Mathematisch-Naturwissenschaftliche Fakultät

Institut für Chemie

Abteilung Anorganische Festkörperchemie

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Vorlesung

Anorganische Chemie VI – Materialdesign

Heute: Proteinkristallographie

Röntgenstrahl

Reflexmuster

gemessene Intensitäten

Position der Reflexe

direkte Methoden/
Patterson Methode

Kristall-Symmetrie

Zelldimensionen

korrigierte Intensitäten

Modell

Fourier-transformation

Amplituden + Phasen (F_o)

Vergleich

neue Amplituden + Phasen (F_o)

Elektronen-dichte

Fourier-transformation

endgültiges Modell

Verbessertes Modell

Fourier-transformation

Phase problem

pPIC9K
9.3 kb

Construct → Pure protein → Crystal → X-ray Diffraction → Electron density → 3D structure

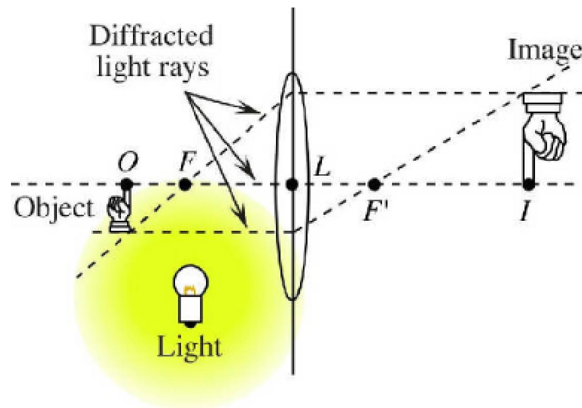
Proteinkristallographie

Nobelpreise für die Aufklärung von Röntgenstrukturen von Biomolekülen:

- 1946 J. B. Sumner (u.a.), Kristallisation von Enzymen
- 1953 DNA-Struktur, Watson & Crick
- 1958 Myoglobin und Haemoglobin, Kendraw & Perutz
- 1958 F. Sanger, Proteinstrukturen, z.B. vom Insulin
- 1962 J. C. Kendrew, M. F. Perutz, Strukturen von Globulin-Proteinen
- 1964 D. Crowfoot-Hodgkin, Kristallstrukturbestimmung biologisch wichtiger Substanzen
- 1982 A. Klug, Kristallographische Methoden zur Strukturbestimmung von Proteinen
- 1985 H. A. Hauptman, J. Karle, Direkte Methoden zur Strukturlösung
- 1988 J. Deisenhofer, R. Huber, H. Michel, Strukturbestimmung des photosynthetischen Reaktionszentrums
- 2000 Röntgenstruktur der Ribosom-Untereinheit

Why X-ray crystallography?

(Light) microscope:



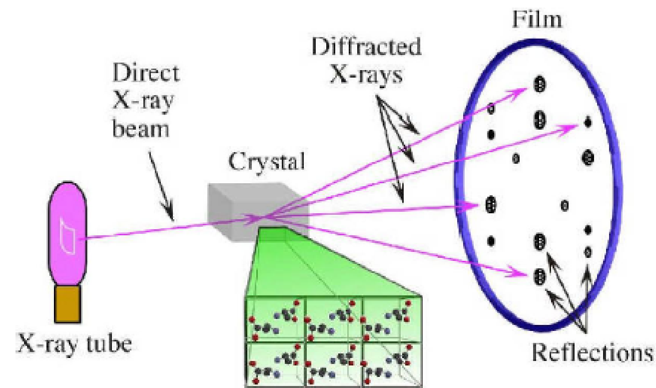
Limitations:

Object needs to be larger than the wavelength of the light (visible light 400-700 nm, atoms = 0.15 nm apart)

