Biophysics

XANES spectroscopy and the crystallographic imperative

from John Galloway

PERHAPS nowhere in the determination of molecular structure is precision more at a premium than in the case of metalloproteins, so that any method that promises to enhance or improve on single crystal protein crystallography is worth investigating. X-ray near-edge structure (XANES) spectroscopy is claimed to represent such promise. The real question, of course, is whether that promise can be realized; in the paper by A. Bianconi *et al.* on page 685 of this issue we might have had the first chance to find out. As it happens, we don't.

What exactly is XANES? Absorption of X-rays takes place chiefly through the photoelectric effect. An electron is ejected from the K shell of an atom (or possibly some other shell). Its chances of escaping are modulated by its being scattered by neighbouring atoms. The resulting fine structure in the absorption cross-section expressed as a function of the energy of the incident X-ray photon contains information about the local atomic geometry. For energies not much in excess of the threshold represented by the absorption edge, say within 50 eV, the photoelectrons are strongly and multiply scattered. These modulations are referred to as XANES.

At higher energies the scattering is weaker and dominated by single scattering events. In this energy regime the fine structure is the rather better known extended X-ray absorption fine structure (EXAFS), which has been widely used in various types of structural study, including some on metalloproteins, since about 1972, the year in which it first became possible to replace the conventional X-ray tube by synchrotron radiation. The domination by single scattering events confers on EXAFS an immediate advantage it shares with a number of other physical probes of structure, namely that it is relatively easily interpreted. But it also confers the considerable disadvantage that it contains information only about two-particle correlation functions, so that only the *radii* of the coordinating shell of atoms and slightly more remote shells in metalloproteins can be extracted.

XANES, by virtue of its strong multiple scattering, contains information about three- and higher-order correlations. Thus, in principle, information about the detailed geometry of arrangements in the shells should be extractable — angles as well as distances. A lot is made of this feature by Bianconi *et al.* The Fe–C–O bond angle is obtained in a single crystal of carboxymyoglobin from the angular dependence of the XANES spectrum, and a difference in this angle between carboxymyoglobin and carboxyhaemoglobin is shown to exist in solution. Unfortunately, the paper gives no indication of the accuracy of these measurements although it is unlikely to be better than ±10°. On the face of it this seems to compare quite well with the angular accuracy that can be achieved by conventional crystallography but the question is not explicitly addressed by the authors.

That XANES might be interesting at all to structural biologists is a consequence of the two cardinal deficiences of single crystal methods. First, that they are obviously only suited to crystals, whereas XANES and EXAFS can and do provide structural information about proteins in solution. Second, that in single crystal determinations the extent and quality of the data limit accuracy; limited resolution and phasing errors typically lead to a precision in atomic position of no better than 0·3–0·5 Å, although with great care standard deviations on atomic positions can be brought well below 0·1 Å, which will be needed for understanding function in metalloproteins, where distances between metal ions and their ligands may need to be known to better than 0·1 Å resolution.

Without a good idea of a method's accuracy, realized or potential, no technique will or should be taken seriously. Early proponents of EXAFS rashly made unfounded claims of its superiority over crystallographic analysis of haemoglobin (Eisenberger, J.B. *et al. Nature* **274**, 30; 1978) and got a bloody nose for their pains (Perutz, M. *et al. Nature* **295**, 535; 1982). But EXAFS has been rehabilitated through very careful comparisons with crystallographic analysis on 2Zn insulin (Bordas, J. *et al. Proc. R. Soc.* B**219**, 21; 1983) and on haemoglobin and insulin (Dodson, G. *Proc. Bioinorganic Discussion Group*, Daresbury, in the press). Dodson concludes that EXAFS has a "clear role in adding precision to the crystallographic picture and hence furthering our insight into the chemical and structural processes that are at the centre of biological phenomena".

Whether the same is true of XANES will be found only in much the same way and will not be easy. It is crucial that the early mistakes made with EXAFS are not repeated with XANES, necessitating rescue operations at a later date. Systematic comparative structural studies of XANES and conventional crystallographic analysis now will pay dividends. The advantage of crystallographic analysis is that production of the electron-density map is not subject to the experimenter's anticipation. XANES is model-dependent — a structure must be imagined and its consequences compared with the experimental data. So the only sound tactic is a heavy investment of effort in alternative structures — a prominent feature of the papers of both Bordas *et al.* and Dodson.

XANES analysis tends to be very heavy on the time of both experimenters and computers. Furthermore, it has inherent limitations, as pointed out by J. B. Pendry (*Comments Solid State Phys.* **10**, 219; 1983). The range of data is small and will not generally be expected to contain many features to be fitted to the model. Indeed, it seems highly optimistic to suppose that the positions of more than three atoms will be found with any accuracy. Therefore, it will not be useful except in harness with other methods and even then its usefulness is likely to lie in choosing between qualitatively different structural alternatives where much is already known. □

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Ecology

Rats as agents of extermination

from Jared Diamond

"TAKE care! Kingdoms are destroyed by bandits, houses by rats, and widows by suitors!" Biologists would emend this insight of the 17th century Japanese poet Ihara Saikaku to note that rats destroy not only houses but also island biotas. The colonization of islands by rats has outweighed all other causes of exterminations of island birds. Rats have also preyed on young Galapagos giant tortoises and exterminated small mammals, large insects, molluscs (Hawaiian achatinellid land snails), and cold-blooded vertebrates (most ground snakes and lizards of Mauritius plus mainland frogs, lizards and tuataras of New Zealand).

Yet it has proved hard to make sense of these gruesome facts. Rats have caused catastrophic extinction waves on some islands, a few extinctions on others, and no visible effect on still others. Somehow, these varying outcomes must depend on the differing susceptibilities of prey species, and on the differing biologies and histories of the three species of rats involved. Ian Atkinson of the New Zealand Department of Scientific and Industrial Research has now made a major contribu-