Protein analysis by microPIXE

Frequently Asked Questions

Elspeth Garman, December 2005, Revised August 2023, May 2024, October 2025

A) How does it work?

The identification and quantification of metals bound to proteins is a crucial problem to be solved in structural biology. The technique of particle induced X-ray emission with a microfocused beam (microPIXE) is a tool for analysing the elemental composition of liquid and crystalline protein samples. The proton beam induces characteristic X-ray emission from all elements in the protein, and the resulting X-ray spectrum can be interpreted to provide the metal content of the protein molecule with a relative accuracy of between 10% and 20%. The compelling advantage of this method is that the sulphur atoms in the methionines and cysteines of the protein provide an internal calibration of the number of protein molecules present, so that systematic errors are minimised and the technique is entirely internally self-consistent. This is made possible by the simultaneous measurement of the energy of backscattered protons (Rutherford backscattering) to enable us to determine the sample matrix composition and thickness, and so correct the PIXE data for the self-absorption of X-rays in the sample.

The theoretical background to the technique is described, and the technical and experimental procedures are outlined in the papers below (all open access).

Leaving no element of doubt: analysis of proteins using microPIXE.
Elspeth Garman. *Structure* (1999) 7, R291-299. https://doi.org/10.1016/s0969-2126(00)88335-5

Elemental analysis of proteins by microPIXE.
E.F. Garman and G.W. Grime.
Progress in Biophysics and Molecular Biology (2005) 89/2, 173-205. DOI: 10.1016/j.pbiomolbio.2004.09.005

The identification and quantification of metal atoms in proteins using microPIXE: a critical evaluation.
G.W.Grime and E.F.Garman, *Nuclear Instruments and Methods, B,* 540, (2023) 237-245
https://doi.org/10.1016/j.nimb.2023.03.025

B) My samples

Which elements can be detected?

All elements in the periodic table with atomic number greater than 9 (sodium and above) give a signal. However, measurements of sodium and magnesium are only possible with some

modification of the equipment, so make it clear when first arranging the analysis if you require quantitative sodium or magnesium results.

What is the limit of detection (LOD) of the technique?

For a wide range of proteins that we have analysed by PIXE, the LOD is typically <0.1 metal atoms per protein molecule. For example, this means that 1 zinc atom in a 100 kDa protein can be detected.

How accurate are the measured stoichiometric ratios?

An accuracy of between 10 and 20% is usually achievable, depending on what elements are present and how much of that element is present.

What happens if there are no cysteines or methionines in my sample to use as an internal standard?

Only one such sample out of around 500 has been presented with this problem so far! Note that the metals in the sample can still be unambiguously identified, but not quantified. Please contact us if you find yourself with such a protein.

Can I measure DNA and RNA too?

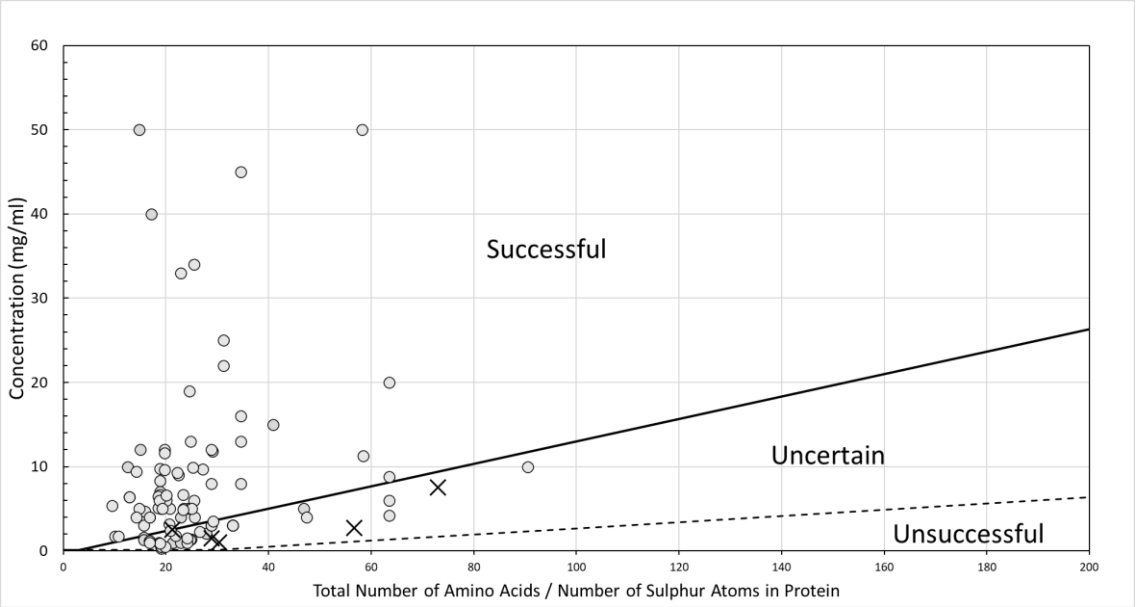
Yes, as the phosphorus can act as the internal standard to give accurate stoichiometric ratios and if you have a protein:DNA complex, the stoichiometry can be determined.