X-rays(0.08-0.6 nm) cannot be focussed by lenses

Molecules are very weak scatterers

X-ray diffraction of a crystal

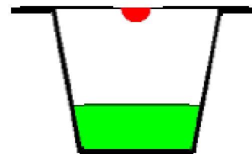


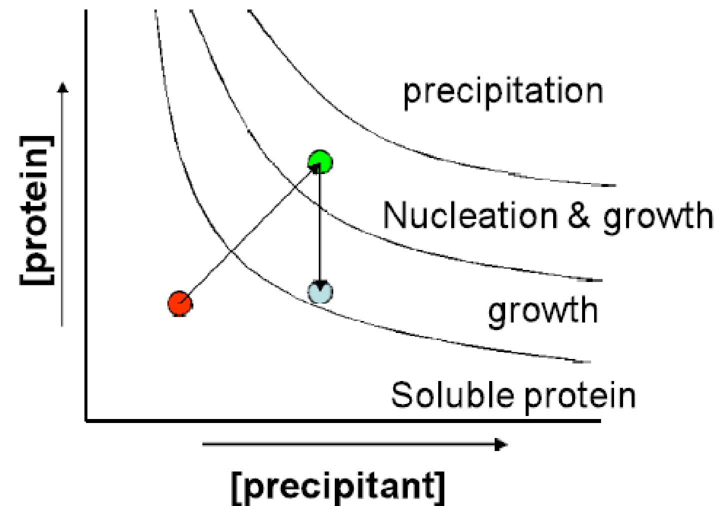
A crystal contains many molecules in identical orientation

Diffracted x-rays of individual molecules 'add up' (positive interference) to produce strong reflections

Computers can simulate a lens and reconstruc the image (Fourier transform)

Growing crystals

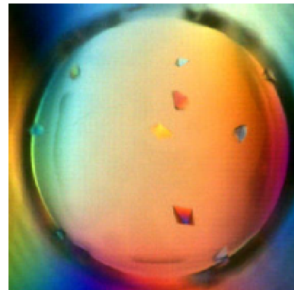


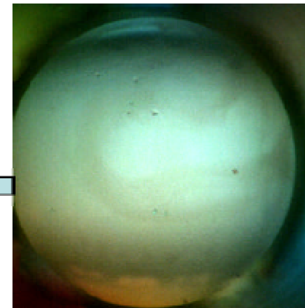


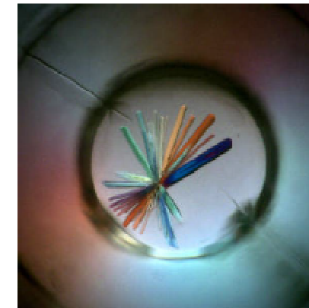
Hanging drop: 1 µl protein solution+ 1 µl reservoir solution

Reservoir: precipitant solution eg. 1 M NaCl or 30% PEG-4k

[precipitant] and [protein] slowly rise as drop equilibrates with reservoir

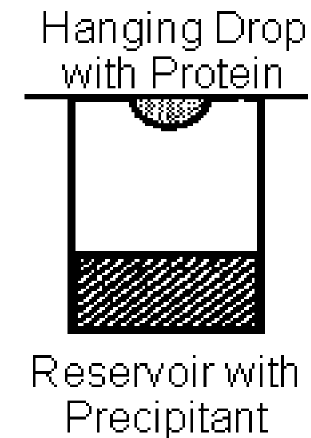






Limitations and Difficulties, Besides the Phase Problem

- Crystallizing Protein:
  - Fragile
  - Requires a crystal with shortest side 0.2 mm

