

# X-ray Crystallography

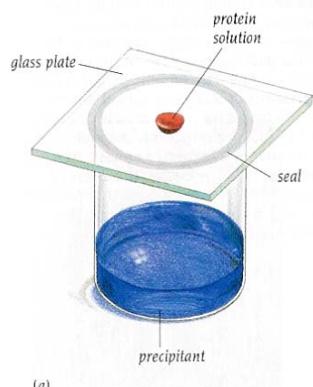
Rongjin Guan

March 7 2008

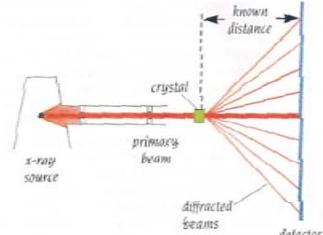
# Purpose of this class

- Introduce the basic principle of X-ray crystallography
  - Symmetry
  - X-ray diffraction
  - Phase problem
  - Methods to solve the phase problem
  - Model building and refinement
- Give an overview of all steps in structure determination by X-ray crystallography
- Help students understand crystal structure papers

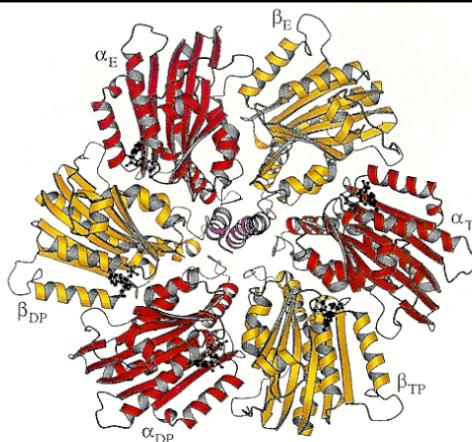
# Overview of X-ray crystallography



1. Grow crystals



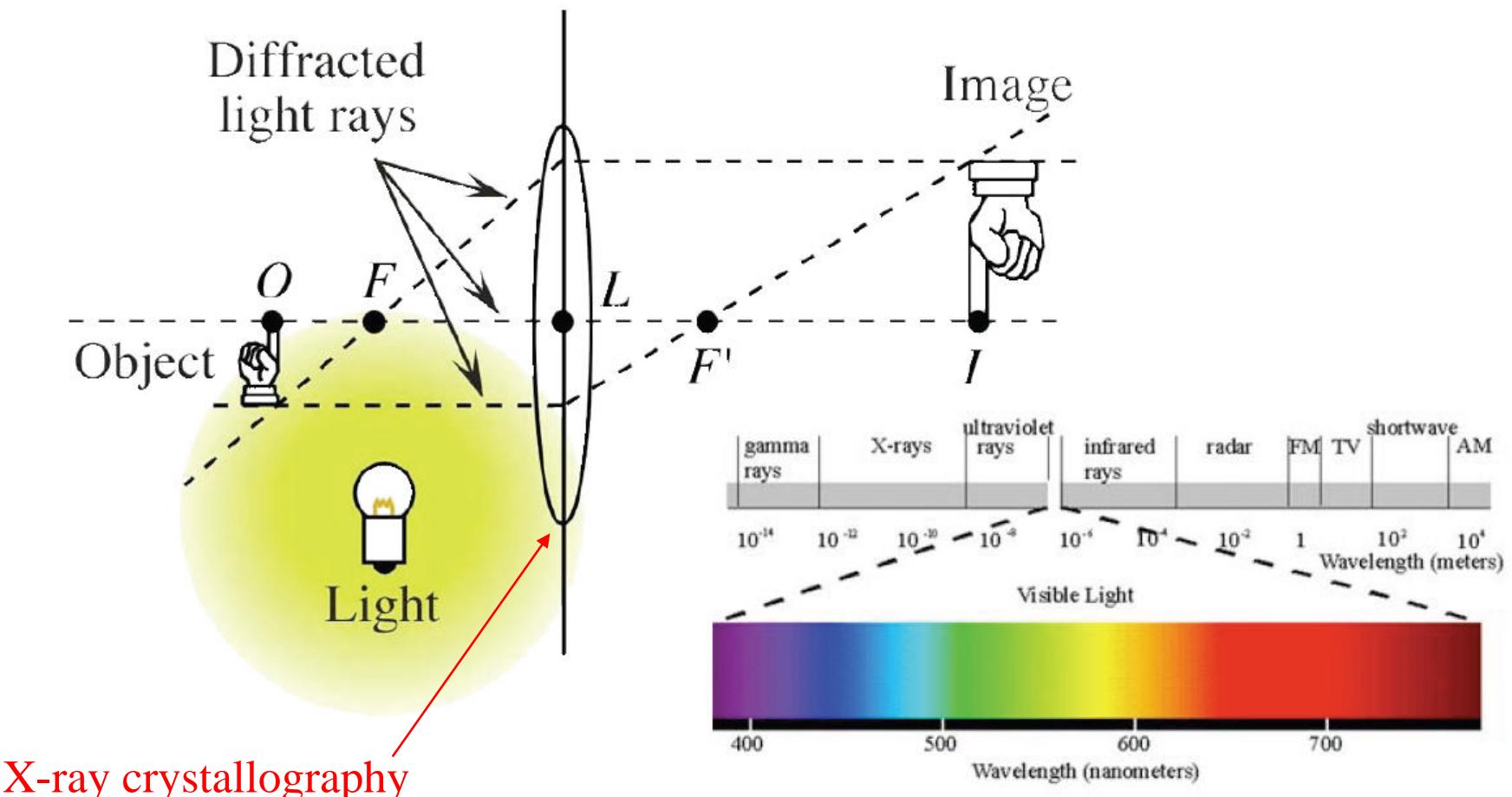
2. Measure diffraction



3. Solve phases and refine structure

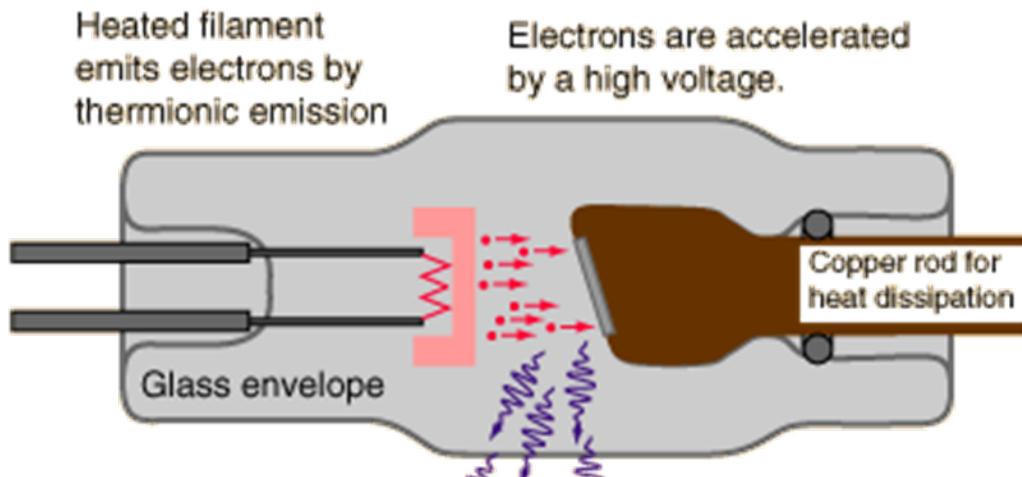
# Why X-rays?

Right wavelength to resolve atoms: C-C bond is 1.54 Å



The electromagnetic spectrum  
from "The Joy of Visual Perception: A Web Book"  
<http://www.yorku.ca/eye/>

# How are X-rays produced?

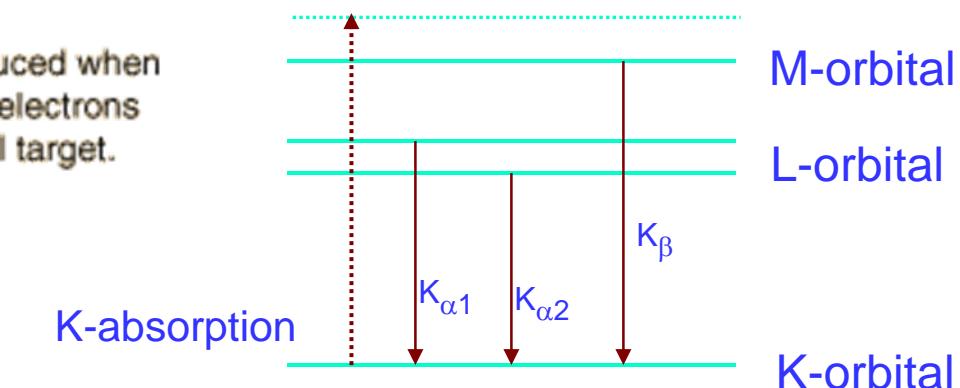


Electrons are accelerated  
by a high voltage.

Glass envelope

x-rays produced when  
high speed electrons  
hit the metal target.

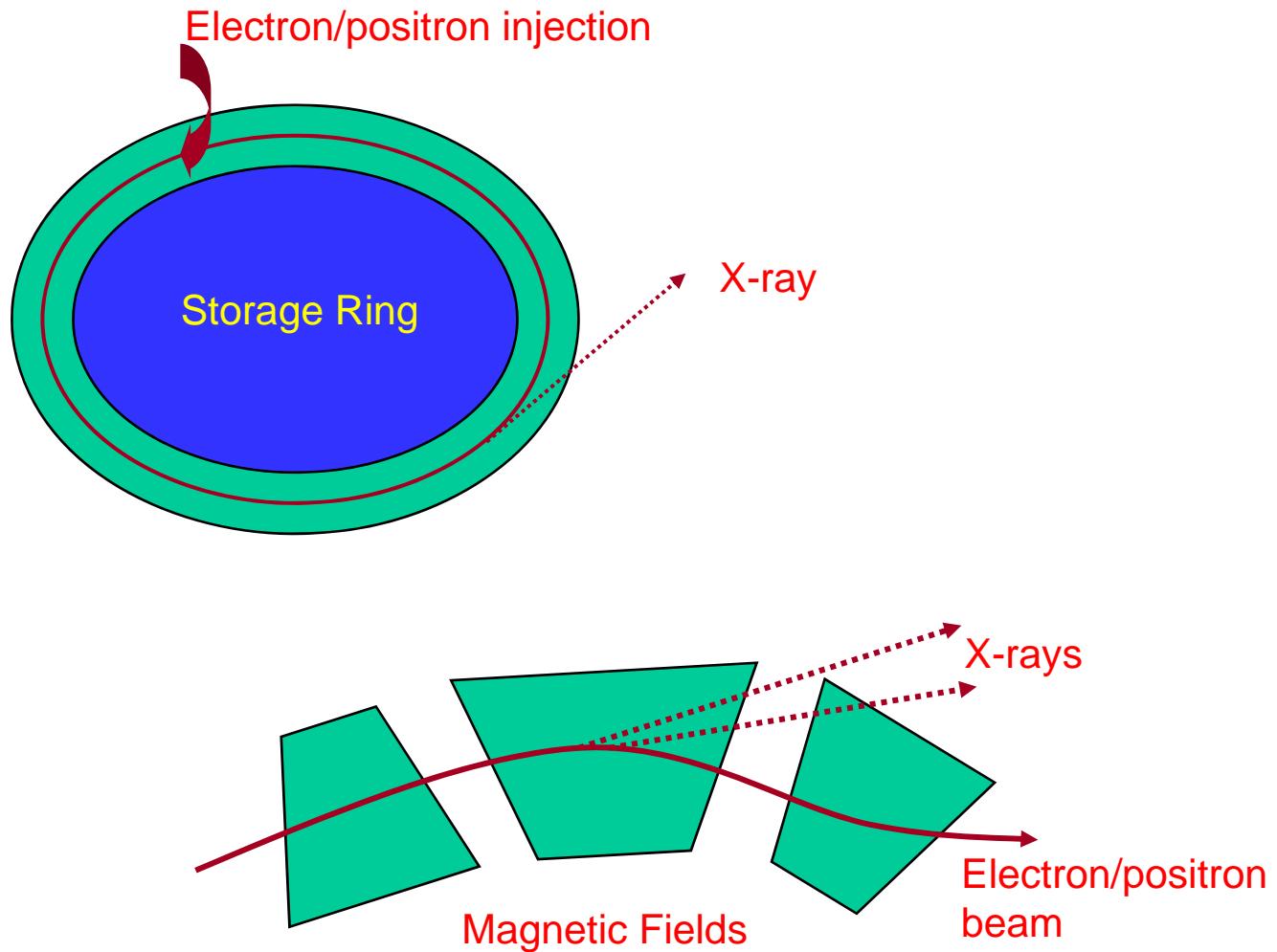
K-absorption



## Wave-lengths

$$\begin{aligned} \text{Cu}(K_{\alpha 1}) &= 1.54015 \text{ \AA}; \text{ Cu}(K_{\alpha 2}) = 1.54433 \text{ \AA} \\ \text{Cu}(K_{\alpha}) &= 1.54015 \text{ \AA} \\ \text{Cu}(K_{\beta}) &= 1.39317 \text{ \AA} \end{aligned}$$

