Resonant elastic X-ray scattering in life science, chemistry and materials science; recent developments

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Abstract. The applications of anomalous scattering for locating metal atoms and discriminating between different elements has increased when optimised with synchrotron X-radiation. A distinct trend now is the introduction of in vacuum diffractometers to serve both the life and physical sciences. Examples of applications are given. Synchrotron source upgrades will allow nanosized X-ray beams to be more widely available. The new X-ray lasers suggest new capabilities too; first applications are described and new ones suggested involving harnessing heavy atom complexes for structure determination.

1. Introduction

Anomalous scattering results for locating metal atoms and discriminating between different elements has increased and found wide application in life science, chemistry and materials science from the optimisation via X-ray wavelength tuning with synchrotron radiation. The polarisation state of the synchrotron X-ray beam also allows dichroism effects to be explored, although the range of applications is less extensively developed as yet. The applications of resonant elastic X-ray scattering include the crystallography of microporous materials, superconductors and magnetic materials as well as in the biological chemistry of metalloproteins within life science.

With inorganic and materials chemistry, the number of wavelengths used is increasing and the ease of measuring diffraction data to usual chemical crystallography diffraction resolution standards has improved, even with longer wavelengths. Various instrumental and technical capabilities and case studies are described in [1]; a recent example reviewed there involved use of eleven X-ray wavelengths, each with diffraction data recorded to a usual, high, standard (i.e. at 0.84Å resolution) to study a zinc substituted gallophosphate, still a record it seems [2]. This ease of measurement of the
resonant scattering signals along with absorption edge shifts indicates an expansion to measurement of multiple data sets to allow monitoring of redox changes, as a function for example of pH or temperature, which has already been done [2] and can expand. Whilst crystal structure determination in biological crystallography has been revolutionised by the synchrotron MAD (Multiple-wavelength Anomalous Dispersion) method [for a review see ref 3 and an example of MAD data measured in minutes see ref 4] MAD is not a requirement for chemical or materials crystallography as other phasing techniques are routine i.e. such as direct methods using single wavelength, non-resonant condition, diffraction data. Further applications to powders, solutions, thin films and amorphous materials indicate a buoyant contribution of Resonant Elastic X-ray Scattering (REXS) to chemistry and materials characterisation of atomic sites. Developments have included the use of high photon energies (upto 100 keV) as well as softer X-rays (2keV). The REXS 2011 article by the author with colleagues [1] reviewed these developments in detail. This article reviews developments and results from 2011 until now, and provides a short overview in the introduction. Other topics to highlight are:- applications of resonance diffraction to proteins and small molecules, including diffraction anomalous fine structure (DAFS) [5]; development of instrumentation and methods for MAD at various synchrotron radiation (SR) facilities [6]; and applications to small molecules and proteins, and an overview of complementary techniques [7].

A distinct trend now is the introduction of in vacuum diffractometers to serve both the life and physical sciences. Examples of applications are given. This trend, a natural one, in a way stems from the previous failure to enhance the anomalous dispersion due to difficulties reaching the longer wavelength absorption edges (see section 2); in chemical crystallography this still leaves the geometric limit of the diffraction resolution being never being able to be better than $\lambda/2$ even at full back scattering ($2\theta=180$ degrees) i.e. by Bragg’s Law. A different technical frontier involves the new X-ray lasers and totally new capabilities beckon especially involving femtosecond time-resolved structural studies; first applications are described and new ones suggested involving harnessing heavy atom complexes to assist structure determination.

2. When do resonant scattering diffraction techniques fade or fail in capability? What is the remedy?

There are several situations where REXS techniques in crystallography are restricted or even fail. These are as follows:-

(i) Cases where lower atomic number metals are of interest (e.g. titanium which has a K-edge at 2.497 Å); even back scattering measurements of the X-ray reflections will ‘only’ yield 1.25 Å
resolution); an alternative solution to obtaining site specific titanium occupancies has
harnessed neutron diffraction of specifically substituted Ti isotopes in silicalite [8]

(ii) The case of even lower atomic number e.g. aluminium (7.948 Å) is interesting; $^{27}$Al Nuclear
Magnetic Resonance (NMR) studies of aluminosilicates and site-specific substitution are
briefly reviewed in the context of REXS in [7];

(iii) Special apparatus for softer X-rays work, up to and including the phosphorus K edge at
5.784Å, was pioneered at the European Synchrotron Radiation Facility (ESRF) by H.
Stuhrmann et al (reviewed in [9] and an in vacuum diffractometer, for macromolecular
crystallography, is being built at Diamond Light Source by A Wagner et al, for example [see
www.dls.ac.uk].

