ROSE-HULMAN INSTITUTE OF TECHNOLOGY

CHEMISTRY AND BIOCHEMISTRY

Protein Structure

(What do proteins ***really*** look like?)

Methods

X-ray Crystallography

NMR

Structure Prediction

- Sequence-based (*Ab initio*)

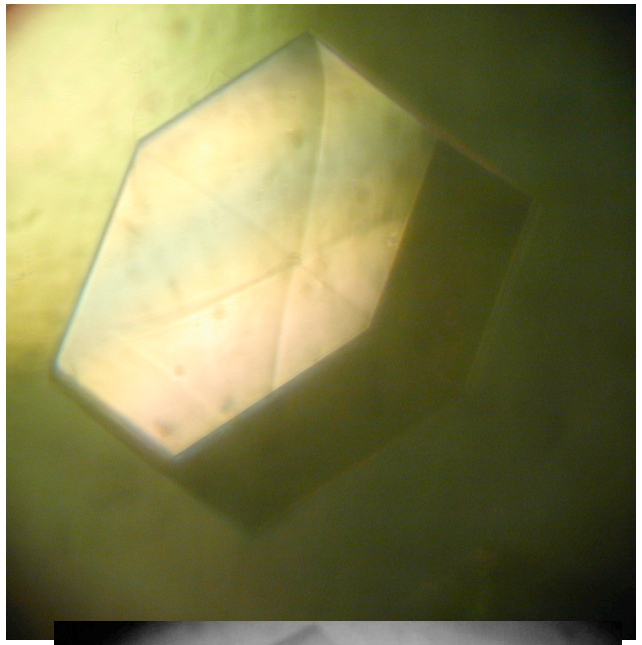
- Homology Modeling

X-ray Crystallography

1. Making protein

2. Making crystals

3. Diffraction quality crystals



Sample: pER3Ø4 fusion +E2 Prep #16-19 Box # 2

Date: 12/12/96

Comments: Screen Kit I 1:2 (25-48)

Name:

[Protein]: 5 mg/ml Temp: Time: 4 pm

Drop Vol: 2+2 µl

	1	2	3	4	5	6
A				28 granular ppt 4:30 still granular 12/13 11:20 AM		36 veil/like ppt 430
B	31 Veil like ppt	chunk + speckles	spindles	chunks + speckles 12/19	[illegible]	[illegible] + chunks
C		sparse clear dirt	Triangular crystalline dirt spider web 6/3/97	40 oil 12/14/96 1:40 pm	dirt on bottom speckles + oil? (sparse) 12/19	42 veil like ppt 430 cleared 12/16 (clear [illegible] + dust) oil droplets 12/19
D	43 granular ppt 12/13 11:25 AM	dust on bottom clear speckles 12/19	45 veil-like ppt 430 in ~½	dust on bottom many speckles 12/19	46 oil? 430 more oil 12/14/96 1:40 pm	dust on bottom many speckles 12/19

Switched solutions 46 and 47

DISCARDED 6/3/97

Note bottom of a number of drops have small amounts of granular material → seems to be increasing w/time

