S. Hasnain, pp. 142–145. Chichester: Ellis Horwood.

Penner-Hahn, J. E., Murata, M., Hodgson, K. O. & Freeman, H. C. (1989). *Inorg. Chem.* **28**, 1826–1832.

Scott, R. A., Hahn, J. E., Doniach, S., Freeman, H. C. & Hodgson, K. O. (1982). *J. Am. Chem. Soc.* **104**, 5364–5369.

Swope, S. K., Chasteen, N. D., Weber, K. A. & Harris, D. C. (1988). *J. Am. Chem. Soc.* **110**, 3835–3840.