Computergestützte Strukturbiologie
(Strukturelle Bioinformatik)

Strukturbestimmung mit Röntgenkristallographie

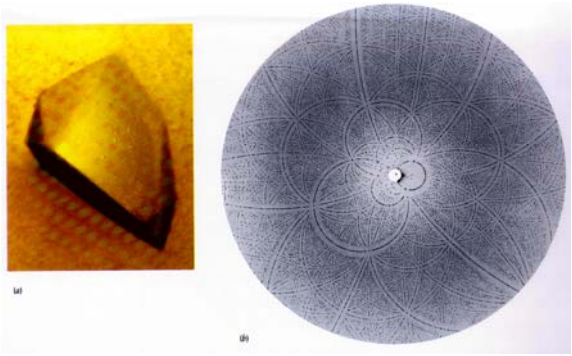
Sommersemester 2009

Peter Güntert

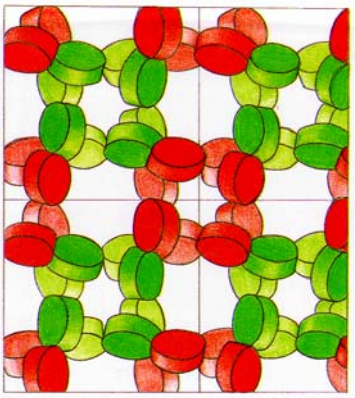
Kristallographie: Geschichte

1839, William H. Miller: Miller Indices für Gitterebenen
1891: 230 Raumgruppen für Kristalle
1895, Wilhelm Conrad Röntgen: Röntgenstrahlung
1912, Max von Laue: Röntgenstreuung
1912, William L. Bragg: Braggsches Gesetz
1914, Bragg: Kristallstrukturen von NaCl und Diamant
1937: Dorothy Hodgkin: Kristallstruktur von Cholesterin
1945: Dorothy Hodgkin: Kristallstruktur von Vitamin B12
1952: Rosalind Franklin: DNA Röntgenbeugungsdiagramme
1955: Rosalind Franklin: Tabakmosaikvirus (TMV) Struktur
1958: John Kendrew: Erste Proteinstruktur (Myoglobin)
2000: Kristallstuktur des Ribosoms
2009: > 49'000 Kristallstrukturen in der Protein Data Bank

Kristall und Beugungsmuster

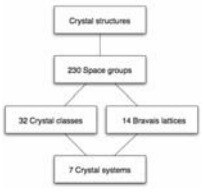


Proteinkristall



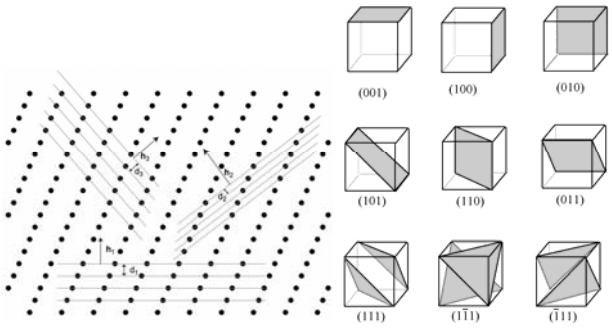
Kristallsysteme

Bravaisgitter



Bravais lattice	Parameters	Simple (P)	Volume centered (I)	Base centered (C)	Face centered (F)
Triclinic	$a_1 \neq a_2 \neq a_3$ $\alpha_{12} \neq \alpha_{23} \neq \alpha_{31}$				
Monoclinic	$a_1 \neq a_2 \neq a_3$ $\alpha_{23} = \alpha_{31} = 90°$ $\alpha_{12} \neq 90°$				
Orthorhombic	$a_1 \neq a_2 \neq a_3$ $\alpha_{12} = \alpha_{23} = \alpha_{31} = 90°$				
Tetragonal	$a_1 = a_2 \neq a_3$ $\alpha_{12} = \alpha_{23} = \alpha_{31} = 90°$				
Trigonal	$a_1 = a_2 = a_3$ $\alpha_{12} = \alpha_{23} = \alpha_{31} < 120°$				
Cubic	$a_1 = a_2 = a_3$ $\alpha_{12} = \alpha_{23} = \alpha_{31} = 90°$				
Hexagonal	$a_1 = a_2 \neq a_3$ $\alpha_{12} = 120°$ $\alpha_{23} = \alpha_{31} = 90°$				