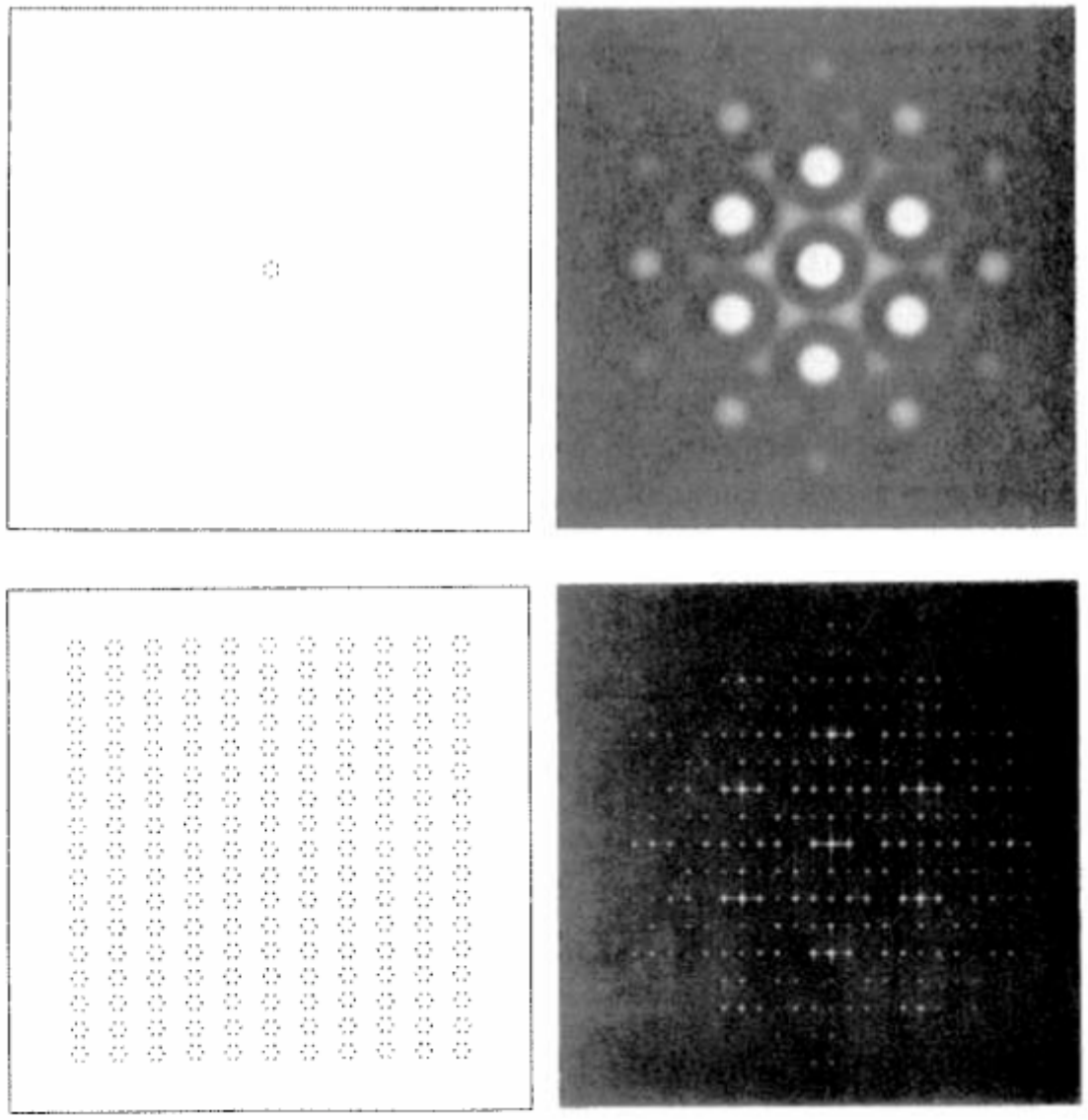
X-ray Crystallography

1. Diffractometers

2. Synchrotron radiation

Aerial View of SLAC. (Photo Courtesy of Stanford Linear Accelerator Center)

Diffraction



From: C.R. Cantor & P.R. Schimmel *Biophysical Chemistry Part II: Techniques for the study of biological structure and function*. W.H. Freeman & Company, 1980