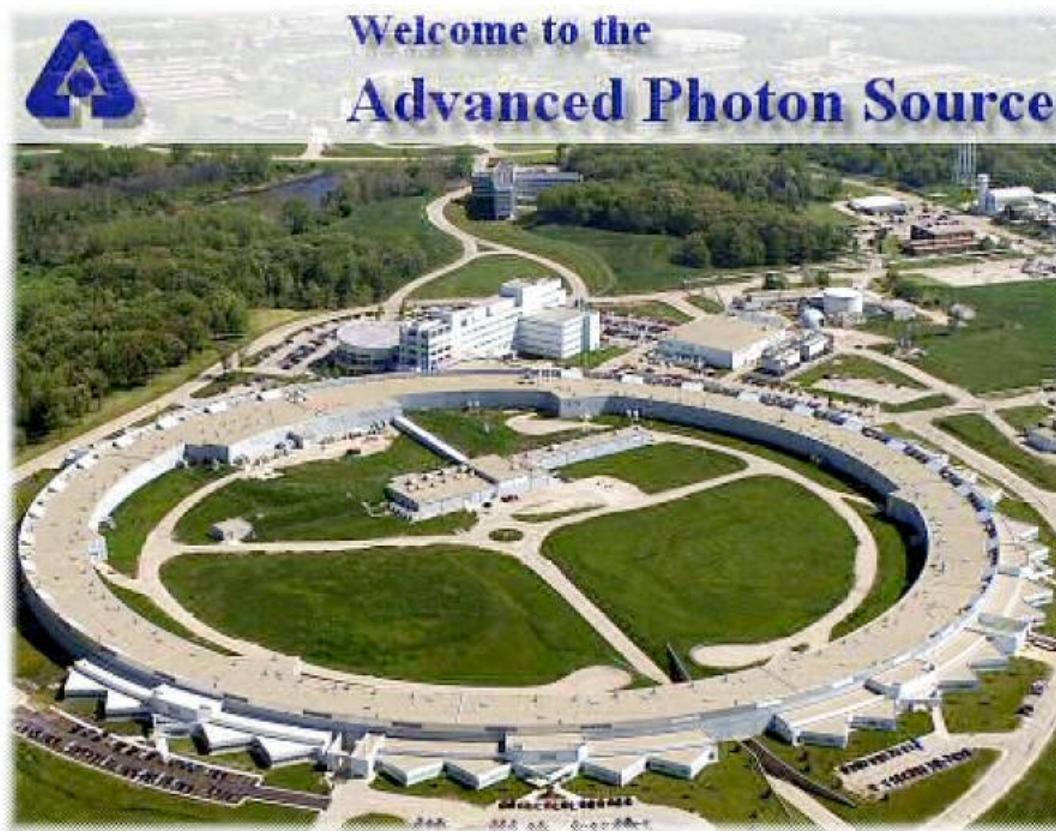
# Synchrotron X-rays



# An example of home X-ray source (Rigaku)



# A synchrotron X-ray source at Argonne, IL



# Symmetry in crystals

Why do we need to know the symmetry of the crystals?

- 1) Reduce the size of the asymmetric unit (a unique part of the crystal)
- 2) Reduces data collection by reducing the number of unique reflections to collect
- 3) Symmetry may be useful for phase determination

International Table (volume 1) is a “Bible” for crystal symmetry.

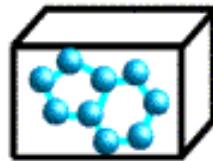
# Periodicity and Symmetry in a Crystal

- A crystal has long range ordering of building blocks that are arranged in an conceptual 3-D **lattice**.
- A building block of minimum volume defines **unit cell**
- The repeating units (protein molecule) are in symmetry in an unit cell
- The repeating unit is called **asymmetric unit** – A crystal is a repeat of an asymmetric unit

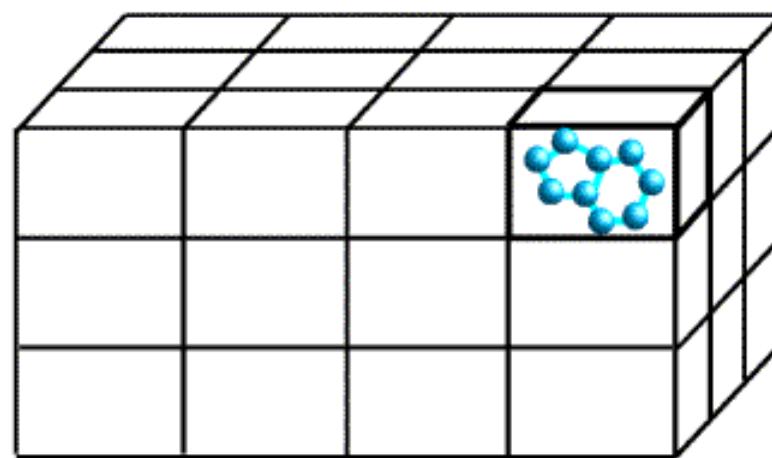
molecule



unit cell



crystal

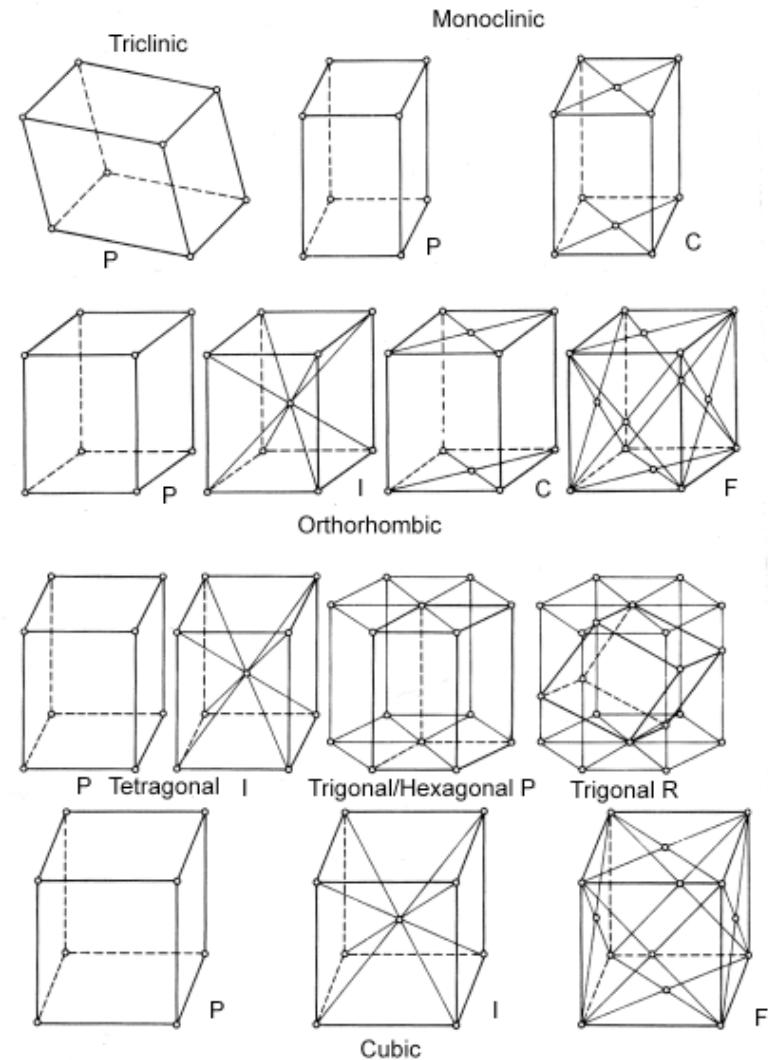


- Arrangement of asymmetric unit in a lattice defines the crystal symmetry.

- The allowed symmetries are 2-, 3, 4, 6-fold rotational, mirror(m), and inversion (i) symmetry (+/-) translation.

- Rotation + translation = screw

- Rotation + mirror = glide



→ 230 space groups, 32 point groups, 14 Bravais lattices, and 7 crystal systems

# Only 65 space groups for chiral molecules

TABLE 4.II  
Enantiomorphic space groups

System	Class	Space group symbols
Triclinic	1	$P\bar{1}$
Monoclinic	2	$P2, P2_1, C2$
Orthorhombic	222	$C222, P222, P_{21}2_{12}, P_{21}2_{12}, P_{222_1}, C_{222_1}, F222, I222, I_{21}2_{12}$
Tetragonal	4	$P4, P4_1, P4_2, P4_3, I4, I4_1$
	422	$P422, P4_22, P_{41}2, P_{41}22, P_{41}2_12, P_{42}22, P_{42}2_12, P_{4_3}2_12, P_{4_3}22, I422, I_{41}22$
Trigonal	3	$P3, P3_1, P3_2, R3$
	32	$P312, P321, P_{31}21, P_{31}12, P_{32}12, P_{32}21, R32$
Hexagonal	6	$P6, P6_5, P6_4, P6_3, P6_2, P6_1$
	622	$P622, P6_122, P6_222, P6_322, P6_422, P6_522$
Cubic	23	$P23, F23, I23, P_{21}3, I_{21}3$
	432	$P432, P4_132, P4_232, P4_332, F432, F_{41}32, I432, I_{41}32$

# Steps in structure determination by X-ray crystallography

- Sample preparation
  - chemical, conformational and aggregational homogeneity
- Crystallization
- Data collection and process
- Phase determination
  - Isomorphous replacement
  - Anomalous scattering
  - molecular replacement, etc
- Model building and refinement
- Structural analysis

# Prepare samples for crystallization

Homogeneous protein samples have the best chance to crystallize. (not only pure in SDS PAGE!!!)

