2   Main Lectures

ML–01.03   TIME-RESOLVED PROTEIN
                         CRYSTALLOGRAPHY

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A complete understanding of mechanism at the molecular
level demands knowledge, not just of long-lived, readily
observable structures, but also of short-lived intermediates
in processes such as catalysis, ligand binding and release,
protein unfolding, and phototransduction.   Can x-ray
crystallography, long regarded as a static technique, in fact
encompass   dynamic   processes?   A   time-resolved
crystallographic experiment (Moffat, K. (1989) *Ann. Rev.
Biophys. Biophys. Chem.* **18**, 309-332.; Time-Resolved
Macromolecular Crystallography (Ed. D.W.J. Cruickshank,
J.R. Helliwell and L.N. Johnson), Oxford University Press
(1992)) has five main components: the x-ray source;
reaction initiation; reaction monitoring by optical or other
techniques; x-ray data acquisition in real time; and data
reduction and analysis. These are illustrated by our recent
work on photoactive yellow protein, on protein unfolding,
and on the acquisition and analysis of crystallographic data
using 120 ps exposures (Szebenyi, D.M.E., Bilderback,
D.H., LeGrand, A., Moffat, K., Schildkamp, W., Smith
Temple, B. and Teng, T.Y. (1992) *J. Appl. Cryst.* **25**, 414-
423).

ML–02.01   DIRECT METHODS IN SUPERSPACE.
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    With conventional direct methods and many other methods
for single-crystal structure analysis, the sample is treated as an
ideal crystal, which possesses exact three-dimensional periodicity.
However an important category of crystals, incommensurate crys-
tals, do not have three-dimensional periodicity. According to the
representation of de Wolff [*Acta Cryst.* A30 (1974) 777-785] and
Janner & Janssen [*Acta Cryst.* A36 (1980) 399-408; 408-415],
incommensurate crystals are regarded as the three-dimensional
hypersection of a higher-dimensional periodic structure. Based
on this representation a multi-dimensional direct-method has been
developed. With this method incommensurate crystals are first
treated in multi-dimensional space to solve the phase problem
and then by cutting the resultant multi-dimensional Fourier map
with the three-dimensional physical space to obtain the structure.
The phasing procedure is divided in two stages. In the first
stage phases of main reflections are developed using a modified
Sayre equation involving only main reflections.   While in the
second stage phases of the satellite reflections are derived based on
another modified Sayre equation, which relates phases of satellites
with those of main reflections. In contrast to other methods of
solving incommensurate structures, no preliminary assumption
on either the corresponding basic structure or the modulation
form is needed through out the process. The method has been
successfully applied to X-ray as well as electron diffraction data
and has been incorporated with an image processing technique
in high resolution electron microscopy. Examples are given on
the study of incommensurate modulation in the Bi-based high Tc
superconductors.

ML–03.01   CONTRIBUTION OF CRYSTALLOGRAPHY TO
                         THE ELUCIDATION OF HYDROLYTIC ENZYME
MECHANISMS. Michael N.G. James, Medical Research
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    Biopolymers such as proteins, nucleic acids and
polysaccharides are stable chemical entities that are
resistant to hydrolytic cleavage into their constituent
building blocks.   However, the dictates of various
cellular metabolic processes requires the breakdown of
the biopolymers once they have served their use.
Enzymes   such   as   proteinases,   nucleases   and
glycosidases have evolved to catalyze the degradation
of those macromolecules. The hydrolytic cleavage of
proteins and peptides is carried out by proteolytic
enzymes.   They have been categorized into four
separate classes, namely the serine, aspartic, metallo-
and cysteine proteinases. There is a wealth of structural
information for various members of each of these
enzyme families. Hydrolytic mechanisms have been
deduced from the vast biochemical and kinetic
literature coupled closely with the structural data
provided by high-resolution X-ray crystallographic
studies of the uncomplexed native enzymes as well as
those of enzyme-inhibitor or enzyme-transition state
analogue complexes.   Both the serine and cysteine
proteinases have covalently attached acyl-enzyme
intermediates on their reaction pathways whereas the
aspartic and metalloproteinases catalyze the hydrolysis
without involving covalently attached intermediates.
Hydrolysis of peptide bonds involves an attack by an
appropriate nucleophile on the carbonyl-carbon atom
of the substrate with the resultant formation of a
transitory tetrahedral intermediate. Generation of the
attacking nucleophile from water or an aminoacid side
chain is assisted by a general base which subsequently
transfers the abstracted proton to the nitrogen of the
leaving group. Electrophilic assistance for the nucleo-
philic attack is also accompanied by the stabilization of
the developing negative charge on the carbonyl-oxygen
atom of the tetrahedral intermediate. The detailed
stereochemistry of the formation and breakdown of the
tetrahedral transition state of the hydrolytic reaction is
established.   Comparison of the structures of the
enzymes in the four proteinase classes shows that the
serine, aspartic and metalloproteinases have converged
on analogous hydrolytic mechanisms in which nucleo-
philic attack of the peptide carbonyl-carbon atom is on
the *re* face. In contrast, the cysteine proteinases of
known structure likely have the thiolate attack on the
si face of the peptide bond. For the proteinases, only
serine residues, cysteine residues and water molecules
are used to generate the attacking nucleophiles.
Imidazole rings and carboxylate groups are used in the
role of general base.   Electrophilic assistance to
hydrolysis is provided by a wide variety of functional
groups; even metal cofactors (*e.g.*, Zn^{++}) have been
recruited to this role. (Research supported by the MRC
Canada.)