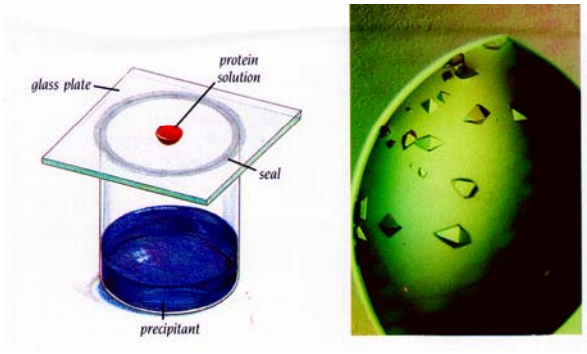
Miller Indizes



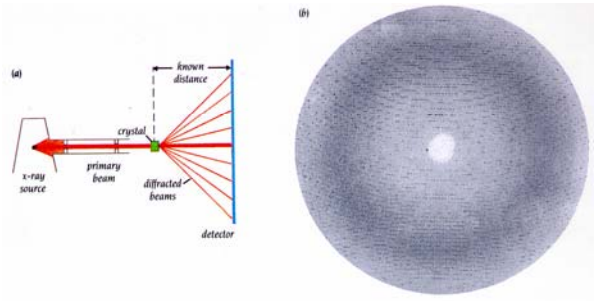
Kristallstrukturbestimmung

1. Kristallisation
2. Messung der Beugungsmuster
3. Datenauswertung
   a) Bestimmung der Einheitszelle und Raumgruppe
   b) Phasenbestimmung
   c) Modellbau
   d) Verfeinerung der Phasen und der Struktur

Proteinkristallisation



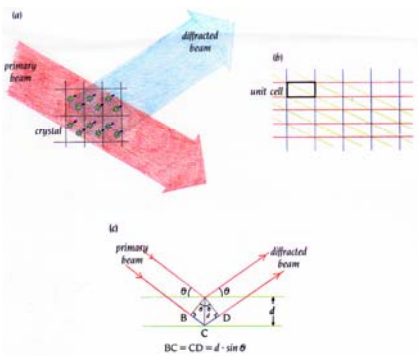
Röntgenkristallographie Messung



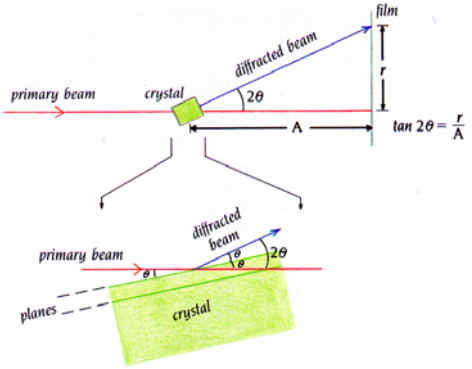
Synchrotron

ESRF Grenoble (France)

