

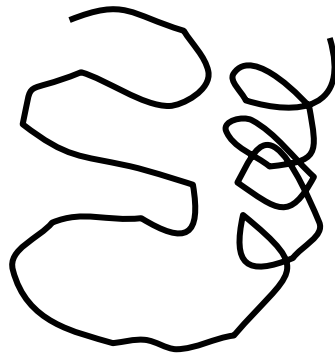
Protein Structure Determination

An Introduction

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What is "Protein Structure?"

...Arg-Lys-Ala-Gln-Trp-Cys-His-Ala-Asp...



What is “Protein Structure?”

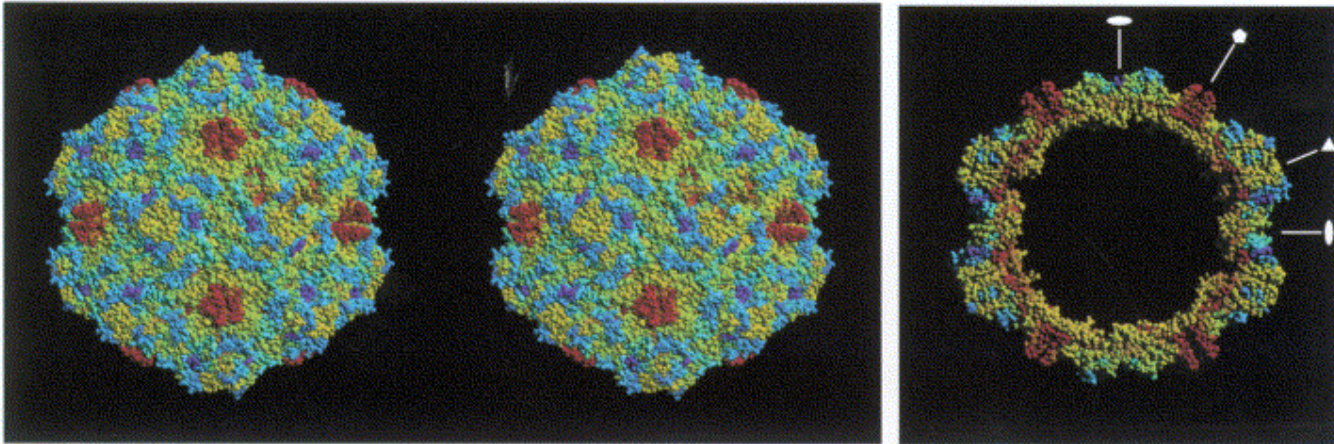
- It is the description of the three-dimensional architecture of a polypeptide.
- Our aim: to know accurately and precisely the position of every atom in a protein. That’s usually between 2000 and 10,000 atoms!

Wherefore Art Thou, Protein Structure?

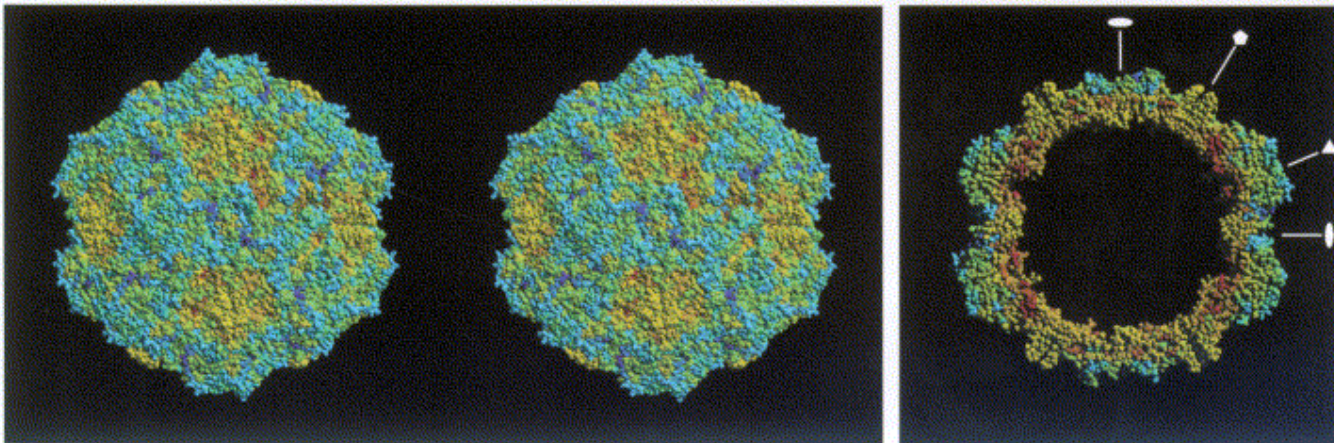
- To know a protein's shape is to get a glimpse of its function.

