Combining Particle Induced X-ray Emission (PIXE), and X-ray Fluorescence (XRF) measurements to probe metalloproteins

Elspeth F. Garman[1], Geoff W. Grime[2], Samuel M. Webb[3], Edward H. Snell[4]

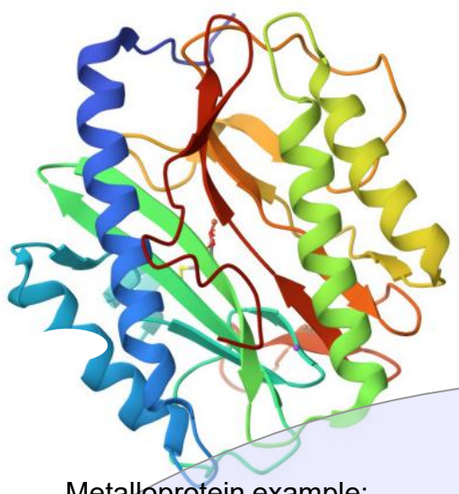
1. Department of Biochemistry, Dorothy Crowfoot Hodgson Building, University of Oxford, South Parks Rd, Oxford, OX1 3QU, UK.
2. Ion Beam Centre, Advanced Technology Institute, University of Surrey, Guildford, Surrey GU2 7XH, UK.
3. Stanford Synchrotron Radiation Lightsource, Menlo Park, California 94025, U.S.A.
4. Department of Materials Design & Innovation, SEAS, University at Buffalo, Buffalo, NY14260-9640, U.S.A.

elspeth.garman@bioch.ox.ac.uk

1. Introduction

Trace elements (particularly metals) often play very important roles in the structure and function of proteins, and their levels can be key to understanding biological pathways. Particle Induced X-ray Emission using a microbeam (MicroPIXE) provides a uniquely accurate way of quantifiably determining the unambiguous identity and stoichiometry of bound metal ions in proteins.

The Protein Data Bank (PDB) contains information on over 200,000 proteins. Of these ~60,000 are metalloproteins, being either proteins in which a small number of metal atoms define the three-dimensional shape (and hence function), or those involved in metal transport or metabolism. For most PDB metalloprotein entries, the metal atom identity has been inferred indirectly from the X-ray diffraction data or by other methods, including the presence of an anomalous diffraction signal.



Metalloprotein example: methionine aminopeptidase PDB: 3mx6, Mn bound

2. Quantitation: Internal Normalisation

MicroPIXE can simultaneously excite significant X-ray emissions over a wide range of emission energies. Since the exact number of sulphur atoms in each protein is known from the number of S-containing amino acids in the primary sequence, we can compare the relative peak areas for sulphur and the, target element directly giving the stoichiometry.
This eliminates many systematic errors [1,2,5]:

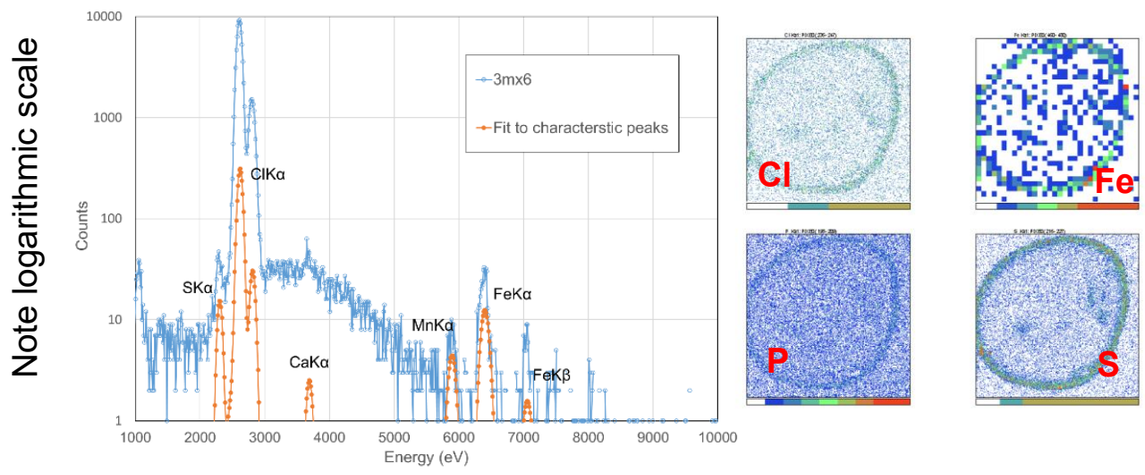
$$\text{Number of atoms of element } x \text{ per protein molecule} = \frac{\text{Concentration of element } x}{\text{Concentration of sulphur}} \times \frac{\text{Atomic weight of sulphur}}{\text{Atomic Weight of element } x} \times \text{Number of atoms of sulphur per protein molecule}$$

Absolute accuracy is 10-20%, but relative accuracy of 5-10% is achievable [1,2,5]. Comparable wet-lab techniques have errors of 50% or more.
X-ray fluorescence (XRF) measurements can also provide elemental identification, but quantitation is more involved [6].