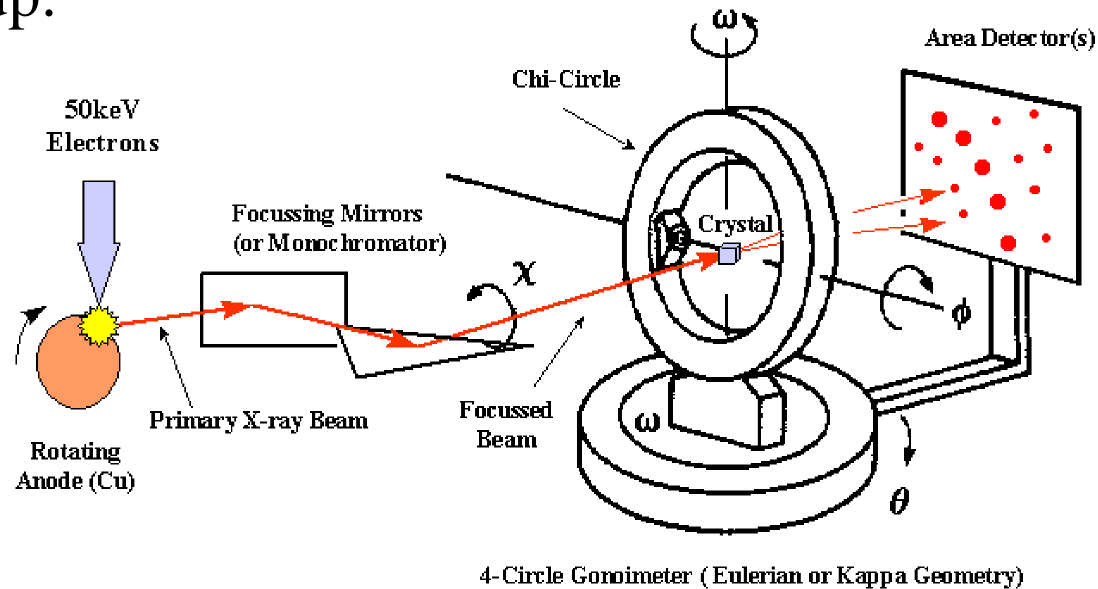


- Flaws of Crystallization:
  - Disorder in Unit Cell
  - Vibrations of molecules
  - Distortion in Crystallization

<u>Fix-Its:</u>

Cryogenic Cooling

The Setup:



- X-ray Source: x-ray tubes, rotating anode tubes, or particle storage rings
- Gonoimeter
- Detectors: X-ray films, CCD cameras, or Multiwire detectors.

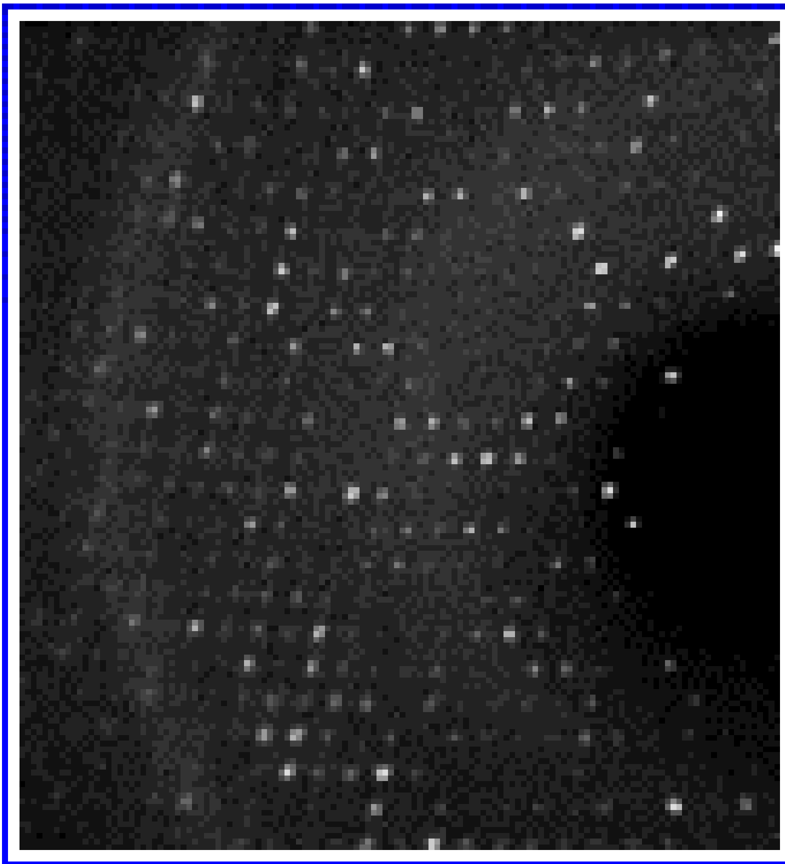
Getting your data

X-ray data are measured on frozen crystals (~100K)

In house X-ray data collection set up

For high quality X-ray data collection extremely intense synchrotron beam lines - like here in Grenoble - are used