Sometimes, Structure = Function

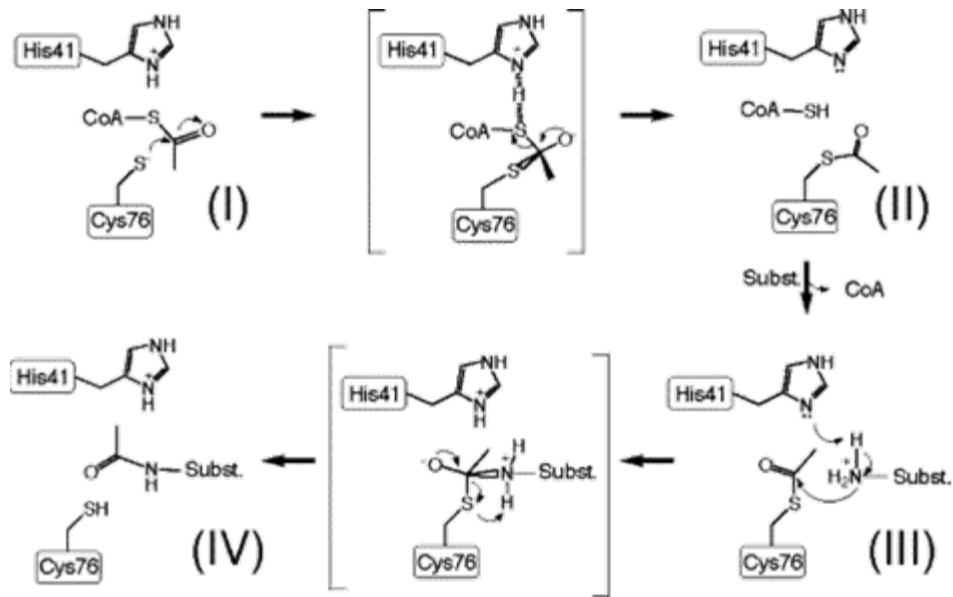
a.



b.



Other Times, Structure Suggests Function



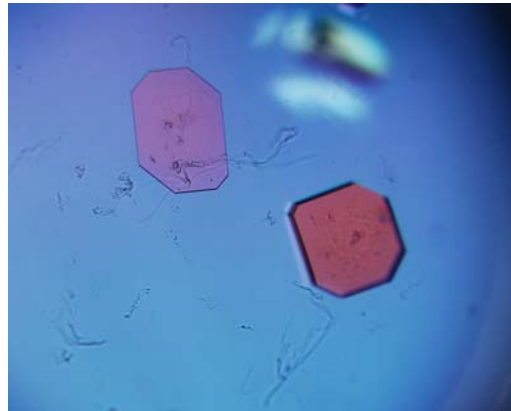
A structure can give us some wonderful things, i.e. testable hypotheses!

How Do We Obtain Protein Structures?

- Nuclear Magnetic Resonance (similar to MRI)
- Electron Crystallography
- Neutron Crystallography
- X-ray Crystallography

In Order to Do Protein
Crystallography, We
Need...

Protein Crystals!