	Method of detection
Chemical homogeneity	SDS PAGE, mass spectrometry
Conformational homogeneity	Native PAGE, protease sensitivity
Aggregational homogeneity	Gel filtration, Native PAGE, light scattering, analytical ultracentrifugation

# How to grow crystals?

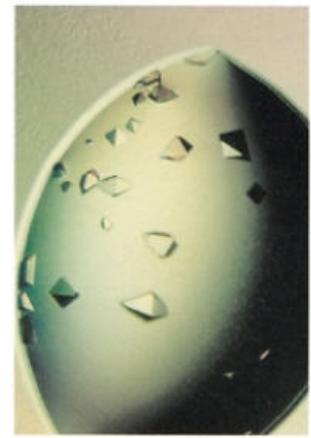
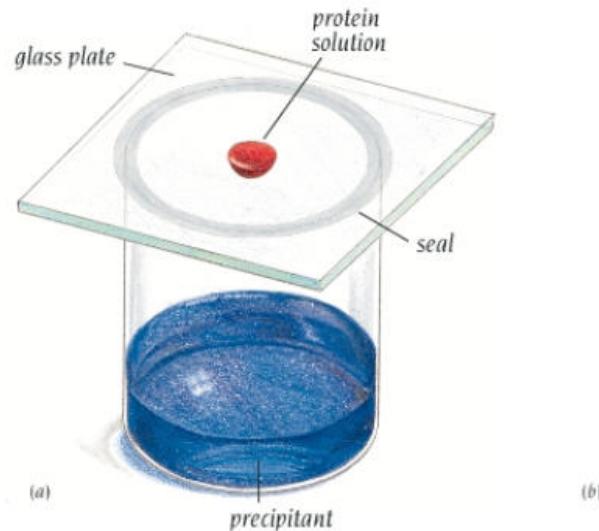
By driving solutions to super-saturation;  
Crystallization is entropically unfavorable, but may be  
energetically compensated by favorable packing forces;  
Incomplete factorial search of crystallization conditions  
combinations of buffers, precipitants (salts,  
polyethylene glycols- PEGs, organic solvents, etc) and  
additives.

Methods for crystallization

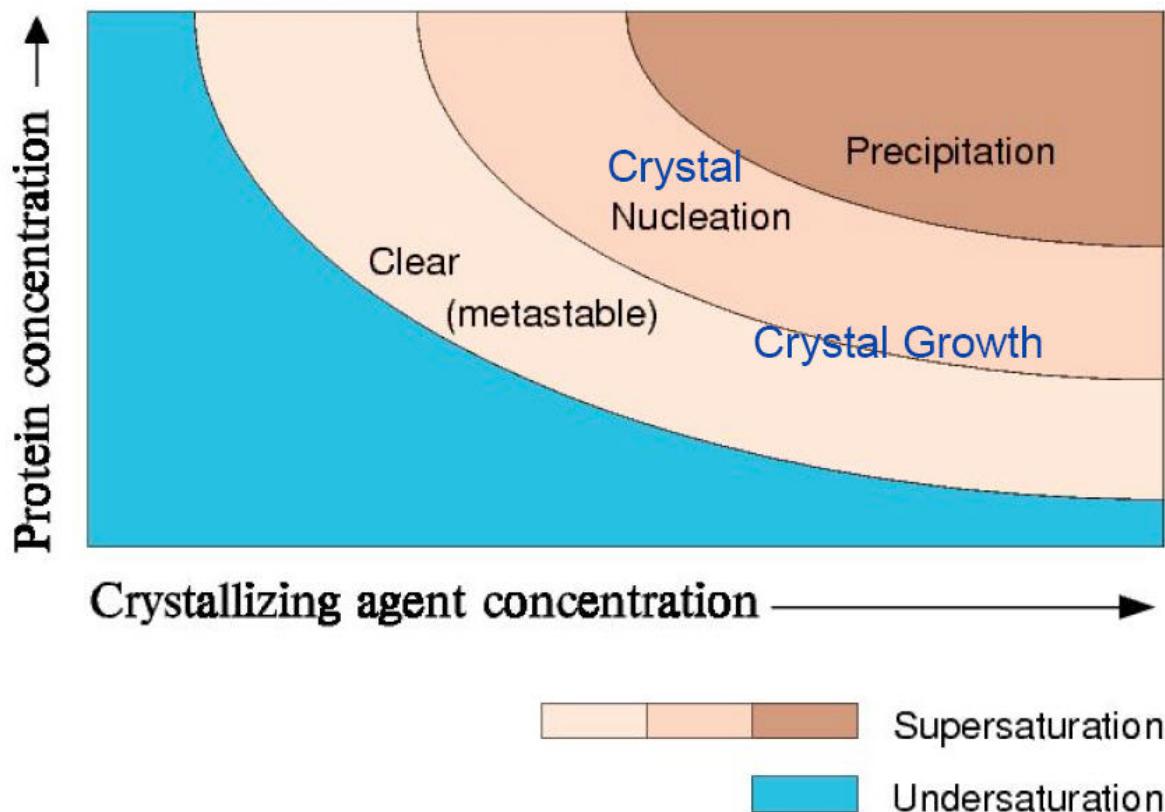
- vapor (phase) diffusion
- hanging drop
- sitting drop

batch

liquid (phase) diffusion



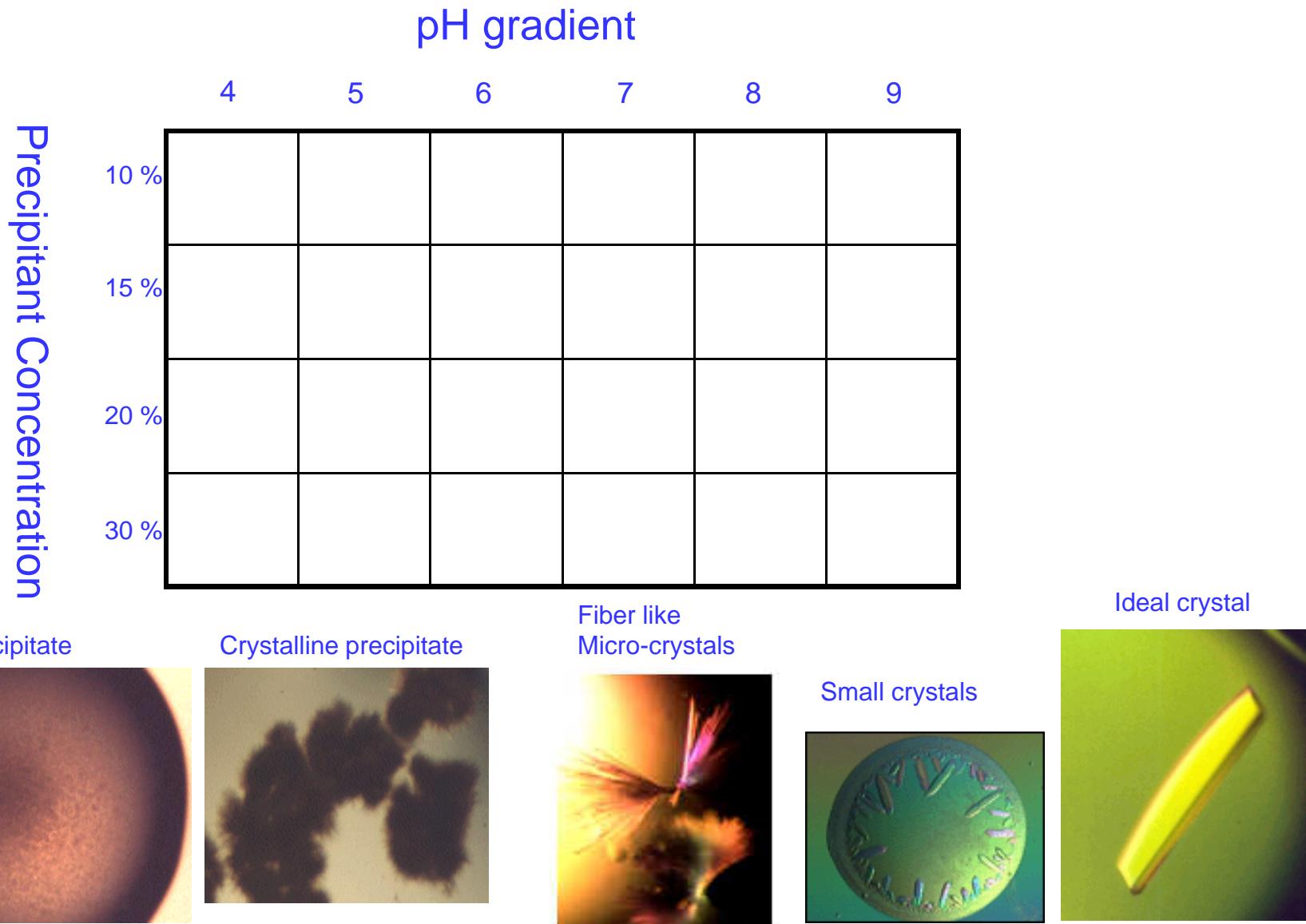
# Phase diagram in crystallization



# Factors that affect crystallization

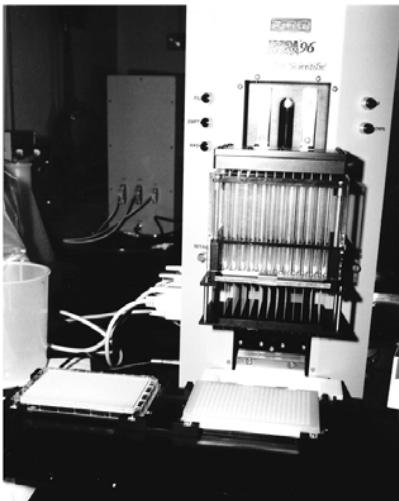
- pH (buffer)
- Protein Concentration
- Salt (NaCl, NH<sub>4</sub>Cl, etc.)
- Precipitant
- Detergent (e.g. n-Octyl-β-D-glucoside)
- Metal ions and/or small molecules
- Rate of diffusion
- Temperature
- Size and shape of the drops
- Pressure (e.g. micro-gravity)