The Raw Data

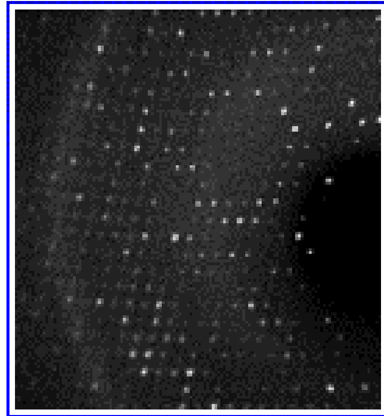


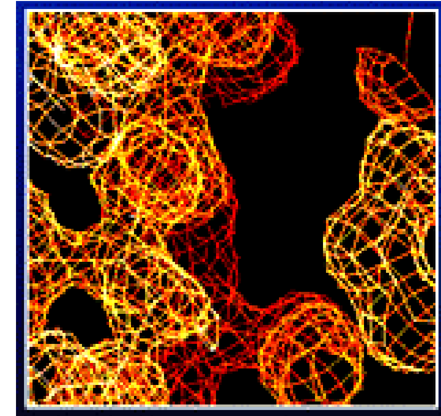
Every atom in a unit cell contributes to every reflection in the diffraction pattern.

<u>Two Pieces of Data</u>

- The position of a reflection point on the reciprocal lattice, given by coordinates h,k,l. Determined by the direction reflected.
- The intensity of the reflection.

From diffraction to electron density map





To get from the diffraction pattern to the electron density, you have to use a Fourier Transform.

$$\rho(xyz) = V^{-1} \sum\sum\sum |F_{hkl}| exp[-2\pi i(hx+ky+lz-\alpha_{hkl})]$$

Square root of reflection Intensity

Phase angle of reflection

Fourier transform
(phase problem)

Raw data: Thousands of intensities of reflections

Electron density

Each diffraction spot (reflection) contains information on the position of every atom!

Solving the Phase Problem

$$\rho(xyz) = V^{-1} \Sigma\Sigma\Sigma |F_{hkl}| exp[-2\pi i(hx+ky+lz-\alpha_{hkl})]$$

Square root of reflection Intensity

Phase angle of reflection

- The diffraction data does not give the phase angle that is needed to calculate the electron density map.
- Have to get the phase angle through other methods.

Isomorphous Replacement: Insert a heavy metal atom into crystal protein, and locate in diffraction pattern and in the cell. Use the location of metal ion to find the phase angle for the other protein atoms.

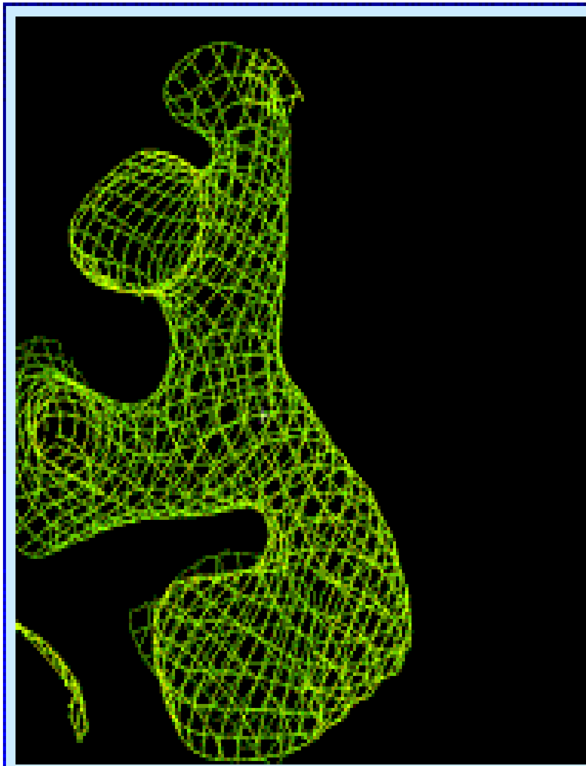
- Requirements:

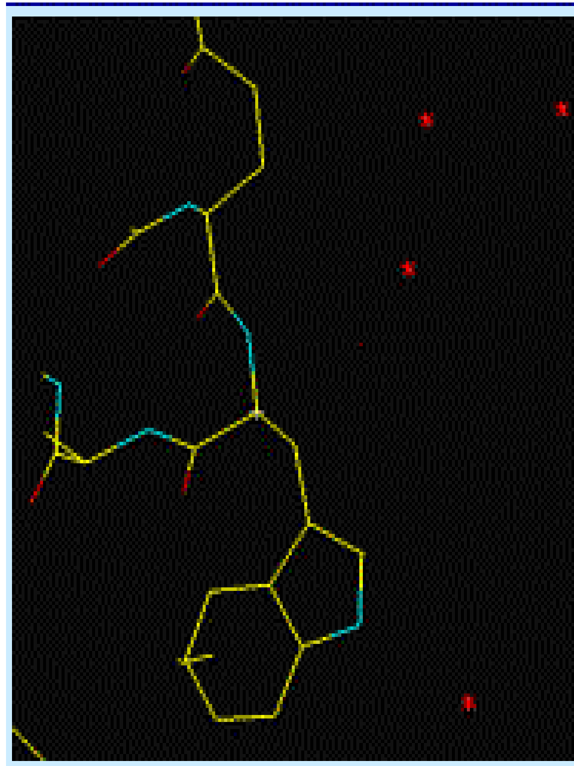
    Add atom with same unit cell size.

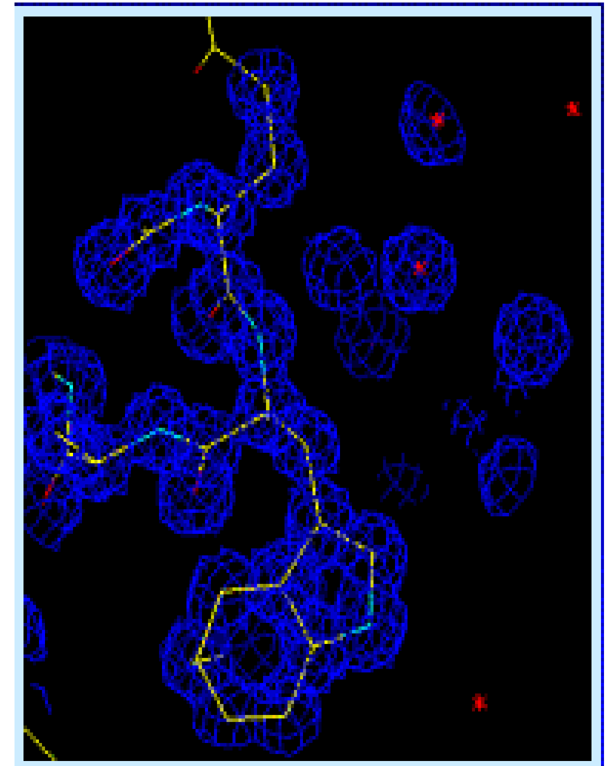
    Cannot disturb protein structure.

Often used Hg, Pt, Au.

Once you have an electron density map, you can begin to fit models to it.







The R-Factor: Measuring Convergence

- To compare the generated electron density map and your model, you have to use the R-factor.
- The R-factor is a measure of convergence between the intensities given off by your model and the observed intensities.

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

R:

0.6-VERY BAD

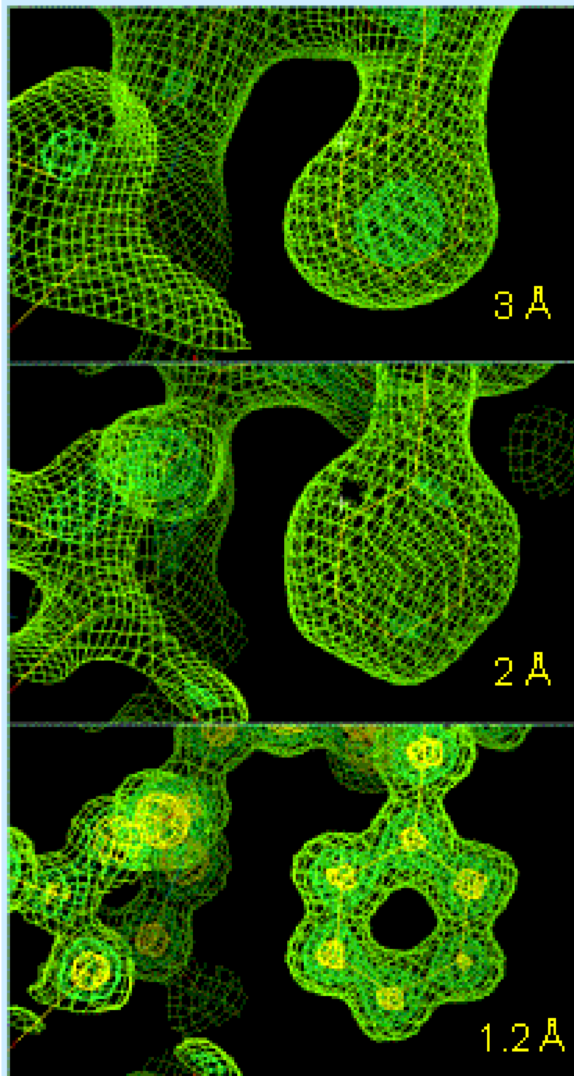
0.5 -BAD

0.4-Recoverable

0.2-Good for Protein

0.05-Good for small organic models

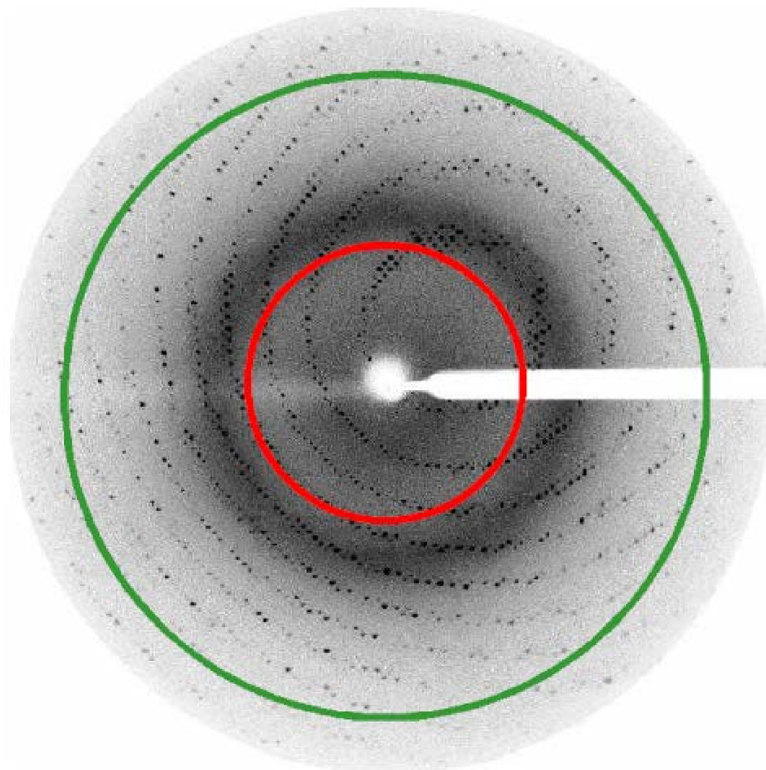
0-PERFECT FIT



Resolution

- Resolution: another measure of how good your model is.
- Resolution gives the size of the smallest molecule you can see or resolve.
- Dependent on the amount of data ultimately phased and used in structure determination.