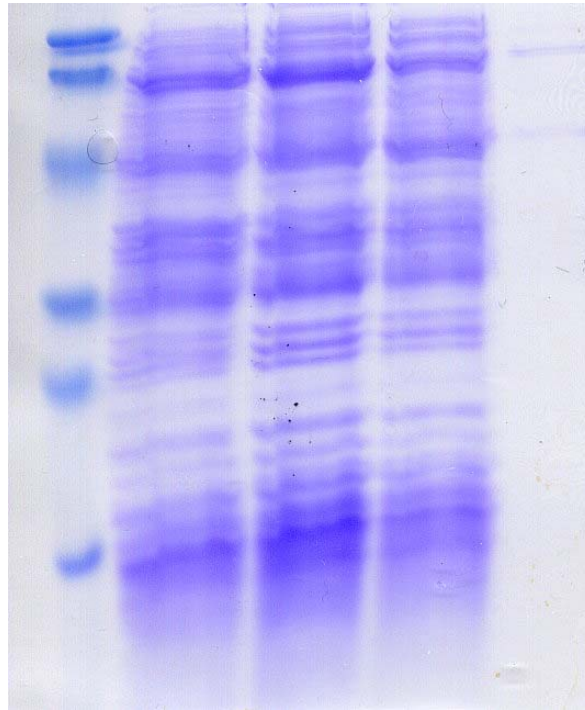
Protein Crystals

In order to obtain crystals of a protein, it is almost always necessary to have **pure protein**.

Protein Sources

- Animal, plant, or microbial tissues or cultures.
- Companies.
- Overexpression.
 - *E. coli*.
 - Insect cells.
 - Mammalian cell culture.

Protein Purification



Protein Purification

- We use the physical properties of the protein of interest to separate it from all contaminants.
- Chief among these properties are:
 - Charge.
 - Size.