C) For liquid samples:

What volume and concentration of protein do I need?

Only a very low volume is required: 0.3 microlitres is plenty, but at least 2 microlitres is recommended so that the sample can be located in the transport container. The necessary concentration in mg/ml depends on the ratio of the total number of amino acids to the number that contain sulphur. We have established a reliable empirical limit for this ratio which is shown on the graph below as a guide to users. This should always be checked before mounting any samples. It may be a waste of time and money to attempt to

measure samples lying below the solid line on the scatter plot: please contact us if this is the case.



Does the protein buffer matter?

Yes! It is crucial that the buffer contains NO sulphur or chlorine (please see next question). Common buffers used in protein preparation which contain sulphur and are thus unsuitable are: BES, DDT, HEPES, MES, MOPSO, PIPES. Suitable buffers are: EDTA, TRIS (make sure you don't use HCl during the preparation, see question below), Tricine, glycine, and TEA. A full list is shown below and can also be found in Table 4 of Garman and Grime, Progress in Biophysics and Molecular Biology, (2005) 89/2, 173-205. DOI: 10.1016/j.pbiomolbio.2004.09.005

Suitability of common biological buffers when submitting samples for microPIXE analysis

Buffer	Chemical formula	Compatible with microPIXE?
ACES	$C_4H_{10}N_2O_4S$	No
ADA	$C_6H_{10}N_2O_5$	Yes
BES	$C_6H_{15}NO_5S$	No
BICINE	$C_6H_{13}NO_4$	Yes
BIS-TRIS	$C_8H_{19}NO_5$	Yes
β-Mercaptoethanol	C_2H_6OS	No
CABS	$C_{10}H_{21}NO_3S$	No
CAPS	$C_9H_{19}NO_3S$	No
CHES	$C_8H_{17}NO_3S$	No
DIPSO	$C_7H_{17}NO_6S$	No
DTT	$C_4H_{10}O_2S_2$	No
EDTA	$C_{10}H_{16}N_2O_8$	Yes
EPPS	$C_9H_{20}N_2O_4S$	No
Ethanolamine	C_2H_7NO	Yes
Glycine	$C_2H_5NO_2$	Yes
HEPES	$C_8H_{18}N_2O_4S$	No
HEPPSO	$C_9H_{20}N_2O_5S$	No
MES	$C_6H_{13}NO_4S$	No
MOBS	$C_8H_{17}NO_4S$	No
Monothioglycerol	$C_3H_8O_2S$	No
MOPS	$C_7H_{15}NO_4S$	No
MOPSO	$C_7H_{15}NO_5S$	No
PIPES	$C_8H_{18}N_2O_6S_2$	No
POPSO	$C_{10}H_{22}N_2O_8S_2$	No
TABS	$C_8H_{19}NO_6S$	No
TAPS	$C_7H_{17}NO_6S$	No
TAPSO	$C_7H_{17}NO_7S$	No
TEA (triethanolamine)	$C_6H_{15}NO_3$	Yes
TES	$C_6H_{15}NO_6S$	No
TRICINE	$C_6H_{13}NO_5$	Yes
TRIS	$C_4O_3H_{11}N$	Yes

My protein is in NaCl. Does this matter?

Yes, as this will give an intense chlorine peak next to the sulphur peak. This gives a shoulder under the sulphur peak which increases the LOD and percentage error for the measurement. An excellent substitute is KBr or NaBr, and we have been successful in a number of cases using them. Sodium acetate is also a good buffer.

D) For crystalline samples:

How good does the crystal have to be?

It can be really awful! MicroPIXE measures the material in the beam, it does not care about diffraction quality, so as long as there is some identifiable object to analyse, it does not matter whether or not it is highly ordered.

How big does the crystal have to be?

Anything over 50 microns is fine. The smallest we have ever measured was 10 microns square and about 2 microns thick (it turned out to be zinc chloride…).

Should I wash my crystal before the measurement?

This depends on whether the crystallisation buffer contains sulphur. If it does, the crystal should be washed 5 or 6 times in milliQ (NOT tap water which adds chlorine to the buffer) by means of a cryoloop. It does not matter if the crystal disintegrates, as long as some of it gets onto the film. If the crystal was washed, it is safest to carry out PIXE measurements on both a washed and an unwashed crystal.

E) Submitting my samples for analysis

Samples should be submitted either as liquid protein solution or crystals in buffer. Full details of how to do this will be provided once the experiment is scheduled.

How long does it take to collect data from a sample?

Each sample takes a maximum of around 30 to 40 minutes to measure (e.g. 1 elemental map to localise the sample and 4 points with the proton beam on it of around 5 minutes each).

How are the results presented?

An analysis report will be provided by the Ion Beam Centre. Raw data is archived and can be provided on request.

How much does it cost?

For further information on access and charges please contact Dr Catia Costa, <c.d.costa@surrey.ac.uk>