# Screening for Crystallization

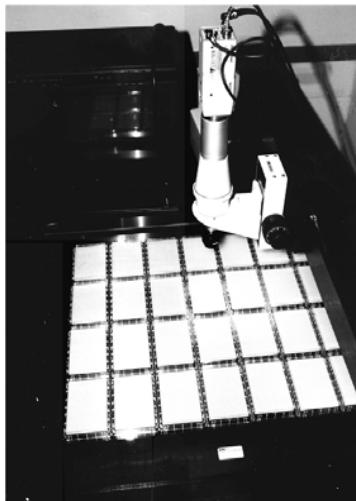


# HTP Crystallization Screening

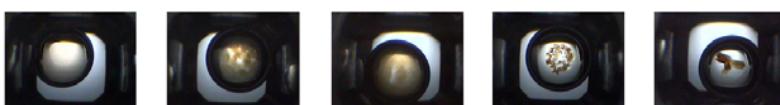
Figure B4.1: The HTP Search Lab



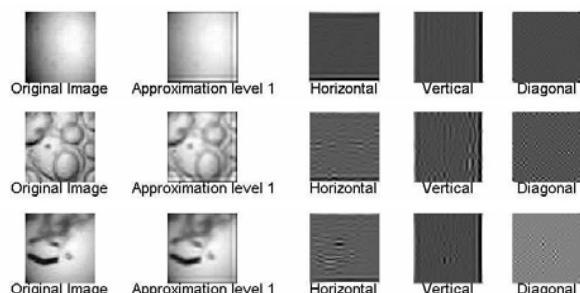
A. Hydra Pipetting Robot



B. Robotic Microscope



C. Robotically-Recorded Images of HTP Search Results  
(Screening of catalase at stock concentration of 21 mg/ml.)



D. Wavelet Transformation of Representative Images

## Monodisperse SeMet Samples

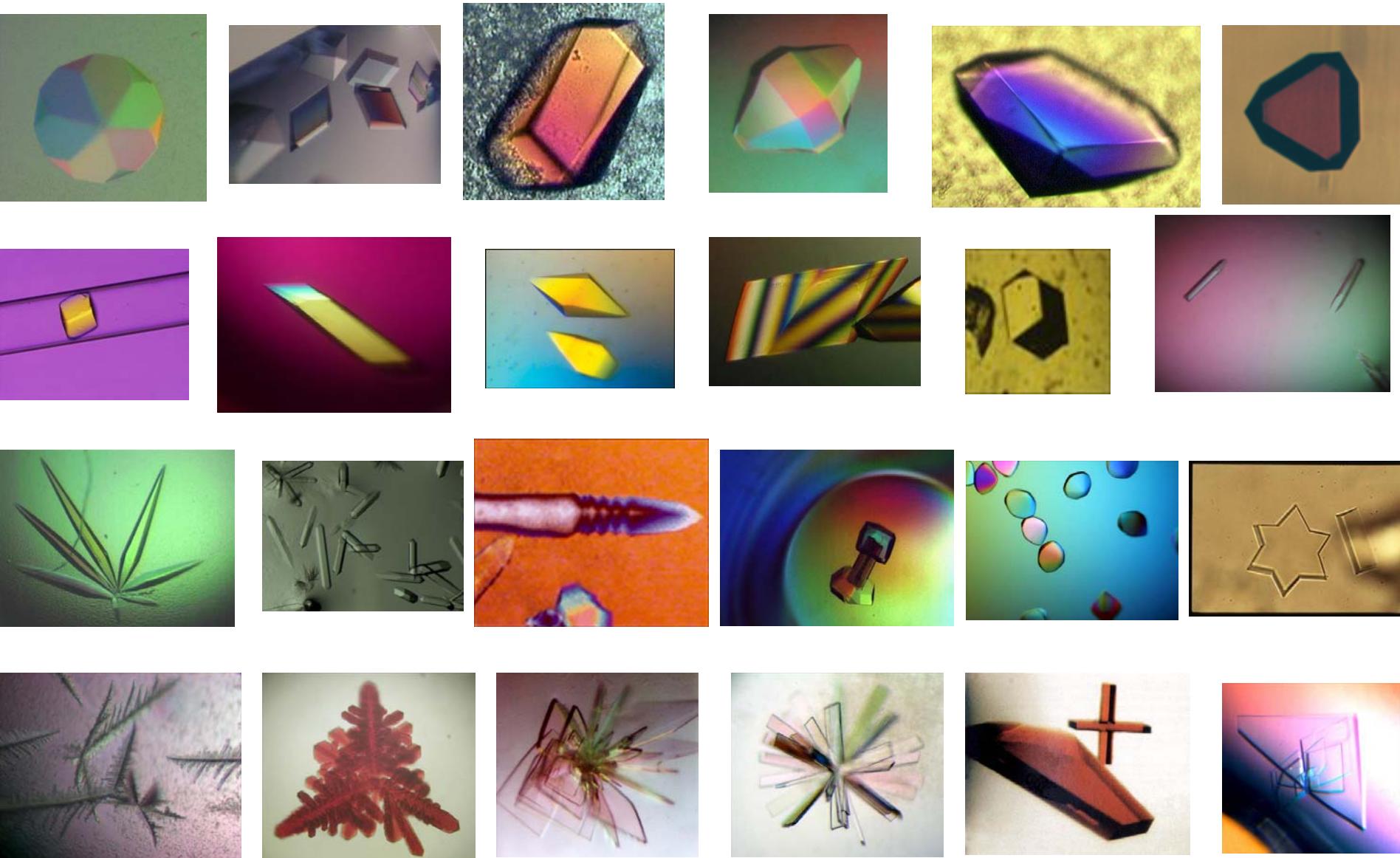
Shipped to both Columbia and Hauptman-Woodward Institute (at HWI) for Crystallization Screening

HTP Robotic Screening (1356 conditions) under oil provides initial hits. DeTitta, Luft, et al

Robotic setup of hanging drop crystallization optimization at Columbia is informed by HWI results. Hunt, Tong, et al

Production crystallization conditions include potential ligands assessed by literature search

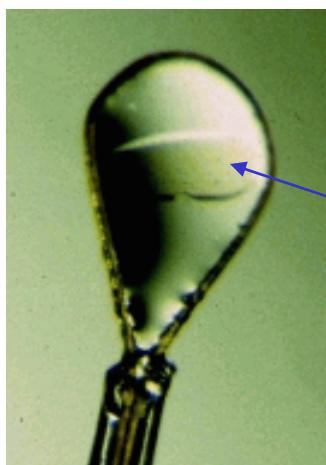
# A protein crystal gallery



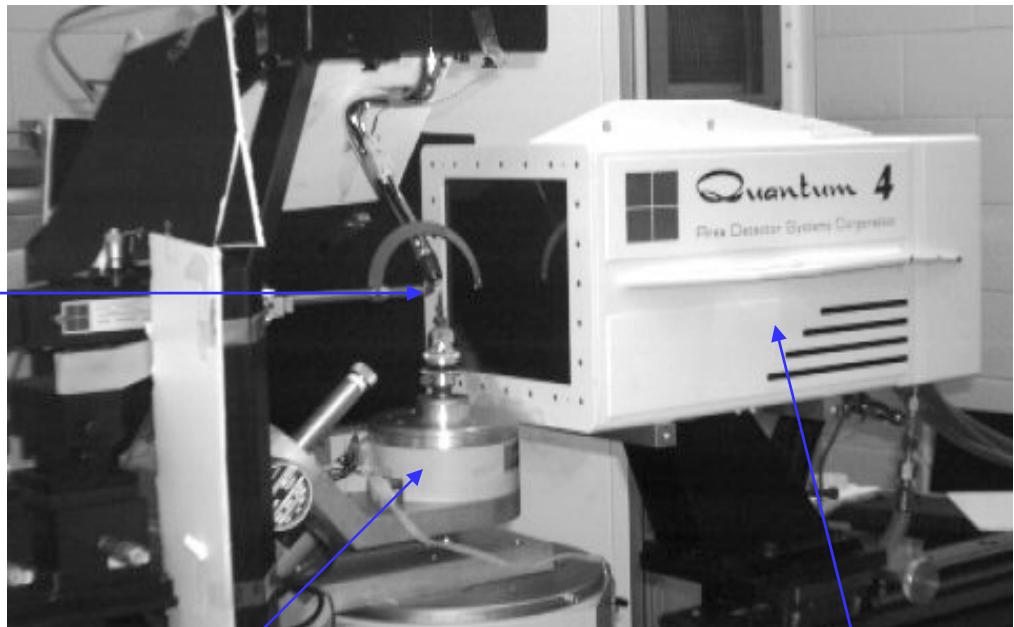
# Steps in structure determination by X-ray crystallography

- Sample preparation
  - chemical, conformational and aggregational homogeneity
- Crystallization
- **Data collection and process**
- Phase determination
  - Isomorphous replacement
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  - molecular replacement, etc
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## Cryo-loop

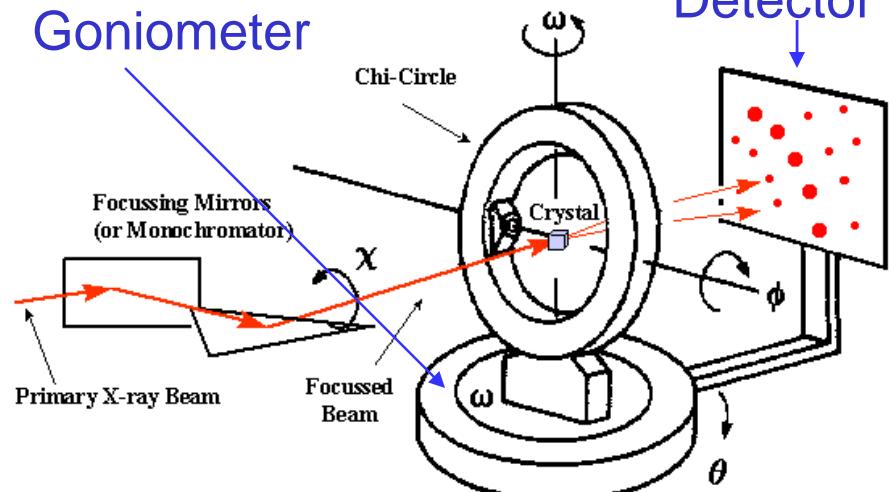


Crystal



Goniometer

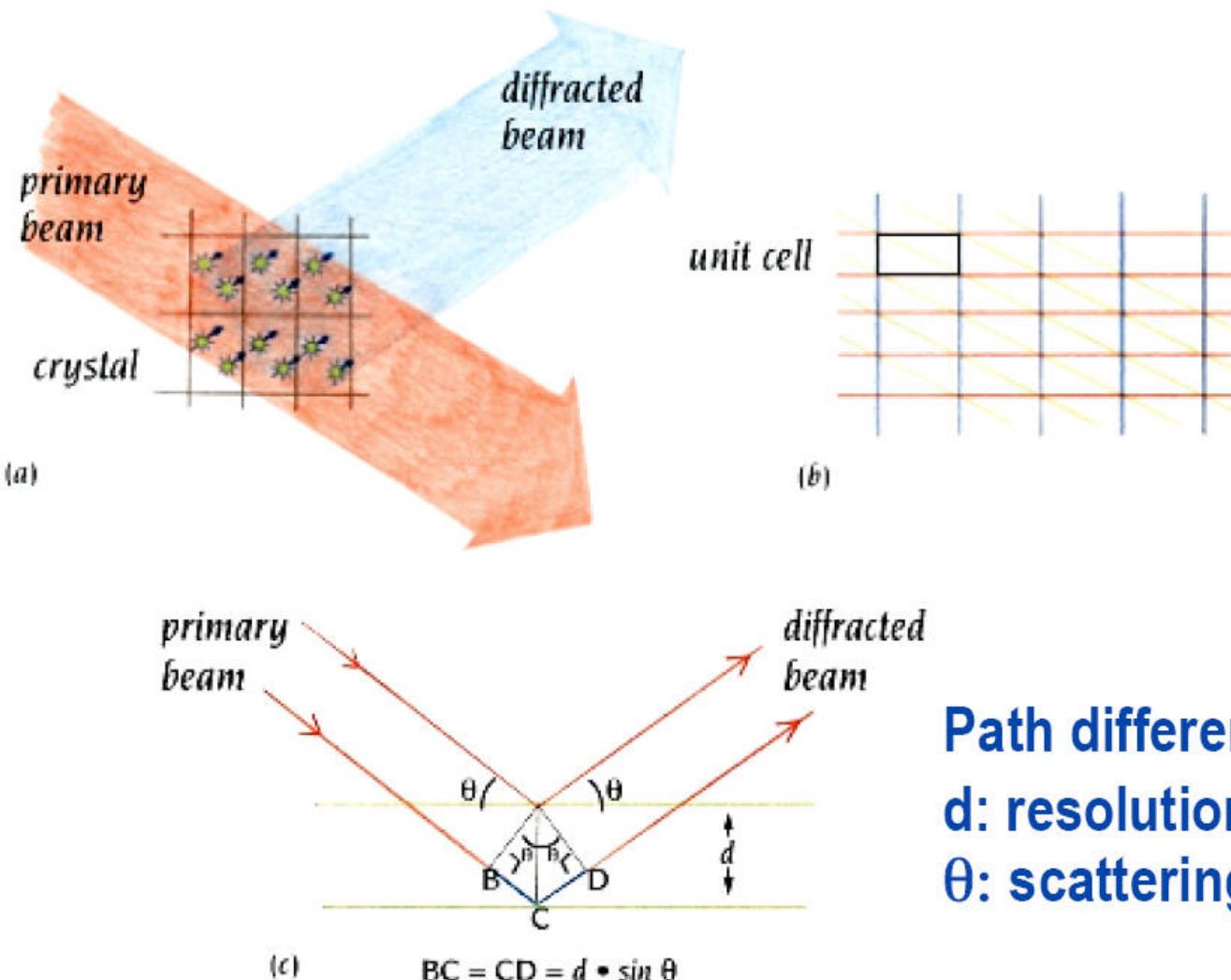
Detector



4-Circle Goniometer (Eulerian or Kappa Geometry)

$$\text{Bragg's law: } 2d \sin \theta = \lambda$$

When this condition is satisfied, a plane with a spacing of  $d$  will 'reflect' X-rays with an incident angle of  $\theta$ , due to constructive interference.