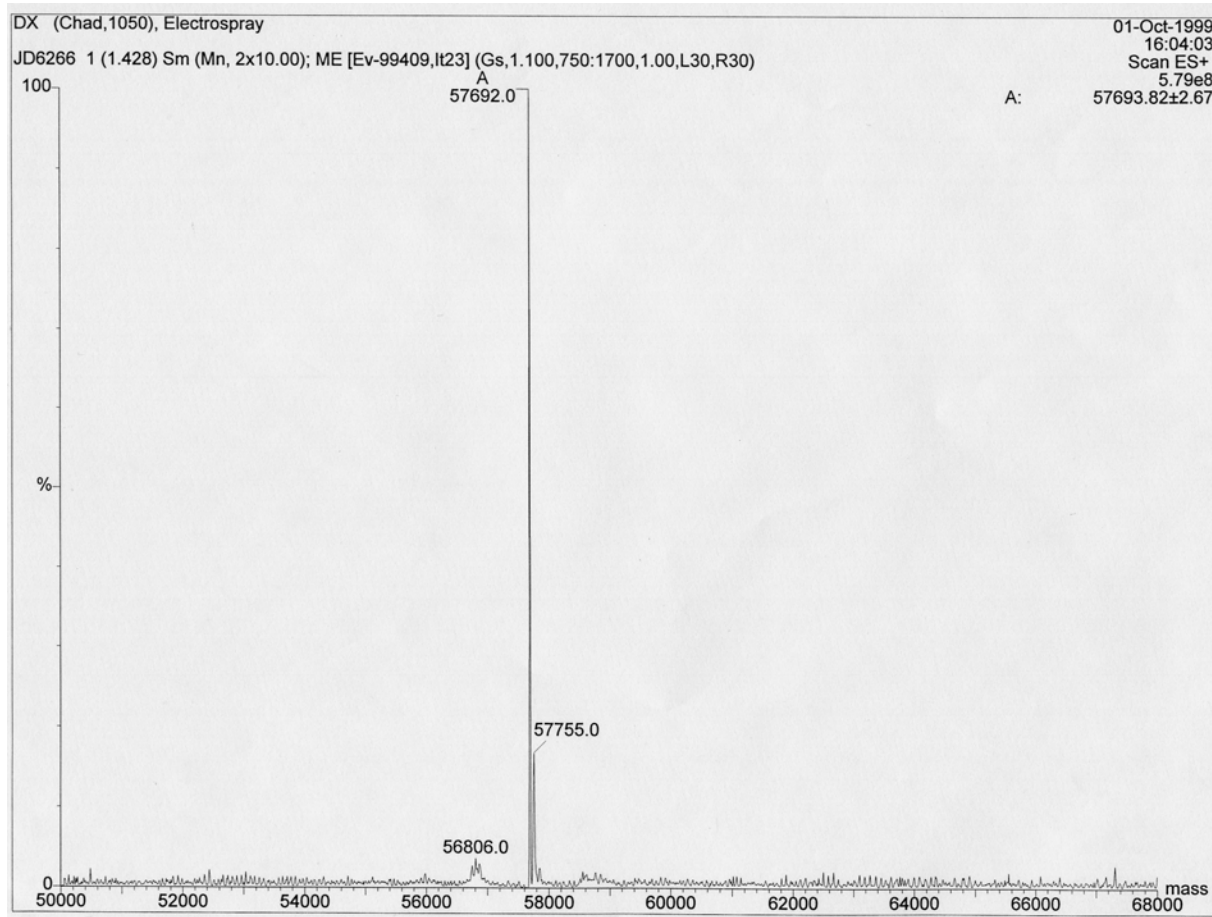
Quality Assessment

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Quality Assessment

