(F1 ATPase)
X-RAY CRYSTALLOGRAPHY

A major technique that has been used to discover the three-dimensional structure of protein molecules, at atomic resolution, is x-ray crystallography.

X-rays, like light, are a form of electromagnetic radiation, but with a very small wavelength. If a narrow parallel beam of x-rays is directed at a well-ordered crystal of a pure protein, some of the beam will be scattered by the atoms in the crystal. The scattered waves will reinforce one another at certain points and will appear as a pattern of diffraction spots when the x-rays are recorded by a suitable detector.

The position and intensity of each spot in the x-ray diffraction pattern contains information about the position of the atoms in the protein crystal that gave rise to it. Computers can use this information to provide a three-dimensional electron density map of the protein molecule, which, together with the sequence of the protein, can be used to produce an atomic model. The complete atomic model is often hard to interpret, so simplified versions are derived that show the essential structural features (see Panel 5-2, pp. 142–143). The protein shown here is ribulose bisphosphate carboxylase, an enzyme that plays a central role in CO₂ fixation during photosynthesis (alpha helices are shown in green, and beta strands in red).
NMR SPECTROSCOPY

Nuclear magnetic resonance (NMR) spectroscopy has been used in the past to analyze the structure of small molecules and now is increasingly used to study the structure of small proteins or protein domains. The technique requires only a small volume of concentrated pure protein solution.

(A) (Courtesy of P. Kraulis.) (B)

The solution is placed in a strong magnetic field and subjected to radio frequency pulses of electromagnetic radiation. Signals from hydrogen nuclei in different amino acids can be identified that allow the distances between interacting pairs of hydrogen atoms to be measured. NMR gives information about the distances between the parts of a protein molecule, and by combining this with a knowledge of the amino acid sequence, it is possible to compute the 3-D structure of the protein. Only the structure of small proteins (20,000 daltons or less) can be determined by NMR spectroscopy.

In (A), a 2-D NMR spectrum derived from the carboxyl-terminal domain of the enzyme cellulase is shown. The spots represent interactions between neighboring H atoms. The resultant structures that satisfy the distance constraints equally well are shown in (B).
HEMOGLOBIN ALPHA CHAIN
HUMAN  142 aa

mvlspadktn vkaawgkvgahageygaearmlfsfpttktyfphfdlshgsaqvkghg
kkvadaltnavahvddmpnalsaldlhhaklrvdpvnflllshcllvtaahlpaeftp
avhasldkflasvstvltskyr

HEMOGLOBIN BETA CHAIN
HUMAN  147 aa

mvhltpeeksavtalwgkvnvedvggealgrllvvpwtqrffesfgdltpdavmgnpkvkahgkkvlgafsdglahdlbnlkgtfatls
elhcdklhvedpenfrllgvnlvcvlahhfgkeftpqpqaaaaqkvvagvanalahkyh

(sickle cell hemoglobin has Valine in place of Glutamic Acid as underlined amino acid in the beta chain)
Figure 4-30  Essential Cell Biology, 2/e. (© 2004 Garland Science)
amino acid side chains

unfolded protein

FOLDING

binding site

folded protein

hydrogen bond

cyclic AMP

(A)

(B)
(A) enzyme binds to two substrate molecules and orients them precisely to encourage a reaction to occur between them.

(B) binding of substrate to enzyme rearranges electrons in the substrate, creating partial negative and positive charges that favor a reaction.

(C) enzyme strains the bound substrate molecule, forcing it toward a transition state to favor a reaction.