Crystals

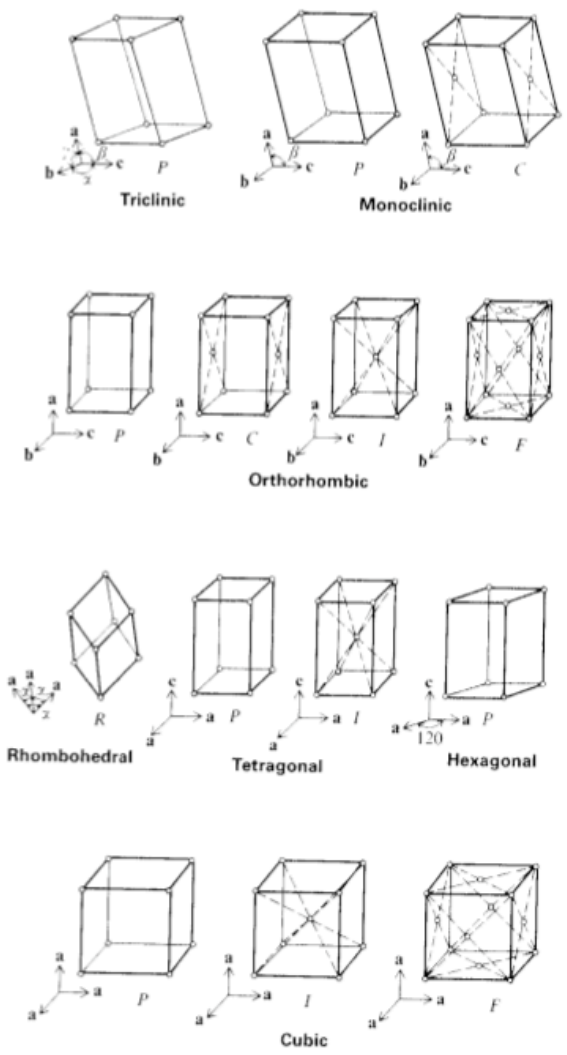


Figure 13-16

The fourteen Bravais lattices. For a list of their properties, see Table 13-1. [After G. H. Stout and L. M. Jensen, *X-Ray Structure Determination* (New York: Macmillan, 1968).]