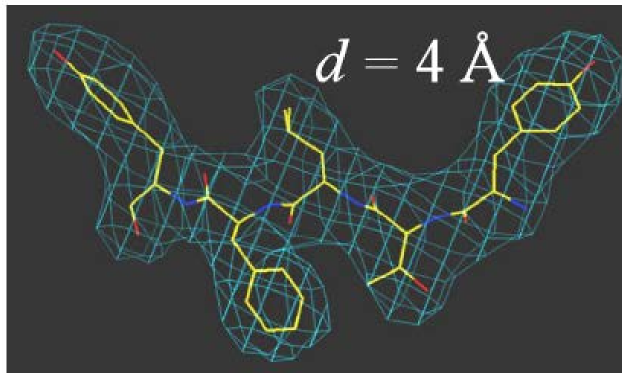
The degree of order in the crystal determines the quality of the diffraction data and ultimately the quality of the final atomic model

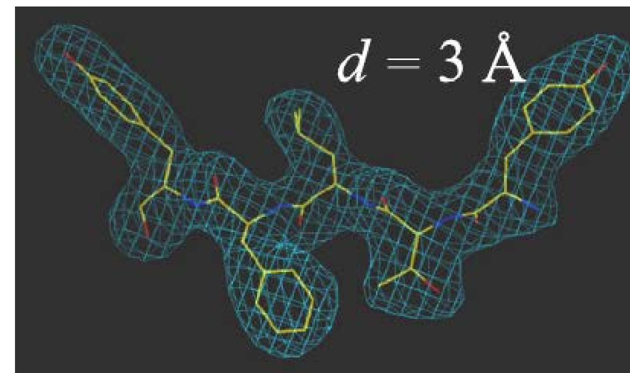


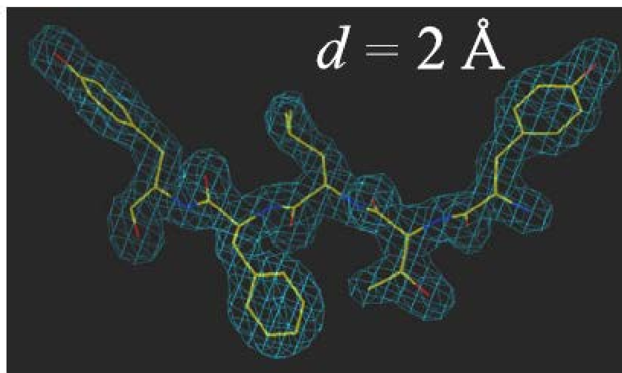
"low resolution"

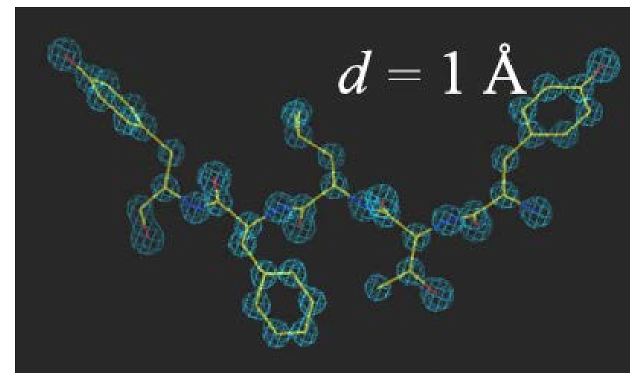
"high resolution"

The precision of the atomic model is mainly determined by the maximal resolution to which the crystal diffracts X-rays



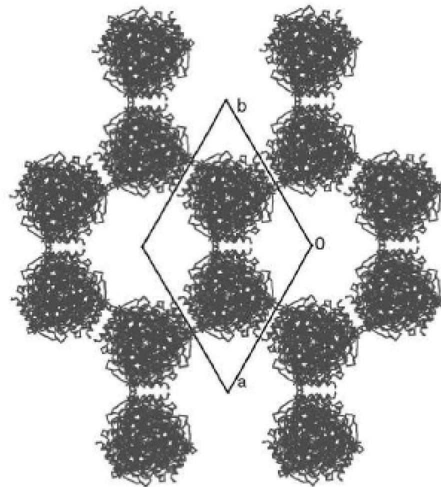




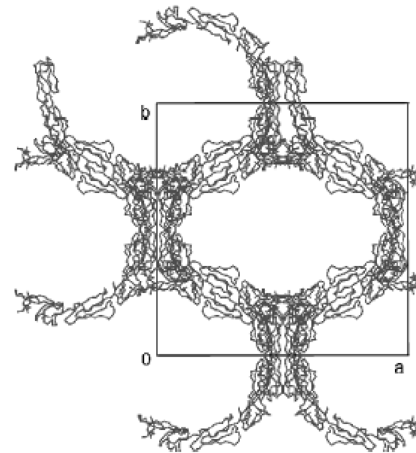


Atomic resolution

Protein crystals contain a lot of solvent and are held together by a limited number of weak contacts between protein molecules