Path difference:  $2ds \in \theta$   
d: resolution  
 $\theta$ : scattering angle

$$BC = CD = d \cdot \sin \theta$$

**Data Collection: measuring positions and intensities of reflections in the diffraction pattern produced by the macromolecular crystal.**

- **Capillary mounting**  
for crystal characterization and data collection above 0°C.
- **Crystal ‘freezing’**  
Soak the crystal in a cryo-protectant solution, then collect data around 100K.  
Commonly used cryo-protectant: glycerol, ethylene glycol, PEG400, sugars, MPD, etc
- Each diffraction image contains diffraction from a small angular sweep (oscillation). Rotate the crystal until a complete 3-D diffraction is completed and collected.
- These diffraction images are recorded by imaging plates or CCD detectors as digital images.

# Crystal characterizations

- Diffraction resolution limit.

$$d = \frac{\lambda}{2 \sin\theta}$$

will determine what kind of detail you may see from the final structure. Usually 3.0 Å or beyond is OK, and 2.0 Å or beyond is good. Note that the resolution limit of most macromolecular crystals does not give “atomic” resolution, i.e. electron density does not resolve into atoms; but the fitting of “atomic models” into an electron density leads to “atomic” resolution.

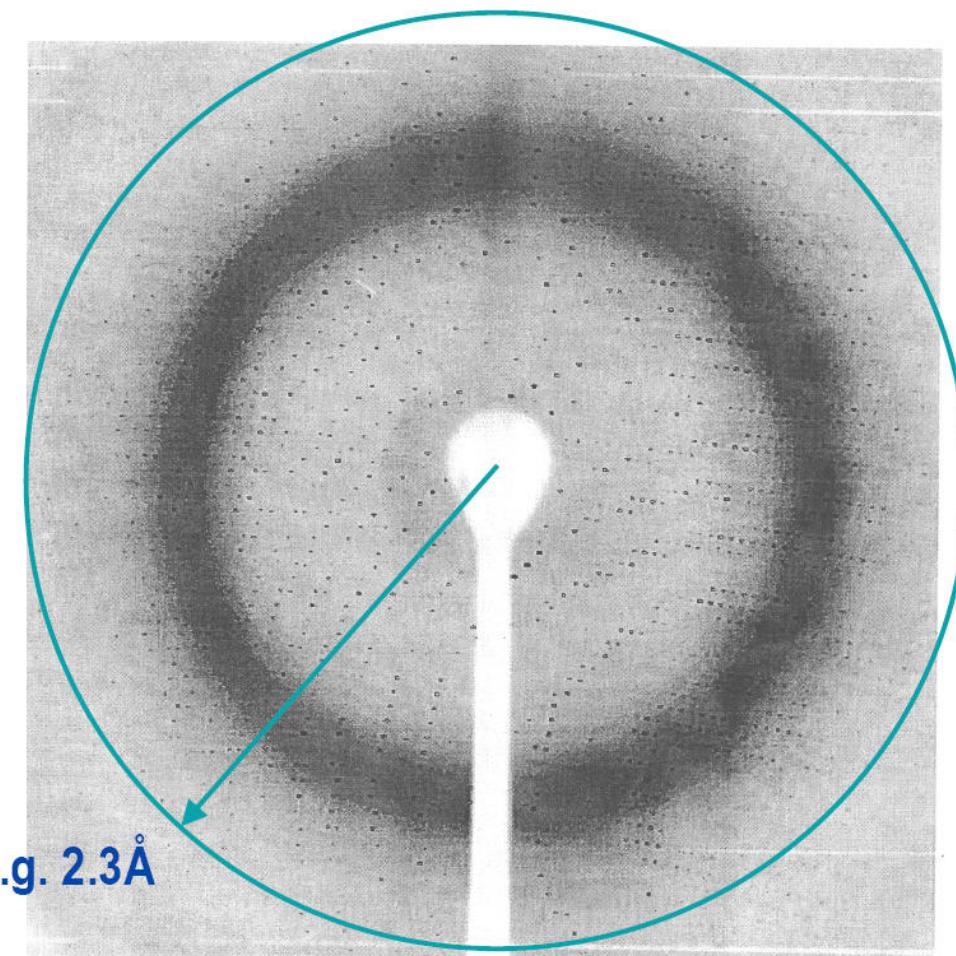
- Cell dimensions.

- Mosaicity: usually 0.3-1.0°. Indication of variability between different unit cells in the crystal. High mosaicity increases the width of reflections, increases the number of reflections on each image and may cause reflection overlap, leading to problems in data collection.

- Space group.

- Data quality: R factor.

# An example of diffraction image



Resolution  
Ice ring  
Mosaicity  
Spots resolved

## Sometimes, there may be problems

- One cell parameter is too long – spots overlaps
- Twinning
- High mosaics
- Water rings
- Radiation damage
- Anisotropic diffraction

# A typical data processing output

Space group p212121

Shell		I/Sigma in resolution shells:								
Lower limit	Upper limit	0	1	2	3	5	10	20	>20	total
99.00	4.09	1.0	2.7	4.1	5.1	6.2	9.6	19.2	80.4	99.6
4.09	3.25	1.0	2.8	4.1	6.0	8.3	14.4	28.0	71.9	99.9
3.25	2.84	1.2	4.3	6.4	8.8	13.7	24.3	45.3	54.7	100.0
2.84	2.58	1.4	7.0	12.1	16.0	24.0	39.7	64.8	35.0	99.8
2.58	2.39	1.5	6.0	12.8	19.4	29.9	48.2	76.7	23.1	99.8
2.39	2.25	2.8	10.9	18.4	26.7	37.6	57.4	82.1	17.5	99.5
2.25	2.14	2.4	11.0	20.5	27.9	40.5	62.7	87.4	12.3	99.7
2.14	2.05	5.0	15.6	27.1	37.8	51.5	73.7	92.3	6.6	98.9
2.05	1.97	5.2	19.9	32.9	45.2	60.4	83.0	96.5	1.8	98.4
1.97	1.90	6.3	25.7	43.6	56.0	70.7	88.4	94.6	0.4	95.0
All hkl		2.7	10.4	17.9	24.5	33.8	49.4	67.9	31.2	99.1

completeness

Summary of reflections intensities and R-factors by shells

R linear =  $\text{SUM}(\text{ABS}(I - \langle I \rangle)) / \text{SUM}(I)$

R square =  $\text{SUM}((I - \langle I \rangle)^2) / \text{SUM}(I^2)$

Chi\*\*2 =  $\text{SUM}((I - \langle I \rangle)^2) / (\text{Error}^2 * N / (N-1))$

In all sums single measurements are excluded

Shell	Lower limit	Upper limit	Average I	Average error	Norm. stat.	Chi**2	Linear R-fac	Square R-fac
99.00	4.09	5589.5	156.6	143.1	0.734	0.038	0.038	0.064
4.09	3.25	4391.0	131.0	109.4	0.693	0.040	0.040	0.053
3.25	2.84	2118.1	82.2	73.3	0.607	0.050	0.050	0.166
2.84	2.58	1164.9	64.3	60.1	0.583	0.065	0.065	0.073
2.58	2.39	839.3	61.9	59.5	0.572	0.083	0.083	0.089
2.39	2.25	695.7	64.8	61.7	0.524	0.100	0.100	0.109
2.25	2.14	643.9	68.2	65.5	0.519	0.115	0.115	0.178
2.14	2.05	496.0	71.9	70.0	0.436	0.135	0.135	0.149
2.05	1.97	394.6	79.6	78.3	0.421	0.170	0.170	0.182
1.97	1.90	296.9	91.6	90.8	0.343	0.202	0.202	0.232
All reflections		1729.2	88.1	81.9	0.562	0.056	0.056	0.077

Rmerge

Intensities of systematic absences				
h	k	l	Intensity	Sigma
0	0	3	-2.9	7.5
0	3	0	3.9	17.4
3	0	0	8.7	9.1
				I/Sigma
				-0.4
				0.2
				1.0

Systematic absences

# Steps in structure determination by X-ray crystallography

- Sample preparation
  - chemical, conformational and aggregational homogeneity
- Crystallization
- Data collection and process
- **Phase determination**
  - Isomorphous replacement
  - Anomalous scattering
  - molecular replacement, etc
- Model building and refinement
- Structural analysis

# Phase problem in X-ray Crystallography

To calculate an electron density map:

$$\rho(xyz) = \frac{1}{V} \sum_h \sum_k \sum_l |F(hkl)| \exp[-2\pi i(hx + ky + lz) + i\alpha_{hkl}]$$

Can measure  
 $|F(hkl)| = I^{1/2}$

Can't measure  
 $\alpha_{hkl}$

The phases are lost during measurement, so electron density cannot be directly calculated. We have to estimate them.

This lack of knowledge of the phases is termed **phase problem**.<sup>31</sup>

# *Importance of Phases*

Karle amplitudes  
with Karle phases



Hauptman amplitudes  
with Hauptman phases



Karle amplitudes  
with Hauptman phases

Hauptman amplitudes  
with Karle phases

Phases dominate the image!  
Phase estimates need to be accurate

# How to Solve the Phase Problem

- **Experimental methods:**

*Multiple/Single Isomorphous Replacement (MIR/SIR)*

*Heavy atoms (Heavy metals, Xe, Br, I, etc)*

*Multi/single Wavelength Anomalous Dispersion (MAD/SAD)*

*Heavy atoms (Se-Met, etc),*

- **Non-experimental methods:**

*Molecular Replacement*

*Homologous templates (similar structure fold)*

*Direct Methods (special cases)*

*high resolution data, small proteins (<2000 atoms)*

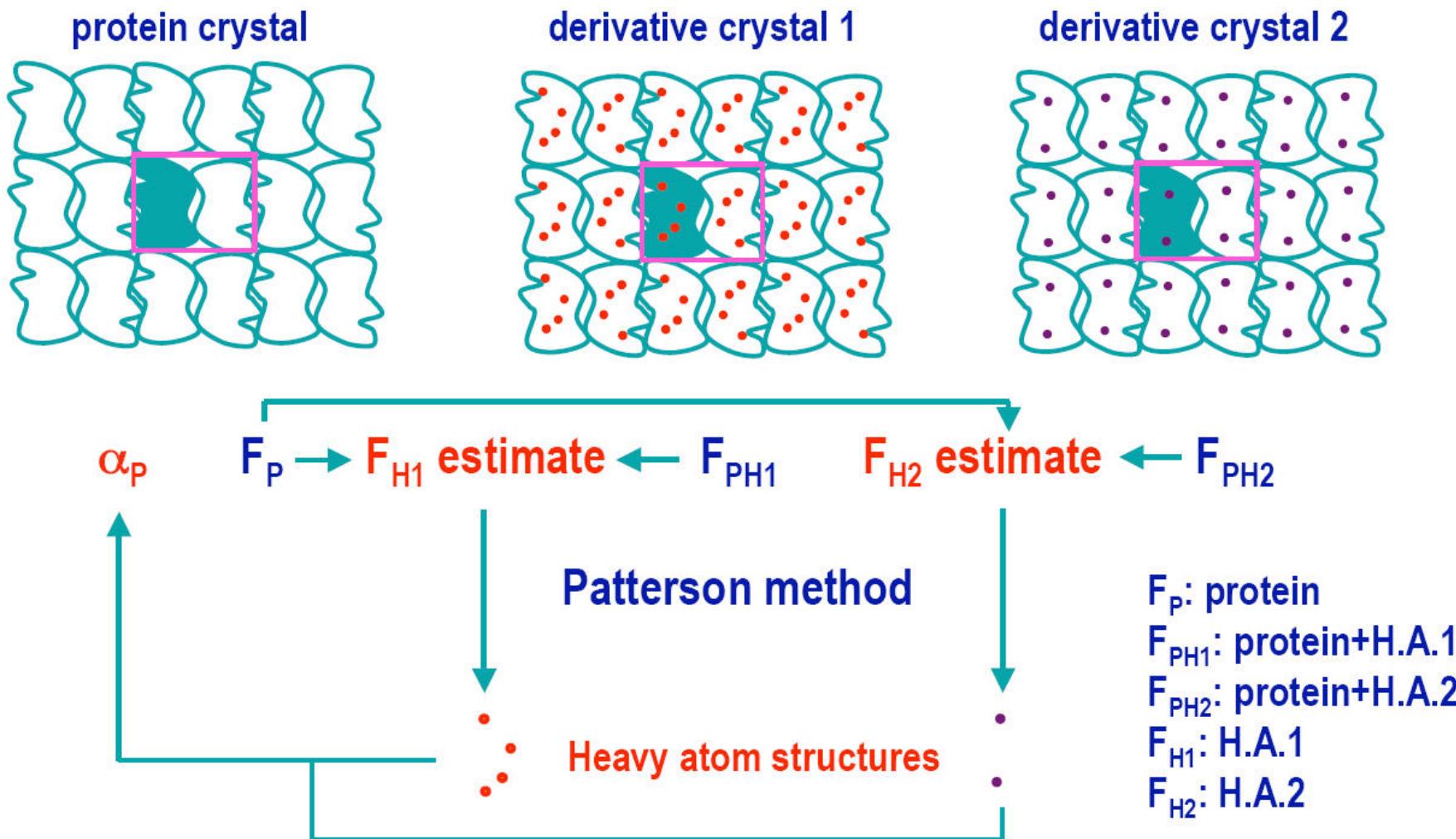
# Isomorhous Replacement (MIR)