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Diffraction

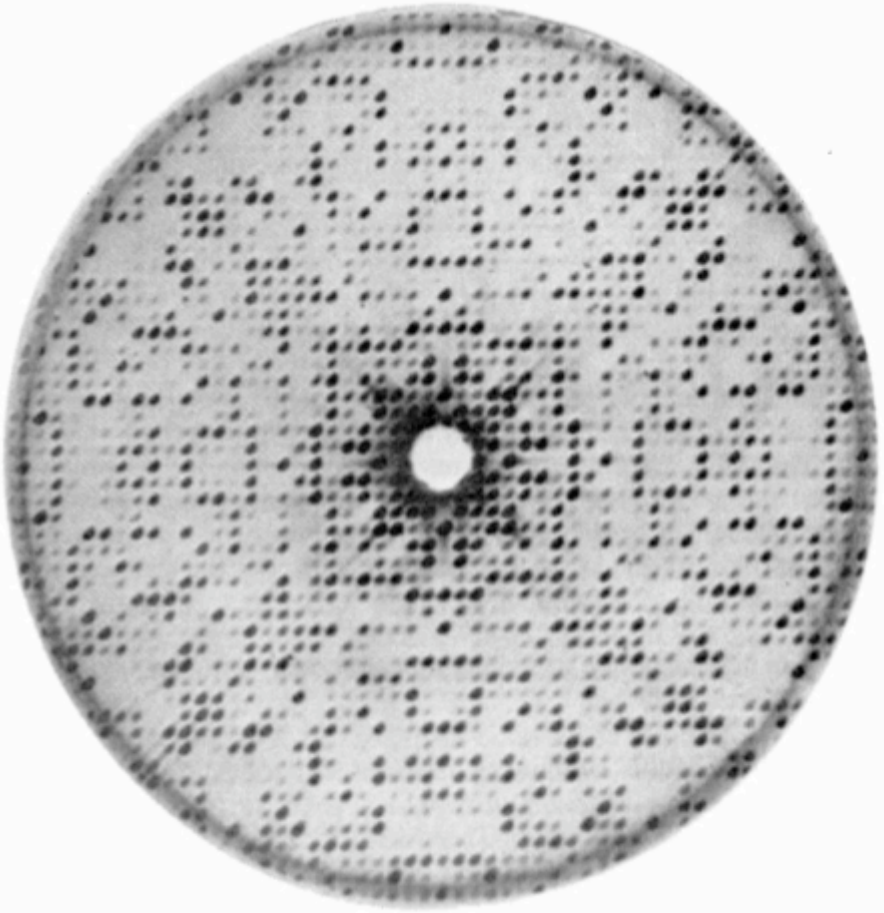

Figure 13-26

A precession photograph. An entire plane of the reciprocal lattice is displayed without distortion. The sample is a tetragonal crystal of lysozyme. Note the presence of a fourfold rotation axis and various mirror-symmetry planes in the diffraction pattern. [Courtesy of C. C. F. Blake.]

Unit cell Structure Factor:

$$F_m(h,k,l) = \sum_j f_j(S) e^{2\pi i(hx_j + ky_j + lz_j)}$$

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Phase

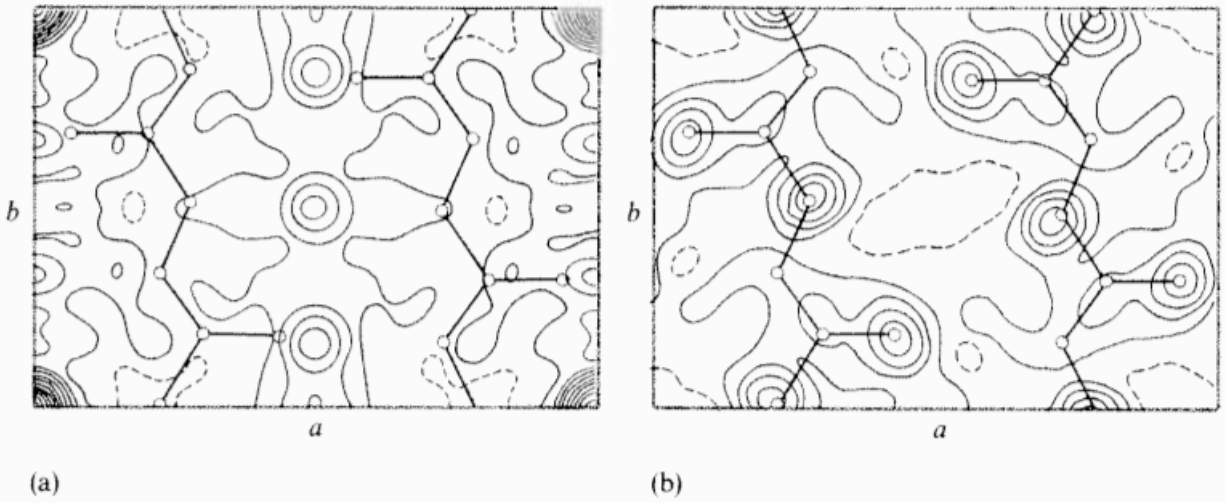


Figure 13-28

Relative importance of intensities and phases in computing an electron density map from diffraction data. The sample and data are the same as those shown in Figure 13-27. **(a)** Correct amplitudes were used in this Fourier synthesis, but all phases were set equal to zero. **(b)** Correct phases were used in this Fourier synthesis, but all amplitudes were set equal to the same average value. [After R. D. B. Fraser and T. P. MacRae, in *Physical Principles and Techniques of Protein Chemistry*, part A, ed. S. J. Leach (New York: Academic Press, 1969).]

$$F = \left|F\right|e^{i\phi} = F_r + iF_i$$

From: C.R. Cantor & P.R. Schimmel *Biophysical Chemistry Part II: Techniques for the study of biological structure and function*. W.H. Freeman & Company, 1980