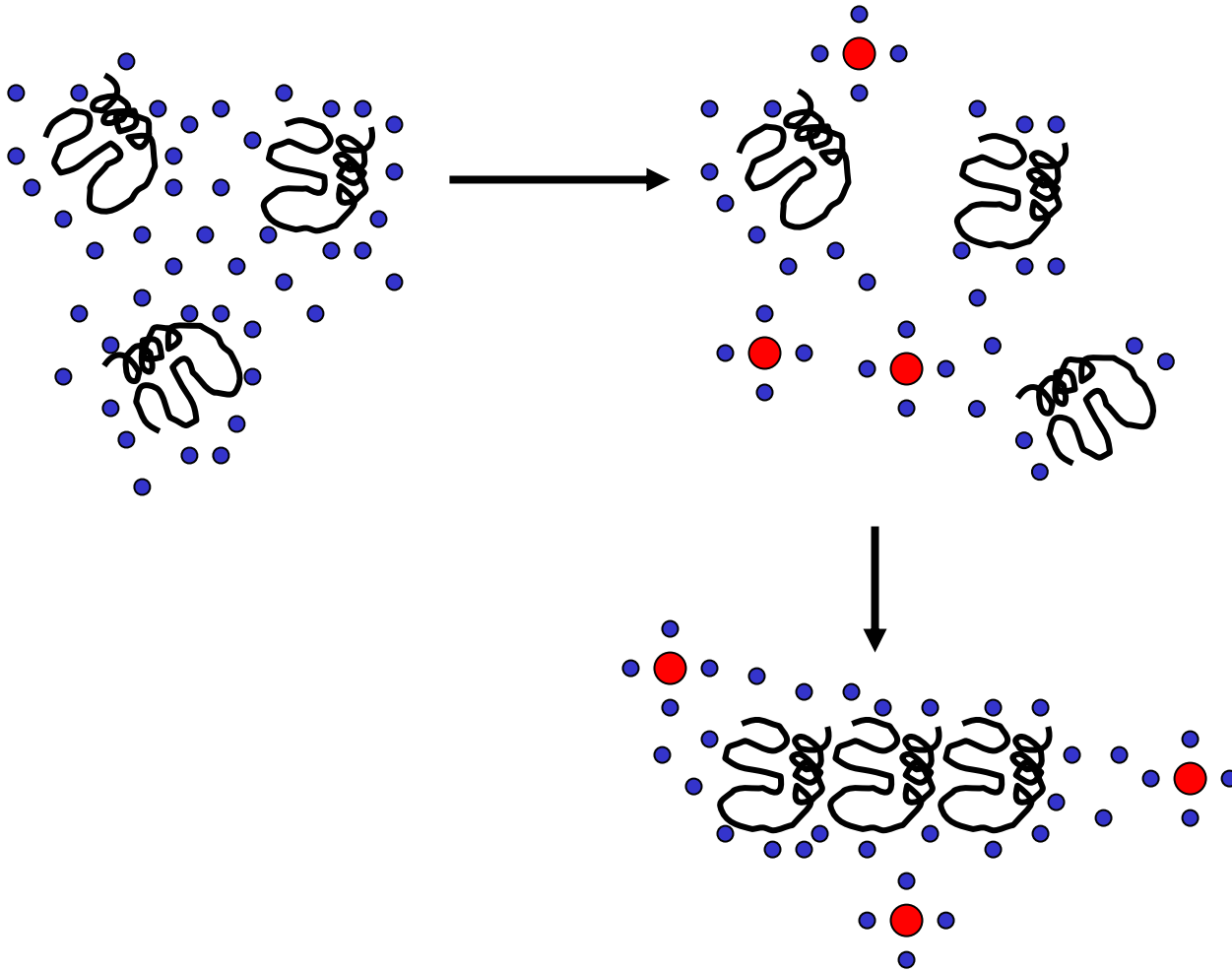
Mass Spectrometry



Now That We Have Pure
Protein...

How do we crystallize it?