(iv) Radiation damage effects, as with all X-ray studies, can be a limitation or a challenge. A
biological samples challenge is as exemplified in the photosynthesis protein ‘PSII’ studies
[10]. In ASAXS (anomalous small angle X-ray scattering) studies of soft matter materials
science radiation damage effects must be anticipated and minimised [11]. In our own previous
research involving a nickel aluminophosphate we observed an X-ray radiation damage effect,
admittedly after prolonged irradiation of 1 week at the National Synchrotron Light Source
(NSLS) [12] and the impact of X-rays on disulphide bridges in proteins [13] is now well
known.

3. Some future, general, trends
Dichroism effects are exploited already but will likely expand in the number of studies undertaken in
the coming years, as appropriate software becomes routinely available [14]. Chemical changes
induced by temperature and/or time-resolved studies should also increase the range and scope of these
REXS applications [15]. In particular as the speed of measuring REXS improves, as more REXS
capable instruments are available and as analysis is more routine then increasing numbers of samples
will be studied per molecular system. Perhaps even sample to sample variations will be allowed for,
although unlikely to be a major concern in general, but maybe of interest in specific cases. Protein
powder diffraction harnessing dispersive differences is also under detailed investigation [16 and
references therein]. A major initiative at 3rd generation SR sources, such as the ESRF Upgrade
programme [see www.esrf.eu], emphasises nanosized X-ray beams, which when coupled with
tunability will expand the REXS techniques to nanosized materials. Such nanosize X-ray beams can
help look for local variations in micro-scans across a sample (although sample thickness will remain an averaging effect).

4. Softer X-rays REXS in life and physical sciences

A distinct trend now is the introduction of in vacuum diffractometers to serve both the life and physical sciences. In the physical sciences a relatively recent 4-circle, in-vacuum, diffractometer for resonant elastic soft x-ray scattering at the Canadian Light Source has been described [17]. It includes 9 in-vacuum motions driven by in-vacuum stepper motors and operates in ultra-high vacuum at a base pressure of $2 \times 10^{-10}$ Torr. Cooling to a base temperature of 18 K is provided with a closed-cycle cryostat. The diffractometer includes a choice of 3 photon detectors: a photodiode, a channeltron, and a two dimensional sensitive channel plate detector. Along with variable slit and filter options, these detectors are suitable for studying a wide range of phenomena having both weak and strong diffraction signals. Example measurements of diffraction and reflectivity in Nd doped La$_{2-x}$Sr$_x$CuO$_4$ and thin film (Ga,Mn)As have been described.

At the Diamond Light Source there is the in vacuum diffractometer for the physical sciences named ‘RASOR’ (See Figure 1) and ref [18 ].

A very recent review of resonant elastic soft x-ray scattering has been given by Fink et al [19]. This review emphasises that “resonant (elastic) soft x-ray scattering (RSXS) offers a unique element, site and valence specific probe to study spatial modulations of charge, spin and orbital degrees of freedom in solids on the nanoscopic length scale. The technique is not only used to investigate single-crystalline materials. This method also enables one to examine electronic ordering phenomena in thin films and to zoom into electronic properties emerging at buried interfaces in artificial heterostructures.”

Equivalently, in the life sciences, the long-wavelength (1.5 to 4 Å) Diamond Light Source I23 project, led by Dr Armin Wagner, will be a user facility to enhance and optimise the anomalous signals from low atomic number elements. These include include sulphur in proteins and/or phosphorous in RNA/DNA (ribonucleic acid/deoxyribonucleic acid) crystals, needed where protein labelling to introduce anomalous scatterers is not feasible, such as involving Se methionine via molecular biology gene expression or heavy atom chemical derivatisation. In addition, for the Diamond Light Source I23 the beamline's wavelength range will provide access to the M-edges of
elements, e.g. of uranium, with huge anomalous signals. A large semi-cylindrical detector and a multi-axis goniometer will be available for crystal alignment and orientation. X-ray tomography to obtain the crystal sample shape and volume for an analytical absorption correction will be possible.

Figure 1 The in vacuum ‘RASOR’ diffractometer at the Diamond Light Source [18]. Courtesy of the website of the Diamond Light Source and to whom we are grateful.

The figure 2 below illustrates with chlorine as the element the long wavelength, for crystallography, of the absorption edge and the $f'$ and $f''$ variations.