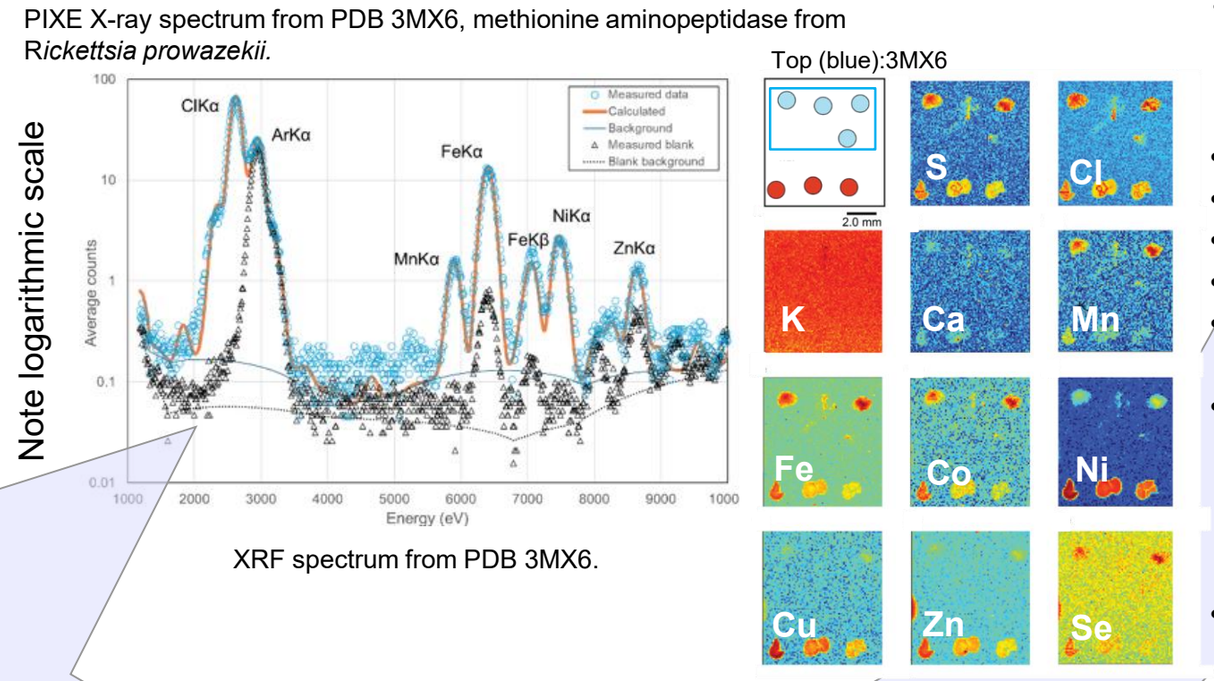
Objectives

To use PIXE and synchrotron XRF spectroscopy to experimentally identify metals in protein samples before crystallization, validate metalloprotein structures, and improve the accuracy of Protein Data Bank models.

Why? Because we suspect that >20,000 metalloproteins may have the WRONG METALS built into their models.



PIXE X-ray spectrum from PDB 3MX6, methionine aminopeptidase from R*ickettsia prowazekii.*



XRF spectrum from PDB 3MX6.

3. The Experiments

PIXE:

- 2.5 MeV 2-μm sized proton beam
- Energy dispersive silicon drift X-ray detector
- Proton detector recording the Rutherford backscattered protons (RBS) to get gross matrix composition & sample thickness for absorption correction.
- Scan in *x* and *y* to obtain elemental maps (left).
- Take point spectra on sulfur ring.
- Analyse with OMDAQ [3] & GUPIX [4]

XRF:

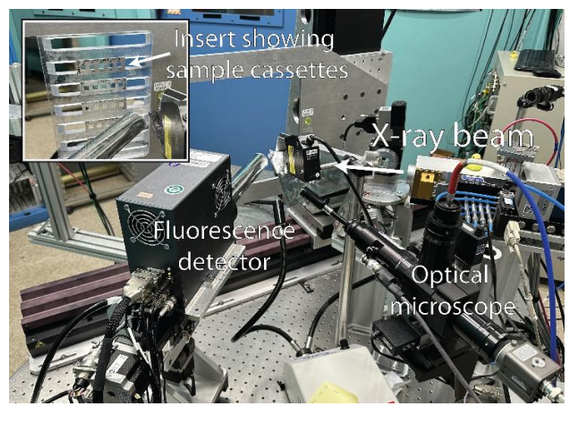
- Synchrotron 13.45 keV X-ray beam
- Same sample holders as for PIXE
- Move sample across beam.
- Collect X-ray spectra.
- Scan in *x* and *y* to obtain elemental maps (left).
- Each pixel in the XRF elemental map (far left) is an individual energy spectrum, all averaged across the drop pixels to obtain the spectrum (centre bottom).
- Quantitation from standards