- Heavy atom derivatives are prepared by soaking or co-crystallizing
- Diffraction data for heavy atom derivatives are collected along with the native data

$$F_{PH} = F_P + F_H$$

- Patterson function  $P(u) = \frac{1}{N} \sum |F(h)|^2 \cos(2\pi u \cdot h)$   
 $= \int_{\mathbf{r}} \rho(\mathbf{r}) \times \rho(\mathbf{r}') d\mathbf{v}$   
→ strong peaks for in Patterson map when  $\mathbf{r}$  and  $\mathbf{r}'$  are two heavy atom positions

# Phase determination by MIR



# The Patterson Function

- Obtained by setting  $F(h,k,l) = |F(h,k,l)|^2$  in the electron density Fourier equation,

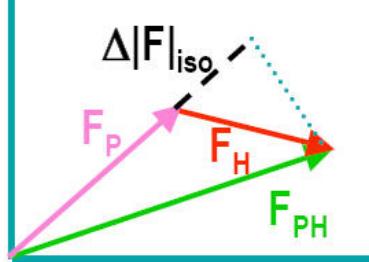
$$P(u, v, w) = \frac{1}{V} \sum_{hkl} |F(h,k,l)|^2 \cos[2\pi(hu + kv + lw)]$$

where u, v, and w are fractional coordinates in the unit cell.  
It is a Fourier summation with intensities as coefficients and phase angles equal to zero.

- Patterson peaks corresponding to vectors between atoms, i.e. inter-atomic distances.

# Obtaining $F_H$ estimate: the isomorphous difference

When diffraction data for protein and a derivative crystal are available, one can obtain the amplitude difference between the protein and the derivative:

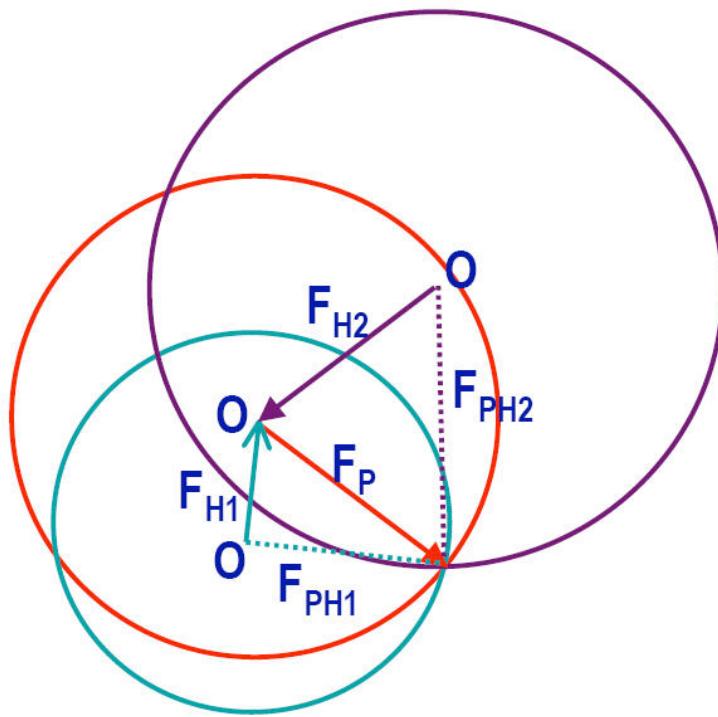


$$\Delta|F|_{iso} = |F_{PH}| - |F_P|$$

This isomorphous difference between a heavy atom containing crystal and a native crystal, is an estimate of  $|F_H|$ .

It can be used in the Patterson Function for determining the heavy atom structure.

# Phase determination by MIR once the heavy atom structures are known



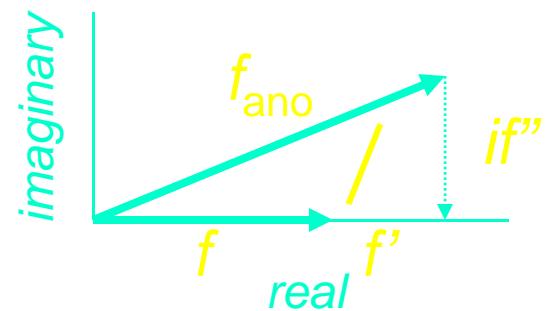
Because the amplitudes and phases of  $F_{H_1}$  and  $F_{H_2}$  are known, the three origins can be defined, which are then used to draw the three circles with radii that correspond to the amplitudes of  $F_P$ ,  $F_{PH1}$  and  $F_{PH2}$ .

# Multiple Anomalous Dispersion (MAD)

At the absorption edge of an atom, its scattering factor

$$f_{\text{ano}} = f + f' + if''$$

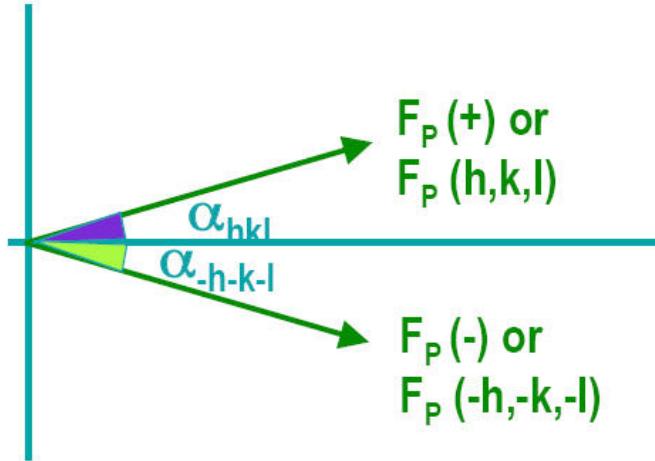
Atom	$f$	$f'$	$f''$
Hg	80	-5.0	7.7
Se	34	-0.9	1.1



- $F(h,k,l) = F(-h,-k,-l) \rightarrow$  anomalous differences → positions of anomalous scatterers → Protein Phasing

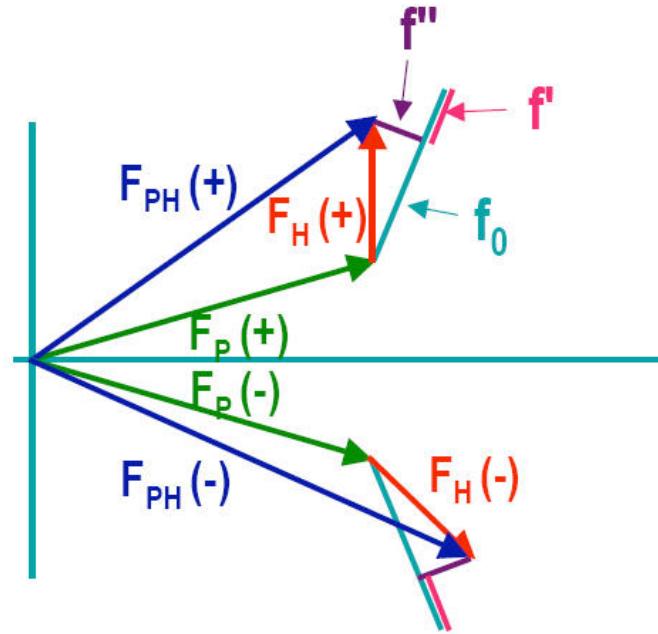
# Anomalous Scattering breaks the Friedel's Law

No anomalous scattering



$$|F_P (+)| = |F_P (-)|$$

with anomalous scattering



$|F_{PH} (+)|$  and  $|F_{PH} (-)|$  are now different

# Obtaining $F_H$ estimate: the anomalous difference

$$\Delta|F|_{ano} = [|F_{PH}(+)| - |F_{PH}(-)|]$$

Similarly as for  $\Delta|F|_{iso}$ ,  $\Delta|F|_{ano}$  is an estimate of  $|F_H|$ .

It can be used in the Patterson Function method for determining the heavy atom structure.

# Se-Met MAD

- Most common method of *ab initio* macromolecule structure determination
- A protein sample is grown in Se-Met instead of Met.
- Minimum 1 well-ordered Se-position/75 amino acids
- Anomalous data are collected from 1 crystal at Se K-edge (12.578 keV).
- MAD data are collected at Edge, Inflection, and remote wavelengths

# Resolve the SIR or SAS phase ambiguity (Handedness) by Solvent Flattening

The ISAS process is carried twice, once with heavy atom site(s) at refined locations (+++), and once in their inverted locations (---).

Data	FOM <sup>1</sup>	Handedness	FOM <sup>2</sup>	R-Factor	Corr. Coef
RHE	<b>0.54</b>	<b>Correct</b>	<b>0.82</b>	<b>0.26</b>	<b>0.958</b>
	0.54	Incorrect	0.80	0.30	0.940
NP With I <sup>3</sup>	<b>0.54</b>	<b>Correct</b>	<b>0.80</b>	<b>0.27</b>	<b>0.955</b>
	0.54	Incorrect	0.76	0.36	0.919
NP With I & S <sup>4</sup>	<b>0.56</b>	<b>Correct</b>	<b>0.82</b>	<b>0.24</b>	<b>0.964</b>
	0.56	Incorrect	0.78	0.35	0.926

<sup>1</sup>: Figure of merit before solvent flattening

<sup>2</sup>: Figure of merit after one filter and four cycles of solvent flattening

<sup>3</sup>: Four Iodine were used for phasing

<sup>4</sup>: Four Iodine and 56 Sulfur atoms were used for phasing

Heavy Atom Handedness and Protein Structure Determination using Single-wavelength Anomalous Scattering Data, ACA Annual Meeting, Montreal, July 25, 1995.