We have recently published on another practical situation where the raw X-ray diffraction data proved valuable and are now briefly reviewed. This is the example [20] of locating a low Z anomalous scatterer at fractional occupancy in a pharmaceutical chemistry crystallography study. Tanley et al 2014 [20] describe a partial chemical conversion of carboplatin to cisplatin under a high (10%) NaCl condition. This meant that they had the crystallographic challenge to see a partially occupied chlorine at the wavelength used for X-ray diffraction data collection of 1.54 Å (i.e. with a quite small $f''$ of 0.7 electrons for a fully occupied Cl). This study showed the importance of open archiving of the
diffraction data images and which allowed a wider comparison of software results from them than the original study (for details see [21]).

Figure 2 The variation of the REXS $f''$ and $f'$ values with X-ray wavelength and photon energy for chlorine (theoretical values) as an example low Z element of specific interest in the life sciences for e.g. structural studies of protein salt (NaCl) interactions or pharmaceutical compounds like cisplatin (see Figure 3). [Courtesy of the website http://skuld.bmsc.washington.edu/scatter/ and Prof Ethan Merritt, to whom we are grateful.]

Figure 3. The two binding sites on the His-15 residue of hen egg white lysozyme (HEWL). The Fo-Fc omit electron density maps are in green and the anomalous difference electron density maps are shown in orange at 3σ cut off; the diffraction data for the Protein Data Bank code 4dd7 study were processed by different software namely (a) XDS [22] and (b) EVAL [23]. From [20] with the permission of the IUCr (http://journals.iucr.org/).

Low Z atomic number elements are especially challenging for protein X-ray crystallography. The classic case is the problem of trying to identify a sodium ion versus a bound water. Such a case in the structural chemistry details can be resolved by the putative sodium ligand distances (~2.2 Å) and the
expected octahedral environment versus a hydrogen bonding interaction for a bound water (~2.8 Å) provided adequate structural precision is available, which of course is not always the case. Besides software improvements for diffraction data image processing of course experimental developments continue apace. Thus the expansion into use of longer X-ray wavelengths is a real help too in such cases e.g. see [24] for a short summary.

Finally, W A Hendrickson and colleagues [25] have recently devised robust procedures for enhancing the signal-to-noise ratio in the anomalous scattering from native biological structures by combining diffraction data measured from multiple crystals at lower-than-usual X-ray energy, both for structure determination and element identification. At the upcoming new National Synchrotron Light Source ‘NSLS2’ there is a proposed beamline for longer wavelengths’ data collection (W A Hendrickson presented at European Crystallography Meeting (ECM) 28).

5. Applications with the X-ray laser

This seems challenging territory for X-ray diffraction in general and REXS in particular. We must ask firstly, most basically, how might the X-ray atomic scattering factor be affected with time in the X-ray laser beam? We take guidance from [26] and Figure 4, which shows the population dynamics for several selected charge states of iron, as an example, during an X-ray pulse; how the X-ray laser pulse affects the scattering factors in a sample is a matter of future practical research in structure determination.
Figure 4  Population dynamics for several selected charge states of Fe during an X-ray pulse. The thin dotted line indicates the X-ray pulse envelope assumed Gaussian. Reprinted figure 1 with permission from ref 26 namely S.-K. Son, H. N. Chapman, and R. Santra, Phys. Rev. Lett. 107, 218102, 2011. Copyright (2011) by the American Physical Society and with permission from the authors to whom we are grateful.

The X-ray laser has been used to study the visibility of the anomalous dispersion signal from protein disulphides [27a]. This is a particularly telling example for life scientists and for which specific X-ray damage has been carefully investigated over the last couple of decades. Barends et al [27a] successfully saw the anomalous signal from sulphur atoms in protein crystallographic data measured with an X-ray free-electron laser; see figure 5. Barends et al commented that “the results presented clearly demonstrate that despite the obvious challenges associated with the analysis and evaluation of diffraction data collected with (X-ray) free-electron laser sources, anomalous (difference f”) data can be obtained by serial femtosecond (X-ray) crystallography.” In a most recent development Barends et al 2014 [27b] study the ‘Single-wavelength Anomalous Dispersion (SAD) phasing’ effectiveness for a gadolinium derivatised lysozyme, using X-ray laser diffraction data from micron sized crystals and who also make a comparison with a ‘zero X-ray dose’ home laboratory X-ray source single ‘normal sized’ crystal dataset. The gadolinium anomalous signal seen within the X-ray laser diffraction data is remarked upon by Barends et al 2014 as being considerably lower than expected. The authors attribute this to an insufficient number of X-ray laser diffraction images being measured, due to a limited beamtime, rather than an effect on the anomalous scattering signature itself. Further X-ray laser experiments are obviously needed to investigate this aspect further.