Phasing

1. Multiple Isomorphous Replacement (MIR)

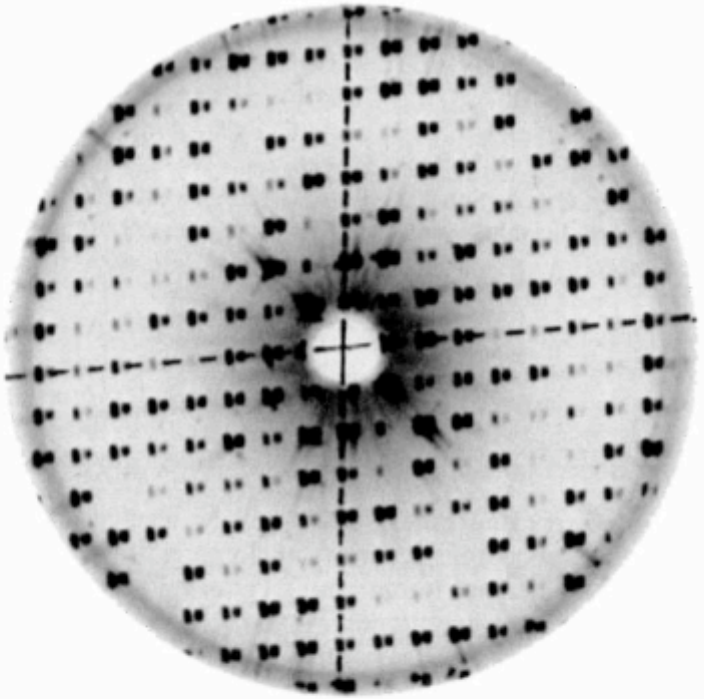


Figure 13-33

Isomorphous replacement. Two precession photographs of triclinic lysozyme crystals are superimposed, slightly out of horizontal register. The left spot of each pair is from a native lysozyme crystal; the right spot is from a crystal after diffusion of $HgBr_2$. This is a photograph of the $(0, k, l)$ plane of the reciprocal lattice. Note the differences in intensities. [From R. Dickerson, in *The Proteins*, 2d ed., vol. 2, ed. H. Neurath (New York: Academic Press, 1964).]

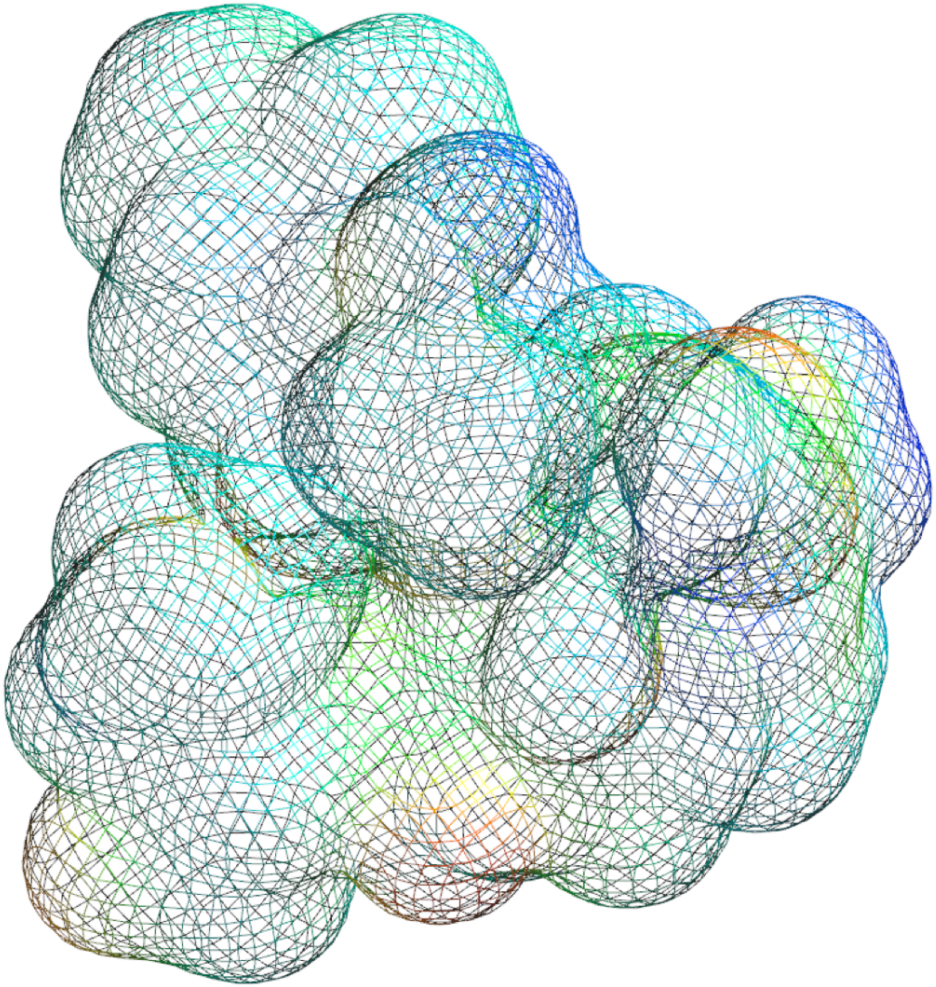
From: C.R. Cantor & P.R. Schimmel *Biophysical Chemistry Part II: Techniques for the study of biological structure and function*. W.H. Freeman & Company, 1980

