

ML.13-2 SITE-DIRECTED MUTAGENESIS AND STRUCTURAL STUDIES TO PROBE AND MODIFY ENZYME ACTION. By D.M.Blow, Blackett Laboratory, Imperial College, London SW7 2BZ, England.

Throughout biological history, until this decade, proteins have evolved by evolution and natural selection. For the first time, proteins can now be created or modified at will, limited only by our own powers of understanding.

Most of the understanding of protein structure has come from protein crystallography. Protein engineering provides a means to extend and deepen understanding of protein structure, protein folding and protein function.

The catalytic function of an enzyme is a particularly sensitive property, because enzyme kinetic parameters can be interpreted in terms of free energy changes during the catalytic process. A change in these parameters, caused by engineering a single amino-acid change, gives a direct measure of a change in the free energy of interaction at some point in the catalytic process. 'Calorimetry' of individual hydrogen bonds and other specific interactions is possible. Larger changes, involving whole sections of chain or domains, can also be studied; similarly the energy of quaternary interaction between protein monomers can be changed.

These interpretations rely on assumptions about the structure of the factitious mutant which can be checked crystallographically. Many small changes produce molecules which crystallise isomorphously with the wild-type enzyme. More radical changes will result in altered crystal structures which can be solved by molecular replacement techniques.

Protein engineering can be used to assist crystallography, by the elimination of a mobile domain or a site of glycosylation to produce better crystals, or by introduction of specific amino acids to provide sites for heavy atoms.

Work on tyrosyl-tRNA synthetase (with A.R.Fersht and G. Winter) is used to illustrate these points. Some of the possible useful applications of protein engineering are discussed.

ML.14-1 SURFACE ATOM LOCALIZATION BY X-RAY METHODS. By G. Materlik, Hamburger Synchrotronstrahlungslabor HASYLAB at DESY, Hamburg, F.R. Germany

For decades x-ray photons were thought of as being of far less use in surface crystallography than electrons or ions. Their comparatively low interaction cross section with atoms was the main reason for this judgement. The availability of highly intense synchrotron radiation sources, however, has drastically changed this situation. Techniques which were formerly only reserved for bulk studies, such as kinematical diffraction and diffuse scattering, have become applicable for surfaces as well and there are in addition even ways to study in a continuous fashion the geometry change from surface to bulk by adding to the diffraction process the additional feature of total external reflection.

Besides standard x-ray techniques new experimental approaches were also found and developed such as surface x-ray absorption spectroscopy and x-ray interference fields created by dynamical Bragg diffraction. These methods make use of information which is available in inelastic scattering channels of the photon-matter interaction. Characteristic fluorescence photons and photo- or Auger electrons are measured often in combination with the elastically scattered photons.

In many applications, the low cross section can be applied advantageously. For example, the kinematical theory can be used for many diffraction studies. Because of the large penetration depth, interfaces which are covered by an epitaxial layer or by a liquid can also be investigated.

A comprehensive review of the present status of x-ray surface crystallography will be presented.

ML.14-2 ZEOLITIC CATALYSTS: THE CHEMISTRY OF CAGES AND CHANNELS. By J.M. Thomas, Davy Faraday Research Laboratory, The Royal Institution of Great Britain, 21, Albemarle Street, London, W1X 4BS, U.K.

Zeolites differ from most other heterogeneous catalysts in that their active sites are uniformly distributed throughout the solid. And, in view of their exceptional microporosity, these sites are accessible only to those reactant species possessing the requisite molecular dimension. In many zeolites, most, if not all, of the constituent atoms are situated at interior surfaces so that, for this class of monophasic catalyst, the techniques of solid-state chemistry and crystallography are, by definition, surface techniques. Even so, conventional x-ray crystallographic methods are not, as we shall discuss, always applicable for the identification of new zeolitic catalysts or for evolving strategies for their synthesis. It is profitable to employ a range of other techniques including electron microscopy, solid-state NMR, neutron scattering and computational procedures. The lecture will illustrate, with the aid of specific examples, the advantages of adopting such a multiple approach.

ML.15-1 SMALL MOLECULE CRYSTALLOGRAPHY: INSIGHT INTO BIOLOGICAL ACTIVITY. Jenny P. Glusker, The Institute for Cancer Research, The Fox Chase Cancer Center, Philadelphia, PA 19111, U.S.A.

The manner in which the knowledge of the three-dimensional structures of certain biologically-active small molecules, as determined by X-ray crystallographic studies, has aided in our understanding of the nature of their biological activity is the subject of this lecture. Some techniques that have been used to analyze such structural results in terms of biological relevance include factor analysis, distance matrix analysis, accessibility studies, molecular mechanics and energy minimization studies. These will be described and their usefulness assessed.

Examples that illustrate these techniques include analyses of some citrate-utilizing enzymes (absolute configuration and fluorine versus hydrogen or hydroxyl substitution), dihydrofolate reductase (absolute configuration and "wrong-way binding"), some steroid-utilizing enzymes (comparisons of substrates and inhibitors), peptide conformation and binding (enkephalins and their comparisons with morphine, thyrotropin-releasing hormone analogs) and vitamin B₁₂ (steric effects and bond stability). Work on nucleic acids to be described includes recent studies of base-pair mismatches that lead to mutations and of nucleic acid interactions with drugs (such as ledakrin analogs) and carcinogens.

The overall insight gained from structural studies of small molecules by X-ray methods can lead to some general principles for the modelling of ligand-receptor interactions, for the design of new biologically-active agents and for an understanding, on an atomic scale, of the stereochemistry of some biologically significant processes.