6. Discussion

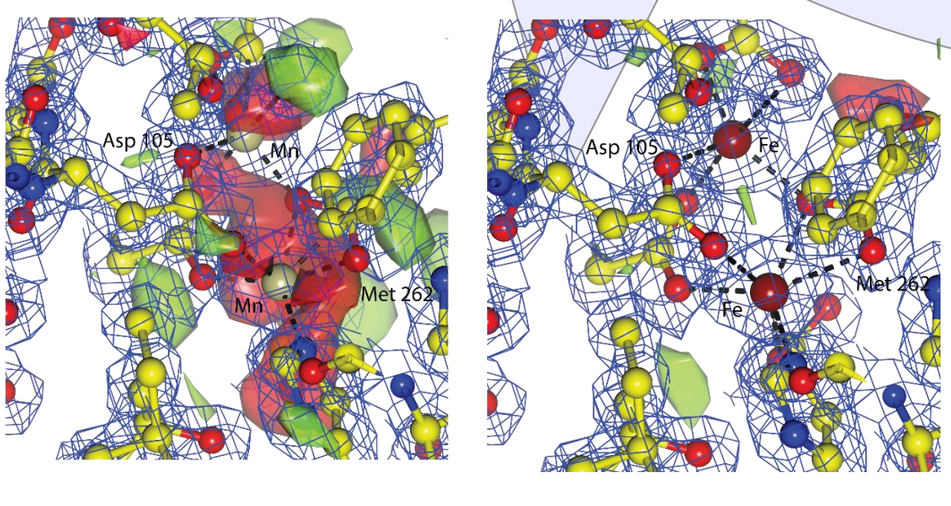
- **Clearly we need to better identify metals in protein models!**
- Need for further high-throughput microPIXE and XRF analysis of micro-arrays to experimentally validate data for a wide range of metalloprotein samples
- Can identify suspect metal assignments from the difference electron density between the PDB model and diffraction data from which the model was derived
- Can use the anomalous diffraction signal to remove ambiguities in metal assignments, and this should be made an integral part of structure determination data collection

4. Results

- **Metal content of 102 metalloproteins analysed [5,6]**
- **>30% of the PDB deposited models had misidentified metals**
- **Another 30% contained additional metals, some explainable from the crystallisation buffers**
- **93% agreement between PIXE and XRF in identifying the top 3 metals present in any sample [6]**



5. Example



Methionine aminopeptidase

Left: PDB model of 3MX6 with Mn. Red/green -/+3σ electron density.

Right: Corrected model with iron instead of Mn. No difference density [6].

References

{1] Garman, EF & Grime GW. Elemental analysis of proteins by microPIXE. (2005) *Progr.Biophys.& Mol. Biol.* **89** 173–205
[2] Grime,GW, & Garman, EF. The identification and quantification of metal atoms in proteins using microPIXE: A critical evaluation (2024) *Nuc.Instr. Meth. B* **40,** 237-245
[3] Grime, GW. OMDAQ data acquisition system (Oxford Microbeams Ltd., UK)
[4] Maxwell, J, Campbell, J & Teesdale, W. The Guelph PIXE software package. (1989) *Nuc.Instr. Meth. B* **43** 218-230
[5] Grime,GW….Snell,EH & Garman, EF. High-Throughput PIXE as an Essential Quantitative Assay for Accurate Metalloprotein Structural Analysis: Development and Application (2020) *J.Am.Chem.Soc.***142,**185
[6] Snell,EH,Grime,GW…& Garman, EF Assessing Metal Ion Assignment Accuracy in Protein Data Bank Models via Elemental Spectroscopy (2026) *J.Chem.Info.& Modeling*. DOI: 10.1021/acs.jcim.6c00352

Acknowledgements

Metalloprotein samples from Northeast Structural Genomics Consortium and Seattle Structural Genomics Center for Infectious Disease. PIXE beamtime at Surrey Ion Beam Centre (IBC) from a pump priming grant and from the RADIATE program (EU) proposal 21002583-ST. Use of the Stanford Synchrotron Radiation Lightsource, SLAC National Accelerator Laboratory, was supported by the U.S. Office of Basic Energy Sciences under Contract No. DE-AC02-76SF00515.