Phasing

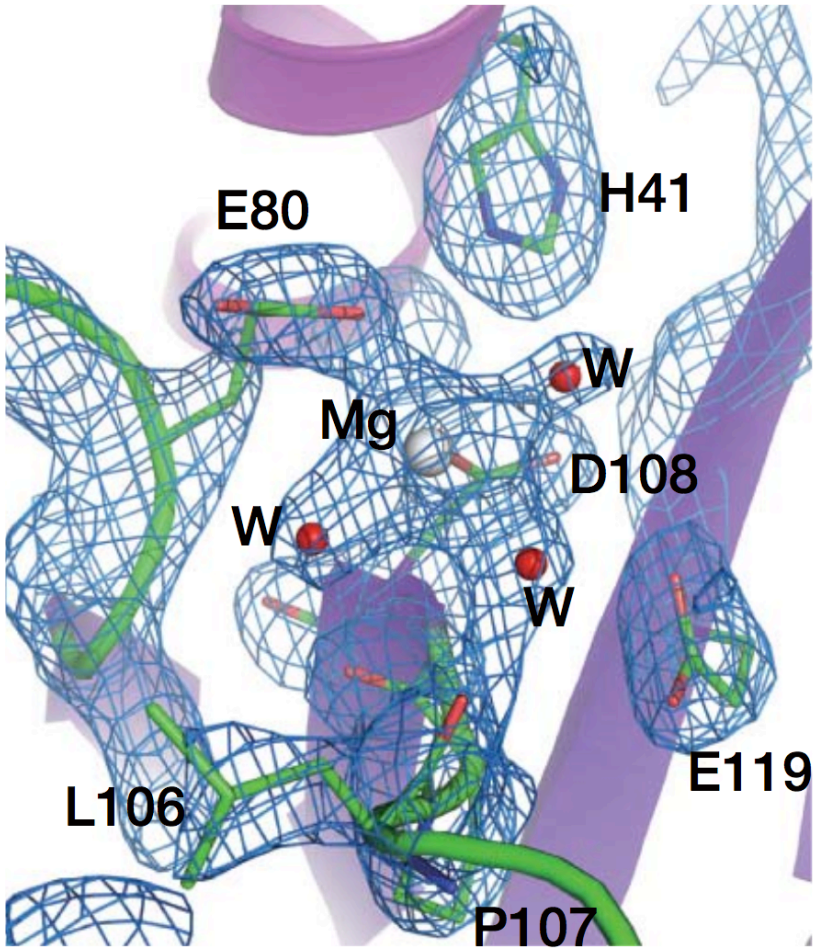
2. Multiwavelength Anomalous Diffraction (MAD)

3. Molecular Replacement

Model Building



Model Building



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R-factor

$$R = \frac{\sum \left| \left| F_{obs} \right| - \left| F_{calc} \right| \right|}{\sum \left| F_{obs} \right|}$$