

Analysis of Liquid and Crystalline Proteins by Particle Induced X-ray Emission (PIXE)

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Unique identification of *metals* bound to macromolecules is an interesting challenge in structural biology, and an unambiguous assignment is often problematic. microPIXE (particle induced X-ray emission) with 2-3MeV protons on liquid and crystalline proteins has been used very successfully in both identifying elements and in measuring their stoichiometric ratio (calibrated per protein molecule by using the sulphur peak to give an internal normalisation of the sulphur atoms from the known cysteines and methionines) to an accuracy of between 10 and 20% on over 50 samples [1,2].

Measurements using the technique have informed a wide range of questions, including the degree of seleno-methionine incorporation into a proteins destined for MAD structure determination, the identity of unexpected electron density in solved structures, identifying of metals bound to liquid protein samples to elucidate their function prior to structural studies, determining whether or not DNA is bound to a protein crystal (from the phosphorus to sulphur ratio), checking for paramagnetic species in proteins prior to NMR analysis, and analysing proteins before and after mutation of putative metal binding sites.

The method is now routine and may have potential as a high throughput screening tool in structural biology.

[1] Garman E., *Structure*, 1999, 7, R291-R299. [2] Garman E.F., Grime G.W., *Progress in Biophysics and Molecular Biology*, 2005, *in press*.

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