Protein Crystallization



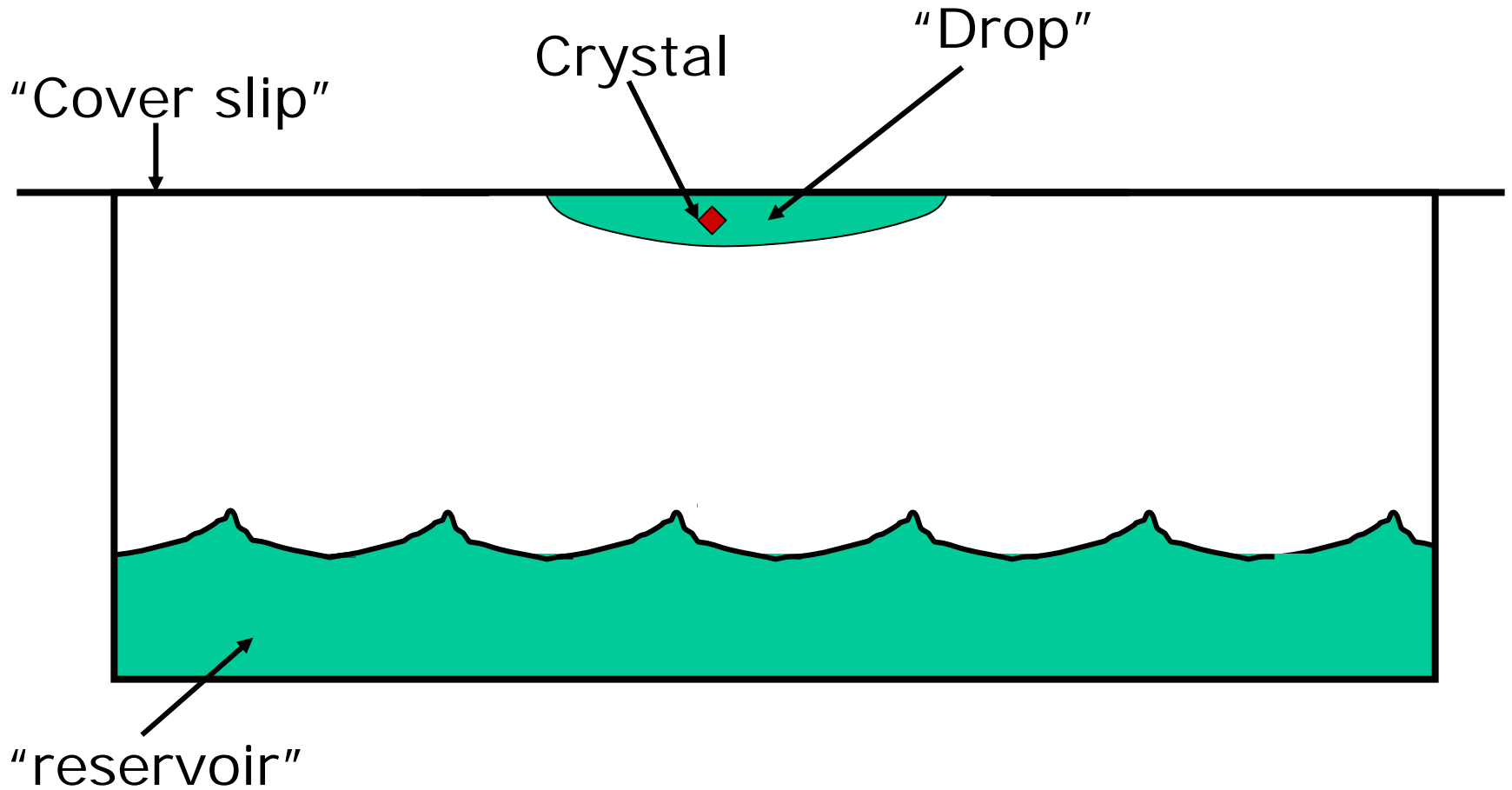
Some Common Water-Withdrawing Chemicals (Precipitants)