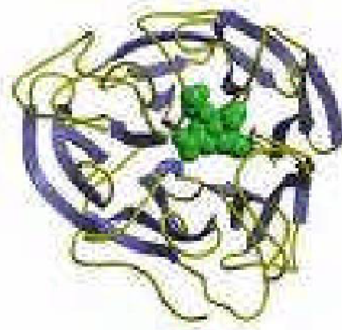
Acetylcholinesterase
~68% solvent

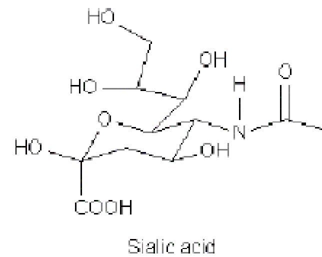


β2 Glycoprotein I
~90% solvent
(extremely high!)

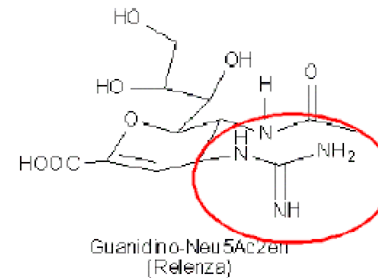
Typical solvent content 40-60%
Solvent channels allow diffusion of compounds into crystal
Often these compounds can reach the active or binding site
Often enzymes are active in crystalline state

The crystal structure of a protein-substrate complex can serve as starting point for structure-based drug design

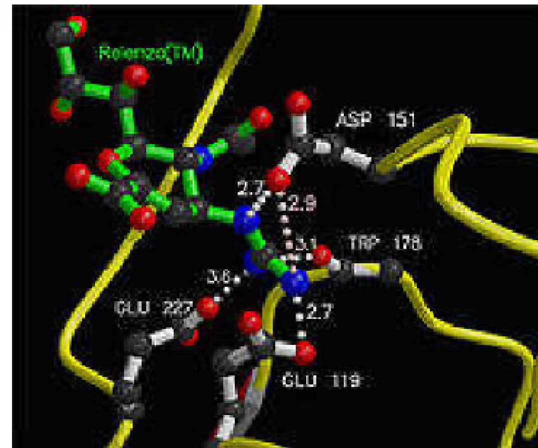




Sialic acid



Guanidino-Neu5Ac2en (Relenza)



Guanidino group provides additional interactions

Relenza was developed starting from the crystal structure of influenza virus neuraminidase with bound sialic acid

Steps of Protein X-ray Crystallography:

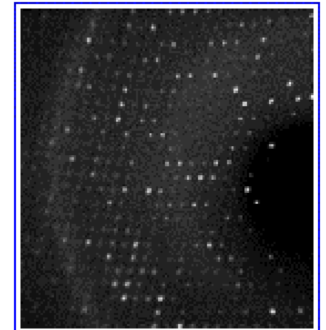
Crystallize your protein.

Cryo-freeze your protein.

Do an X-ray diffraction.

Make a heavy atom derivative of protein.

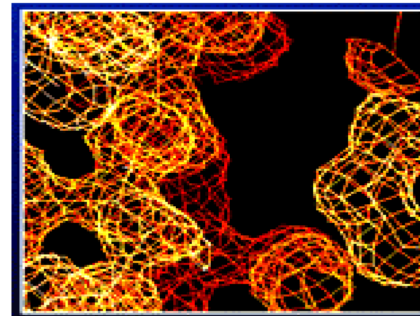
Take X-ray diffraction of the derivative.



Do a Fourier Transform (or let a computer do it).

Create models.

Check R-Factor of models.



Sources:

Rupp, Bernhard. "X-ray Crystallography 101." 21 Nov 2003. http://www-structure.llnl.gov/Xray/101index.html.

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