# Molecular Replacement

- If the structure of a similar molecule **is** known, structure factors of this known model may be calculated, allowing placement of the model in the unit cell by minimizing the R factor as a function of the orientation and position of the model within the unit cell:

$$R\text{-factor} = \sum_{hkl} \frac{\|F_{\text{obs}} - |F_{\text{calc}}|\|}{\sum_{hkl} |F_{\text{obs}}|}$$

The templates for a successful molecular replacement must be **structural homologues** ( $C\alpha$  rmsd < 1.5 Å), not necessarily, though often, homologous in sequence.<sup>44</sup>

# How phases are calculated in MR

- By artificially packing the model into the crystal unit cell of the unknown molecule, we can calculate the structure factor  $F$ :

$$F_{\text{calc}}(hkl) = \sum f_i \exp[2\pi i(xh + yk + lz)]$$

- $F_{\text{calc}}$  contains information about both structure factor amplitude and phase  $\alpha_{\text{calc}}$
- Using  $\alpha_{\text{calc}}$ , the unknown structure can then be computed using the following expression:

$$\rho_{(x,y,z)} = \frac{1}{V} \sum_h \sum_k \sum_l F_{(h,k,l)} \exp[-2\pi \cdot i(hx + ky + lz)]$$

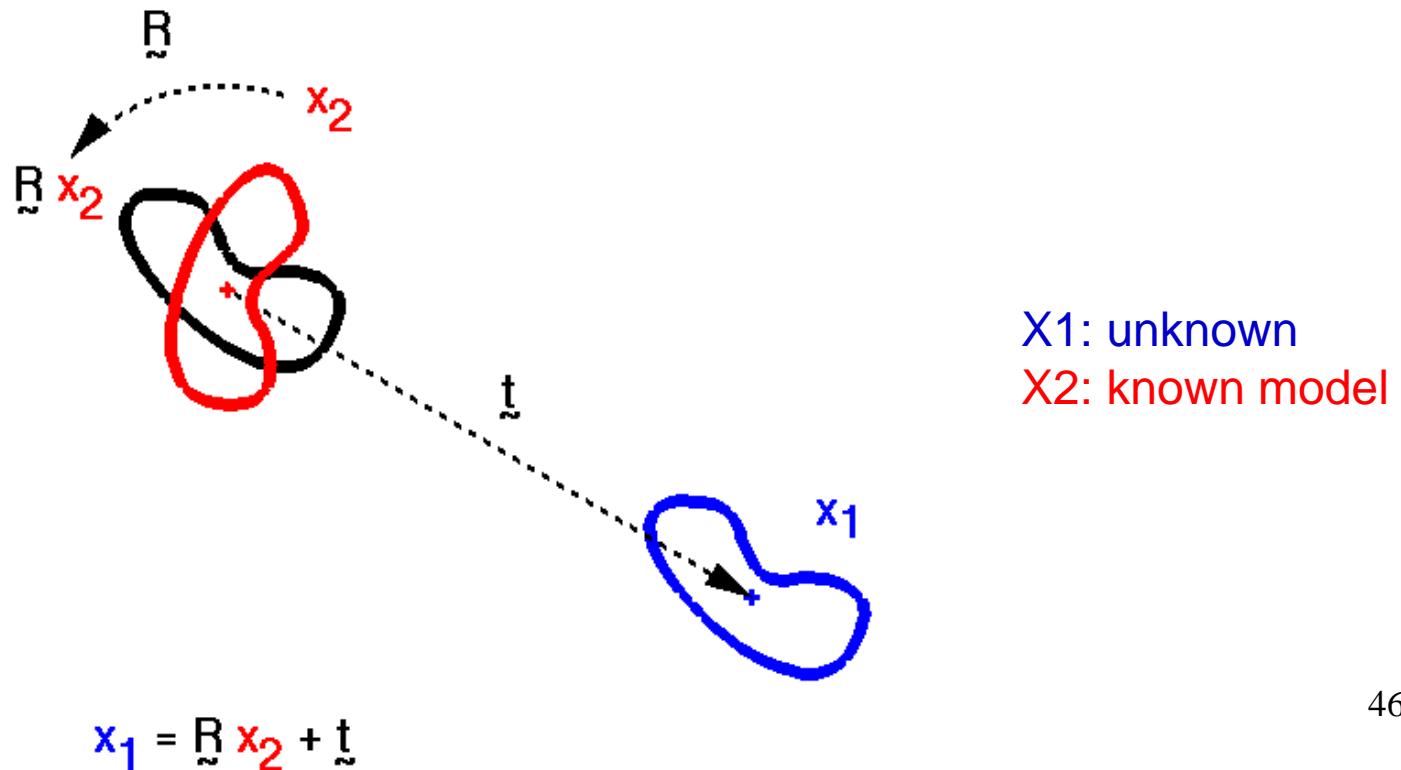
$\downarrow$

$$F_{\text{obs}(h,k,l)} \exp(i\alpha_{\text{calc}(h,k,l)})$$

Molecular replacement provides  $\alpha_{\text{calc}(h,k,l)}$  for electron density calculation

# Two step process in Molecular Replacement

1. find orientation of model (red ==> black)  
Rotation Function
2. find location of orientated model (black ==> blue)  
Translation Function



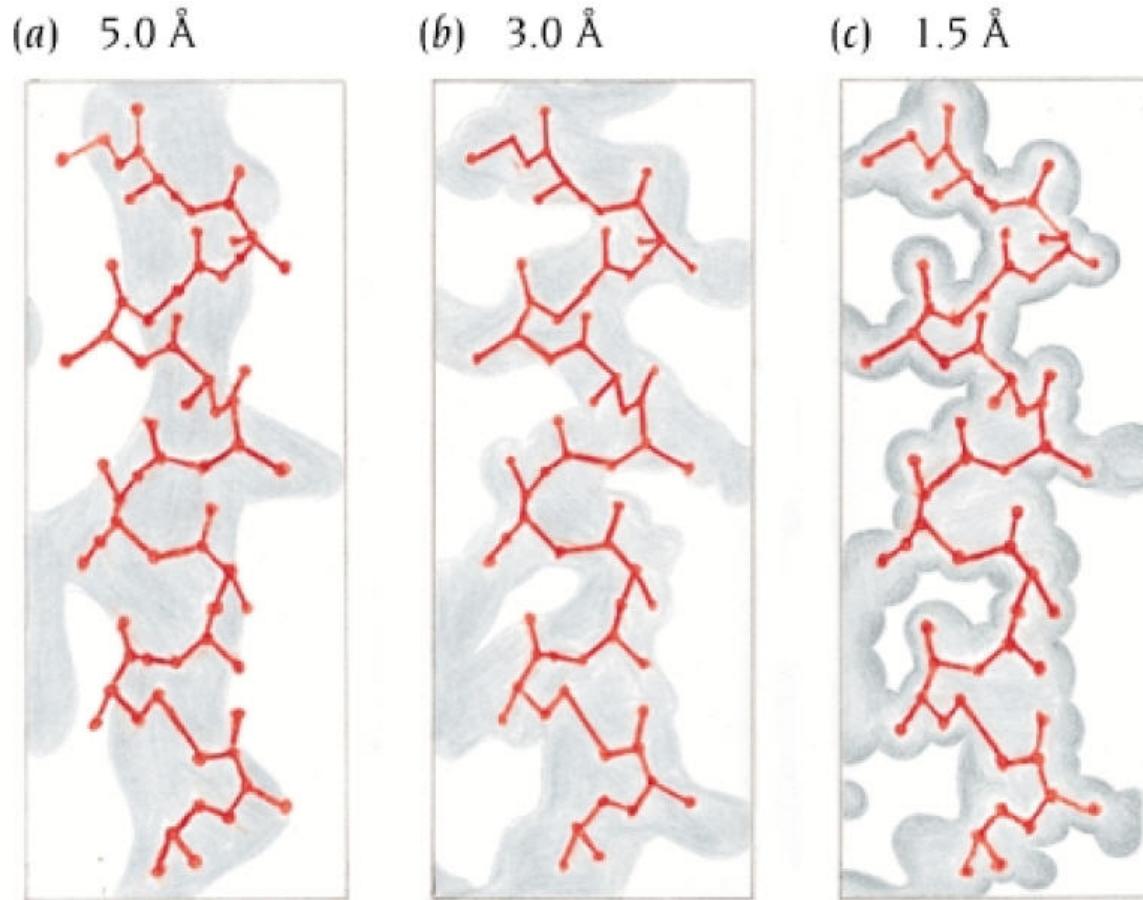
# Once phases are determined, an electron density map can be calculated

- From diffraction  $F(h,k,l)$  to electron density by Fourier Transform:

$$\begin{aligned}\rho(x,y,z) &= \frac{1}{V} \sum_h \sum_k \sum_l F(h,k,l) \exp(-2\pi i(hx + ky + lz)] \\ &= \frac{1}{V} \sum_h \sum_k \sum_l |F(h,k,l)| \exp(i\alpha(h,k,l) - 2\pi i(hx + ky + lz)]\end{aligned}$$

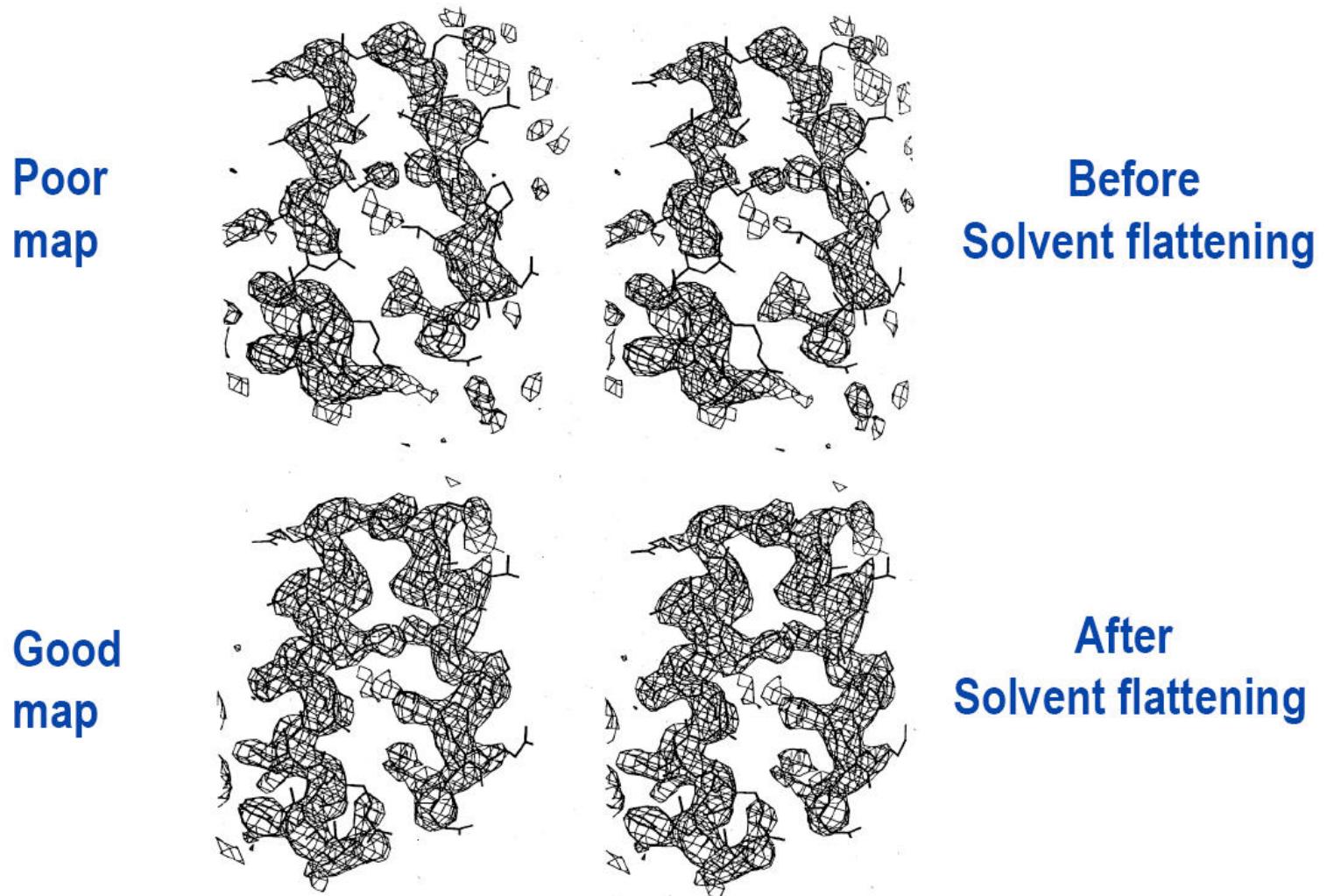
- Amplitudes measured from a native diffraction data:  $|F| = |I|^{1/2}$ .
- Phases computed from MIR, SIR, SIRAS, MAD, SAD or MR.
- For each reflection, a phase reliability coefficient or ‘figure of merit’ (between 0 and 1) may be multiplied to reduce the contribution from less reliable reflections.