In [24] the suggestion was made for use of marker heavy atoms to assist with structure determination with X-ray Free Electron Laser (XFEL) diffraction data. “There may also remain interest in the use of XFEL wavelengths at the xenon L edges e.g. to control the value of xenon f’ values for protein structure determination where differences at two wavelengths in single molecule diffraction data could reveal the location of xenon atoms. A similar two wavelength, delta f’, approach would work for iodine or sulphur to locate their positions in a protein single molecule imaging experiment as well. Such stepwise structural approaches may prove valuable in challenging single molecule protein structure determinations via the XFEL route.” Whilst the focus today is the use of protein microcrystals, and then nanocrystals, at the X-ray laser the ultimate target sample size of single molecules for diffraction [28] is of keen interest. An intermediate step beyond nanocrystals, before the single molecule sample, would be a nanocluster of molecules. A natural extension of marker atoms in
such structural studies with the X-ray laser, presented by J R Helliwell at European Crystallography Meeting (ECM) 28, building on protein two wavelength powder MAD diffraction developments [16], would be to use heavy metal complexes such as the platinum hexahalides, most notably the platinum hexa-iodide and the platinum hexa-bromide, and or tantalum bromide (Ta₆Br₁₂). These would provide recognisable shapes in electron density maps, respectively octahedral for the platinum hexahalides and roughly spherical for tantalum bromide, and also enhance the overall X-ray scattering strength of a single molecule or nanocluster type of sample size challenge. The particular chemistry for their binding of these metal complexes, namely a complementary shape and or electronic charge to a binding site on the protein, would restrict their applicability of course. Another possibility would be iodine triangles [29] albeit less electron rich but another recognisable shape that would be helpful where there was a noisy electron density map to be interpreted; these offer a further range of chemistry binding site types [29].
Figure 5 Anomalous difference Fourier maps (yellow) based on data measured at the LCLS for (a) Met105 contoured at 3σ and (b) the Cys76-Cys94 disulfide bond contoured at 2.5σ. From [27a] and refs therein with permission of the IUCr (http://journals.iucr.org/) and the authors to whom we are grateful.

6. Concluding remarks and future prospects
The field of application of anomalous scattering in structural chemistry and biology is now truly vast. The evolution of capability is breath taking and shows up the pace of modern day research.

Overall, to conclude, the necessary technical advances have been made to harness REXS X-ray anomalous scattering at synchrotron radiation sources these last decades, which have now brought reliable answers to a vast number of life science, bioinorganic, inorganic and materials chemistry research challenges.

The future indicates an expansion of the field as the measurements are more rapid and there are increased numbers of such tunable SR beamlines available. Beamlines providing high and low photon energies (in a range of ~90 to 2 keV) indicate wide chemical element capability. The SR source upgrades towards providing nanosized tunable X-ray beams, such as at ESRF (www.esrf.eu), opens new possibilities of applying REXS techniques increasingly for nanoscience. Also the XFELs for REXS applications in structural chemistry and biology, even perhaps with single molecules and or nanoclusters, are an interesting prospect if the wavelength setting of the X-ray laser can be controlled and the inner shell photoelectron ejection [30] proves not to be limiting.

12. Acknowledgments
J R Helliwell thanks all his past and current collaborators over the years in these fields. Numerous funding agency and SR beamtime awards have been received including from the Science and Engineering Research Council, the Swedish Research Council, the Biotechnology and Biological Sciences Research Council, the Engineering and Physical Sciences Research Council and the Council for the Central Laboratories of the Research Councils, which are the UK government research agencies, and the Synchrotron Radiation Source (SRS) Daresbury, the NSLS, the Sincrotrone Trieste ‘ELETTRA’, the ESRF and Diamond Light Source and to whom JRH is especially grateful. JRH’s anomalous scattering work has evolved over a period of 30 years and so he is grateful to the
Universities of Oxford, Keele, York and Manchester and to the Daresbury Laboratory for providing stimulating environments in which to study and to do experiments. JRH also thanks The Royal Society for a Research Exchange Agreement funding travel and subsistence with Prof V Kaucic and Dr N Zabukovec Logar at the National Institute of Chemistry in Ljubljana, Slovenia.

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