- Polyethylene Glycol.
- Ammonium Sulfate.
- Sodium Chloride.
- 1,4 Methyl Pentane Diol.
- Ethyl Alcohol.

Enough Theory-
How Do We Actually Do
This?

Vapor Diffusion!

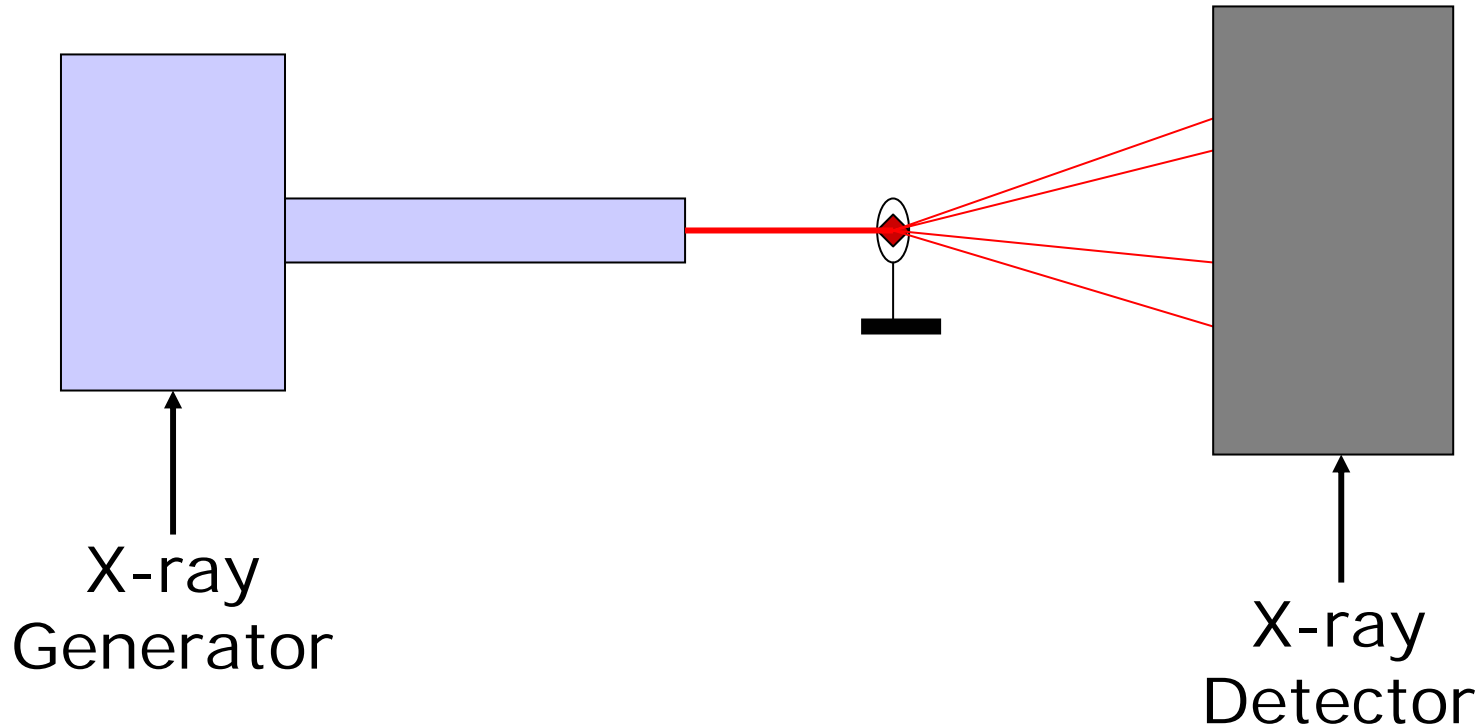
Vapor Diffusion



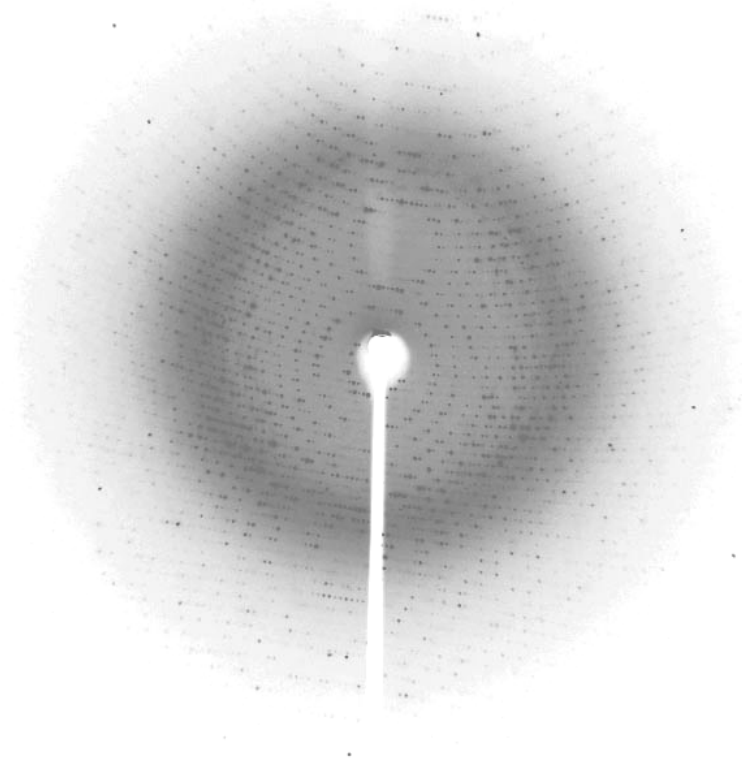
In Most Cases, Protein Crystals Are Not an End

They are the means by which we can determine a protein's structure.