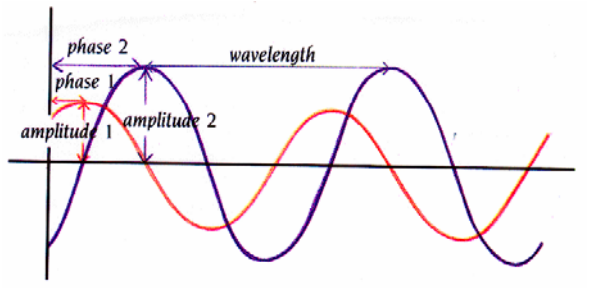
Röntgenstreuung: Bragg-Bedingung



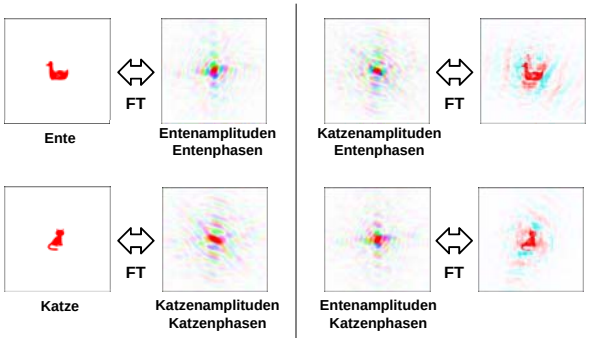
Röntgenstreuung



Superposition of two waves



Fourier Transformation: Phasen und Amplituden

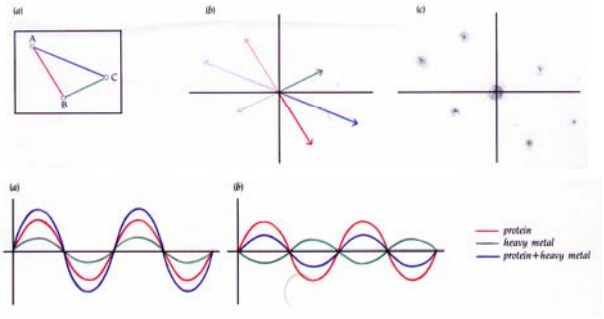


http://www.ysbl.york.ac.uk/~cowtan/fourier/

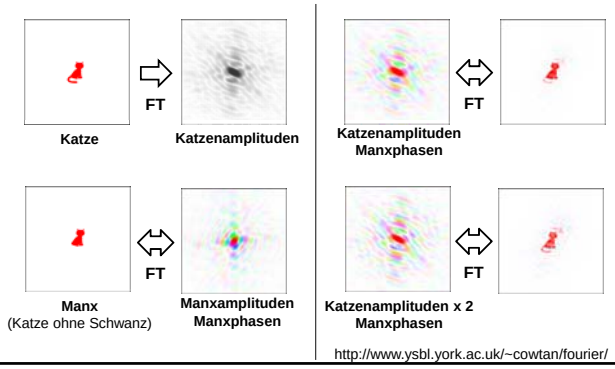
Determination of phases

- **Ab initio phasing (direct methods):** Exploit theoretical phase relationships. Requires high resolution (< 1.4 Å) data.
- **Heavy atom derivatives (multiple isomorphous replacement; MIR):** Crystallize the protein in the presence of several heavy metals without significantly changing the structure of the protein nor the crystal lattice.
- **Anomalous X-ray scattering at multiple wavelengths (multi-wavelength anomalous dispersion; MAD):** Incorporation of Seleno-methionine.
- **Molecular replacement:** Use structure of a similar molecule as the initial model.

Multiple isomorphous replacement (MIR)

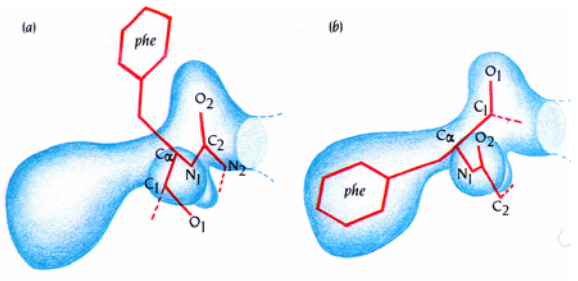


Molecular replacement

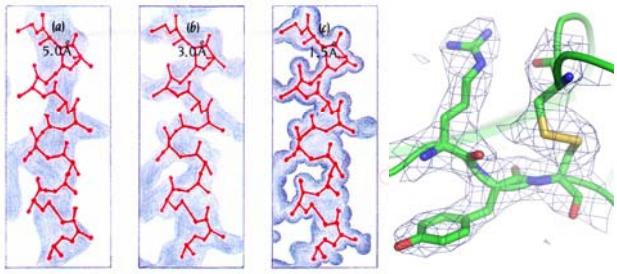


http://www.ysbl.york.ac.uk/~cowtan/fourier/

Interpretation der Elektronendichte

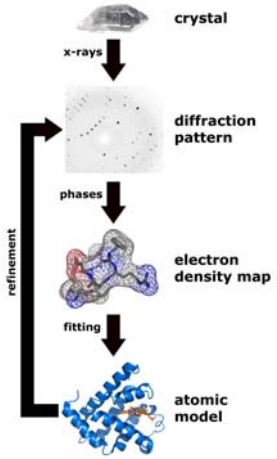


Auflösung



5.0 Å **3.0 Å** **1.5 Å** **2.7 Å**

Struktur-ermittlung



X-ray crystallography: *R*-factor

- Measures agreement between measured data (reflections) and 3D structure
- Definition: Relative difference between structure factors, $F(hkl)$, that were observed (F_{obs}) and back-calculated from the 3D structure (F_{calc}):

$$R = \frac{\sum ||F_{obs}| - |F_{calc}||}{\sum |F_{obs}|} \quad \text{with} \quad I_{hkl} \propto |F(hkl)|^2$$

  I_{hkl} = intensity of reflection (hkl)
- Perfect agreement: $R = 0$
  Good protein X-ray structure: $R < 0.2$
  Random structure: $R \approx 0.6$

X-ray: Free *R*-factor

- Use, say, 90% of the data (reflections) for the structure determination
- Use the remaining 10% to compute the *R* value → "free" *R* value, obtained from independent data
- Detects errors better than conventional *R*-factor
- Each reflection influences whole electron density
- Many reflections → No problem to omit 10% of the reflections from the structure determination

Brünger, A. T. (1992). Free *R* value: a novel statistical quantity for assessing the accuracy of crystal structures. *Nature* 355, 472-475.