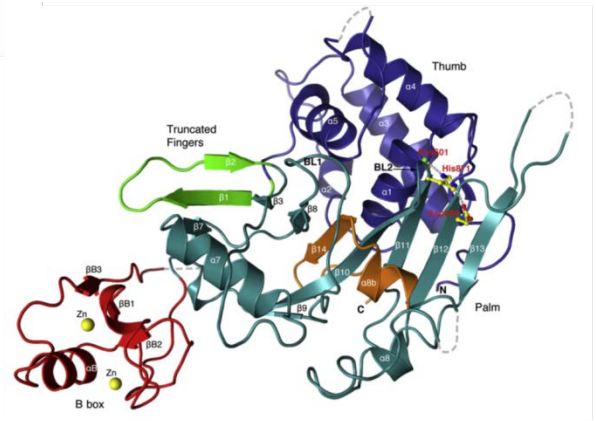
Protein Structure Determination



Structure of the ubiquitin specific protease (USP) domain of the CYLD tumour suppressor gene. The B-box domain is necessary to retain the CYLD gene in the cytoplasm of cells. The two Zn atoms in the B-box were identified and quantified by IBA (part of Figure 1 of Komander et al, *Molecular Cell* **29**, 2008, 451–464).

The Figure (from Komander *et al*, 2008 [1]) shows authoritative work done at the Institute of Cancer Research, using the beamline ID-29 of the ESRF at Grenoble. The IBA was done separately according to the methods described in Garman & Grime, 2005 [2]. The IBA method is absolute, requiring no standards, since the metal is typically quantified relative to the sulfur signal. This latter originates from the methionine and cysteine amino acids in the molecule, each of which contains one sulfur atom. The number of sulfurs is already known from the sequence of the protein. Thus the result is expressed as the number of metal atoms per protein molecule.

It is necessary to collect PIXE and EBS data simultaneously ("Total-IBA") since PIXE cannot be validly quantified absolutely unless the film thickness is known (allowing the self-absorption correction to be correctly calculated). The term "Total-IBA" [3] was proposed quite recently by different workers, but using EBS & PIXE synergistically was the first report of "Total-IBA" [4] and was published in 1996. The use of Total-IBA for protein analysis [5] was first reported explicitly in 1999.

The important example described above is one of many that could have been chosen. We give a few other notable examples for which information crucial to understanding the function of the proteins was obtained by IBA.

1. Woo et al (*Nature Structural Biology*, 2000; Reading & QMC London [6]) present the first definitive crystallographic and biochemical evidence that barley germin and, by inference, the related germin-like proteins, represent a new group of extracellular, manganese-containing bifunctional enzymes and also confirm an evolutionary link with the vicilin seed storage proteins. A single manganese ion is bound per germin monomer by ligands similar to those of manganese superoxide dismutase. Germin has such extreme resistance to heat, and to chemical degradation by proteases or hydrogen peroxide, that its stability has been compared to that of the scrapie form of the prion protein. The manganese ions central to its function were positively identified and quantified by IBA.

2. The homeostasis of biological iron in *Mycobacterium tuberculosis* is controlled by the ferric uptake regulator (FUR). One of the FUR proteins was crystallised and characterised by XRX with the metal (Fe and Zn) ligands identified by IBA (Pohl *et al, Molecular Microbiology* 2003 [7]) in work from the European Molecular Biology Laboratory in Hamburg (EMBL) together with the Laboratory of Molecular Biophysics in Oxford.
3. There is a widespread gene found in eubacteria, archaebacteria, and mammals with a highly conserved sequence. This was previously known as "*ElaC*" but has been shown by Ezraty *et al* [8] to be identical with the enzyme "*RNase BN*". In work also from the EMBL, Vogel *et al* (*J. Biological Chemistry* 2002) [9] show that "*ElaC*" is a binuclear zinc phosphodiesterase (a result novel at that time), where again the metal ligands were identified by IBA.
4. Guanosine triphosphate (GTP) is related to adenosine triphosphate (ATP), and both are essential to life. Work from the UK National Institute for Medical Research (Graham et al, *Chemistry & Biology* 2002 [10]) suggested that magnesium fluoride as a replacement for alumino-fluorides in GTPase-activating proteins may be a reagent of choice for studying biological phosphoryl transfer reactions (these are the reactions that manipulate GTP and ATP). Transition state analogs are widely used in enzymology as kinetic and structural tools. The study of phosphoryl transfer reactions in biological systems has been greatly facilitated by the use of alumino-fluorides as transition state mimics. Again, the XRX would have been useless without the extra information afforded by the IBA, which was capable of distinguishing and quantifying Al and Mg in the proteins.
5. Karkehabadi *et al* (*J. Molecular Biology* 2008; Uppsala and Ghent) [11] solved the detailed structure of "Cel61A", one of a significant class of proteins: glycoside hydrolase "family 61" found in the fungus *Hypocrea jecorina*. This was the first solution of any of the GH family 61 class. The authors identified a cluster of highly conserved residues on the surface of the Cel61B structure, finding a nickel ion bound within this conserved surface (using IBA). The observation of bound Ni is expected to be important for Cel61B activity, stability or function.
6. In further work on the same fungus *H. jecorina*, the same group (Jacobson *et al, PloS one* 2013 [12]) identified and solved a previously unknown protein, denoted "cellulose induced protein 1" (Cip1) which is thought to be likely to function in biomass degradation. A calcium ion binding site was identified in a sequence-conserved region of Cip1: the presence of this ion was found to have a structural role. The calcium ion was unambiguously identified and quantified by IBA.
7. In work from Oxford and to expand our understanding of catalysis by the alkaline phosphatase class of enzymes, Yong *et al* (*Science*, 2014 [13]) determined the structure of the widely occurring microbial alkaline phosphatase PhoX. These play a crucial role in phosphate acquisition by micro-organisms. The enzyme contains a complex active-site cofactor comprising two antiferromagnetically coupled ferric iron ions (Fe3+), three calcium ions (Ca2+), and an oxo group bridging three of the metal ions. These metal ions were identified and quantified by IBA. The presence of iron in PhoX raises the possibility that iron bioavailability limits microbial phosphate acquisition.

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