X-ray Diffraction by Crystals

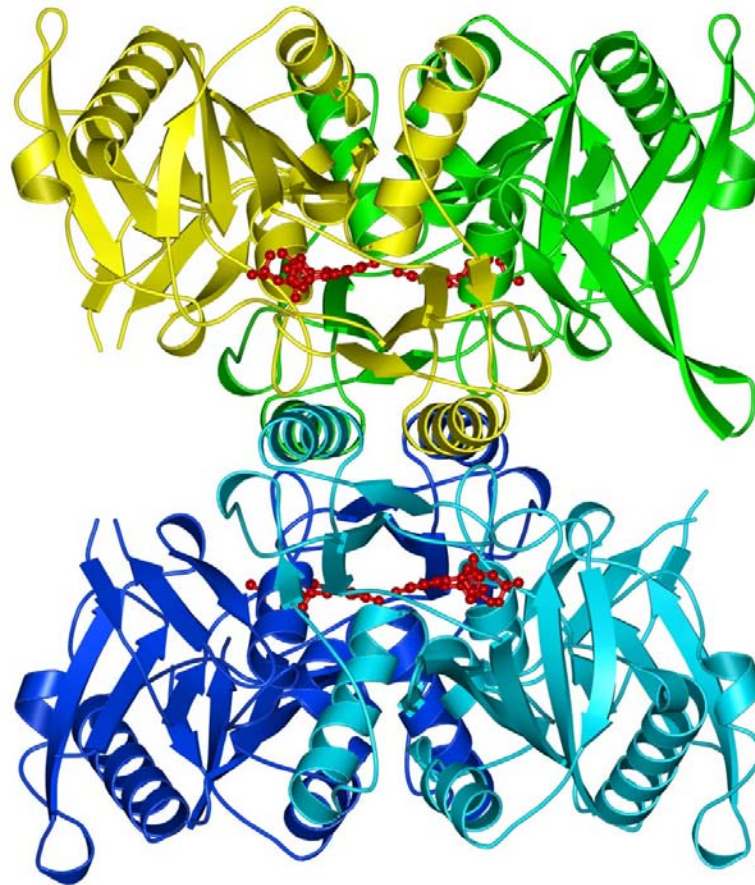


An X-Ray Diffraction Pattern

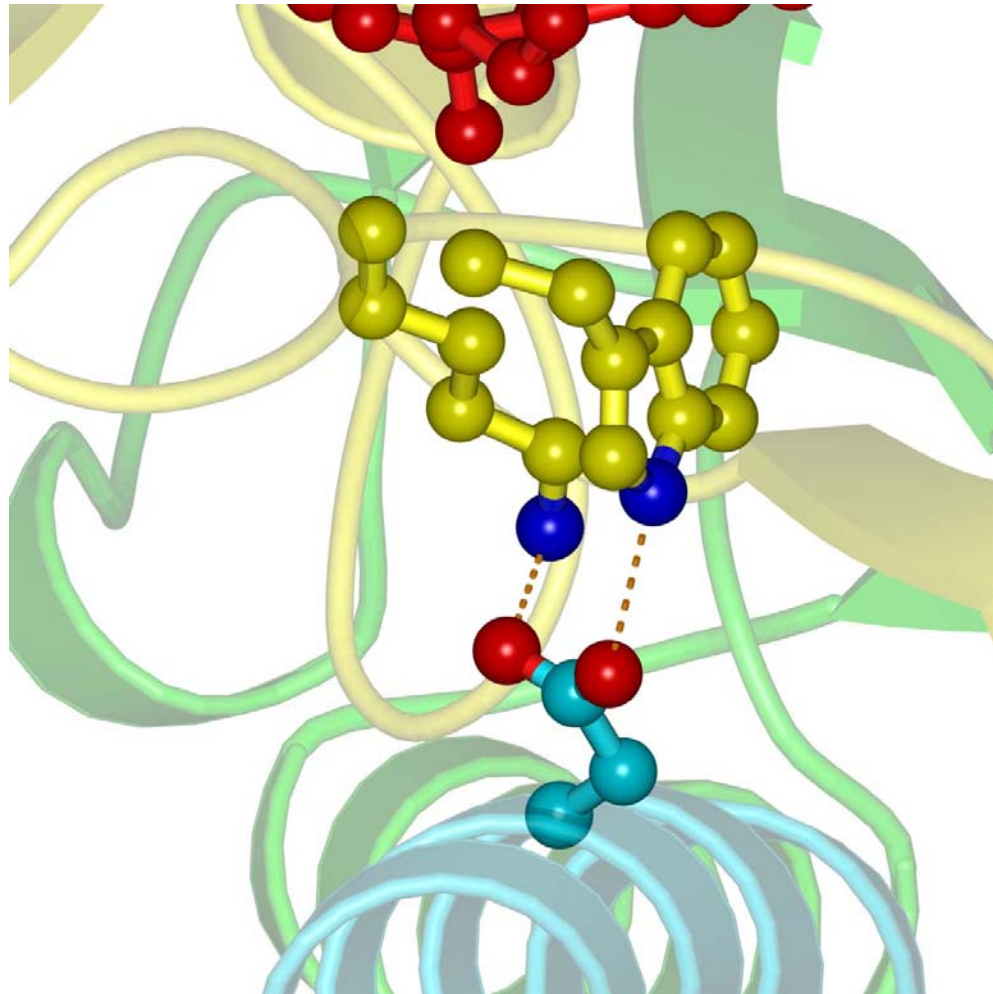


Electron Density

Analysis of the Protein Structure



Analysis of the Protein Structure



Analysis of the Protein Structure