# Maps at different resolution show different levels of details



Maps are contoured on a certain sigma value, such as 1.0.

# Phase refinement by solvent flattening

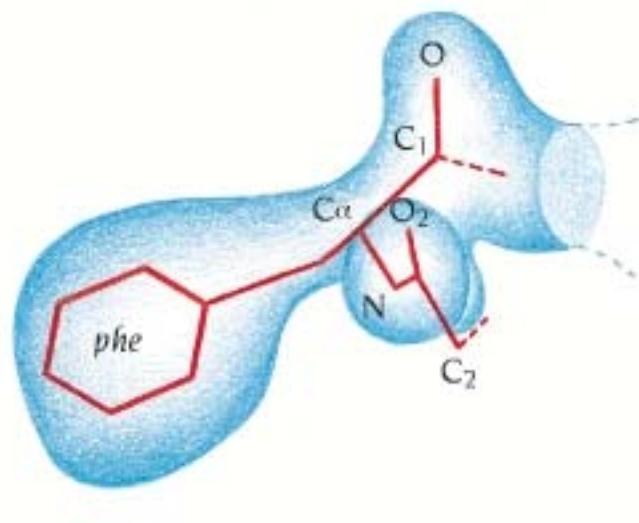
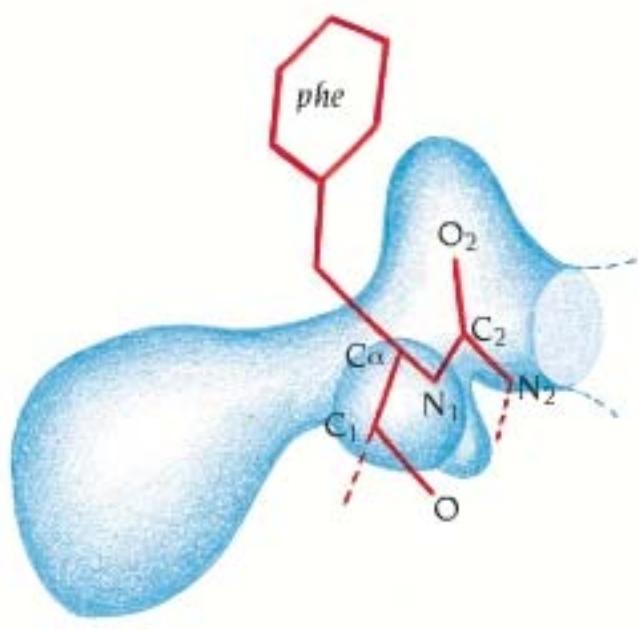


E. coli soluble lytic transglycosylase at 3.3 $\text{\AA}$  resolution.  
Top: MIR map; bottom: solvent flattened map (60% solvent)

# Steps in structure determination by X-ray crystallography

- Sample preparation
  - chemical, conformational and aggregational homogeneity
- Crystallization
- Data collection and process
- Phase determination
  - Isomorphous replacement
  - Anomalous scattering
  - molecular replacement, etc
- **Model building and refinement**
- Structural analysis

# Model building is the fitting of atomic models into electronic density



# Least-Squares Refinement

List-squares refinement of atoms (x,y,z, and B) against observed  $|F(h,k,l)|$

Target function that is minimized

$$Q = \sum w(h,k,l) (|F_{\text{obs}}(h,k,l)| - |F_{\text{cal}}(h,k,l)|)^2$$

$dQ/du_j = 0$ ;  $u_j$ - all atomic parameters

## What to refine?

- Atom coordinates: (x, y, z)s
- B factors and occupancy

## Steps in refinement

1. Rigid body refinement
2. Energy minimization
3. Simulated annealing refinement
4. Model building in graphics by hand
5. Add waters and other molecules
6. B factor refinement

# Geometric Restraints in Refinement

Each atom has 4 (x,y,z,B) parameters and each parameter requires minimum 3 observations for a free-atom least-squares refinement. → A protein of N atoms requires  $12N$  observations.

For proteins diffracting  $< 2.0 \text{ \AA}$  resolution observation to parameter ratio is considerably less.

Protein Restraints (bond lengths, bond angles, planarity of an aromatic ring etc.) are used as restraints to reduce the number of parameters

# R-factor

$$R_{\text{cryst}} = \frac{\sum_{hkl} |F_{\text{obs}}(hkl) - kF_{\text{cal}}(hkl)|}{\sum_{hkl} |F_{\text{obs}}(hkl)|}$$

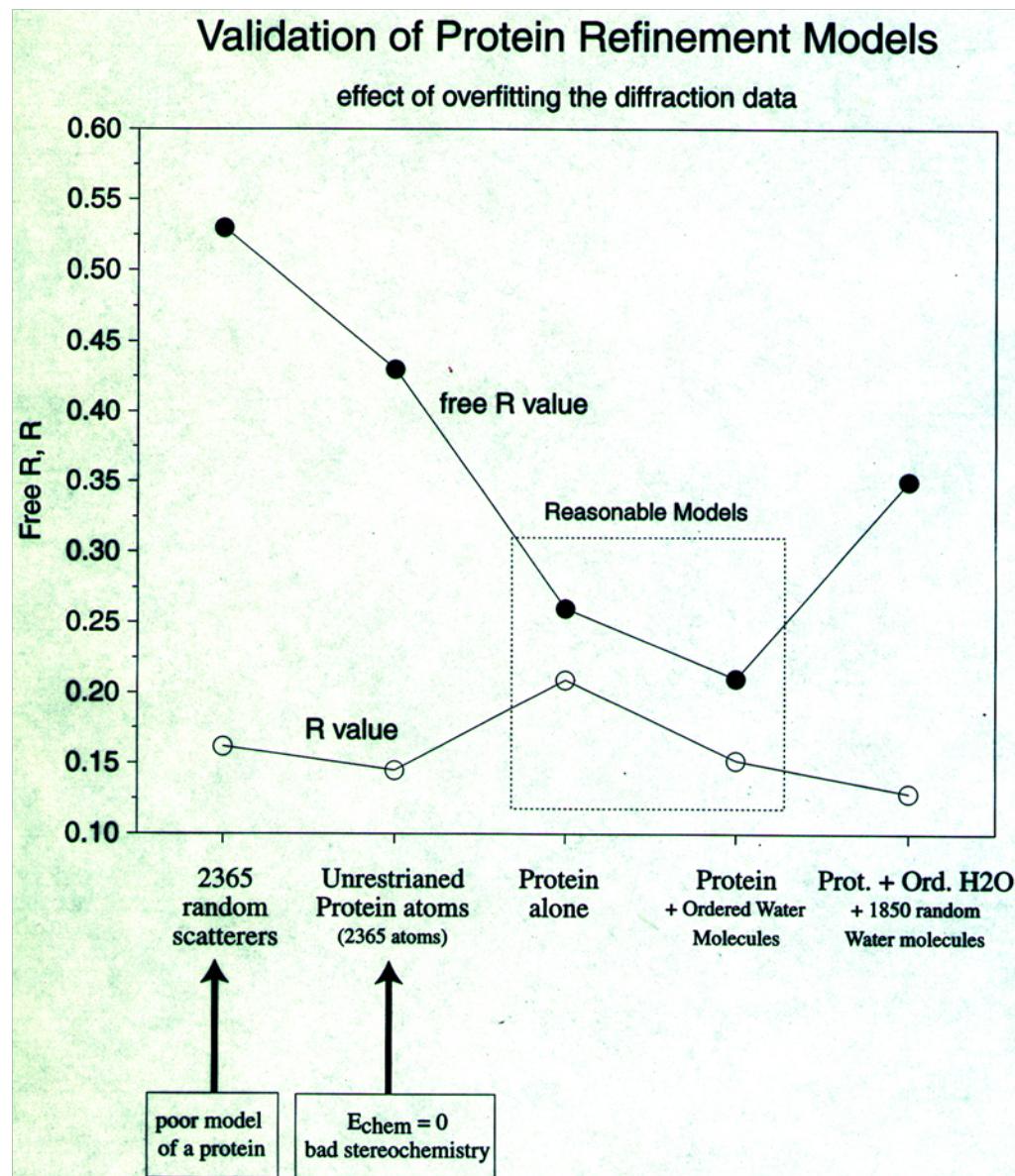
## Free-R

R-factor calculated for a test-set of reflections  
that is never included in refinement.

R-free is always higher than R.

Difference between R and R-free is smaller for  
higher resolution and well-refined structures

# Free R Factor



## Refinement Validation

- Free R factor
- Ramachandran plot
- Luzzati plot
- Molprobity

When you believe you have done the best model building and Refinement, you can deposit your structural factors and atomic Coordinates to PDB: [www.pdb.org](http://www.pdb.org)

# The famous “Table 1”: crystallographic refinement statistics

hTRANCE-R complex	
Crystal	
TRAF6	Residues 346-504
Peptide	342-QMPTEDEY
Space group	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>
Cell dimensions	a=38.0Å, b=45.0Å, c=106.5Å
Diffraction Data	
Resolution	40-2.0Å
R <sub>sym</sub> (last shell)	5.5% (13.9%)
Completeness (last shell)	96.0% (86.9%)
Refinement	
Resolution	20-2.0Å
Sigma cutoff	2.0
Number of protein residues	161
Number of protein atoms	1331
Number of solvent atoms	80
Number of reflections used	12396
R (R <sub>free</sub> )	21.3% (24.2%)
RMSD bond length	0.005Å
RMSD bond angle	1.3°

In this case, even at 2.0 Å resolution, reflection/parameters ratio  
= 12396/4\*(1331+80) = 2.2, very under-determined.

# Structural analysis

- Can you explain biological data from this structure?
- What is the structural basis of the biological function?
- What implication can you get from the structure? Can you design better inhibitor or substrate (drug design, protein engineering...)
- Are what you observed from this crystal structure believable (or artifact from crystallization?)
- Paper writing

# Further reading if you are interested

*Crystallography Made Crystal Clear*, by Gale Rhodes

*Principles of Protein X-ray Crystallography*, by Jan Drenth

*International Tables for Crystallography*, vol F, edited by

M. G. Rossmann & Eddy Arnold

<http://www.ruppweb.org/Xray/101index.html>

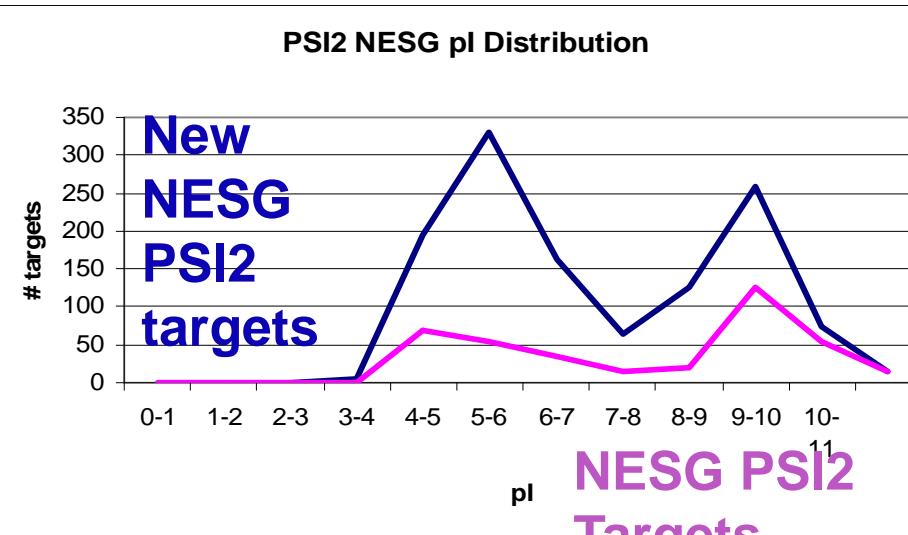
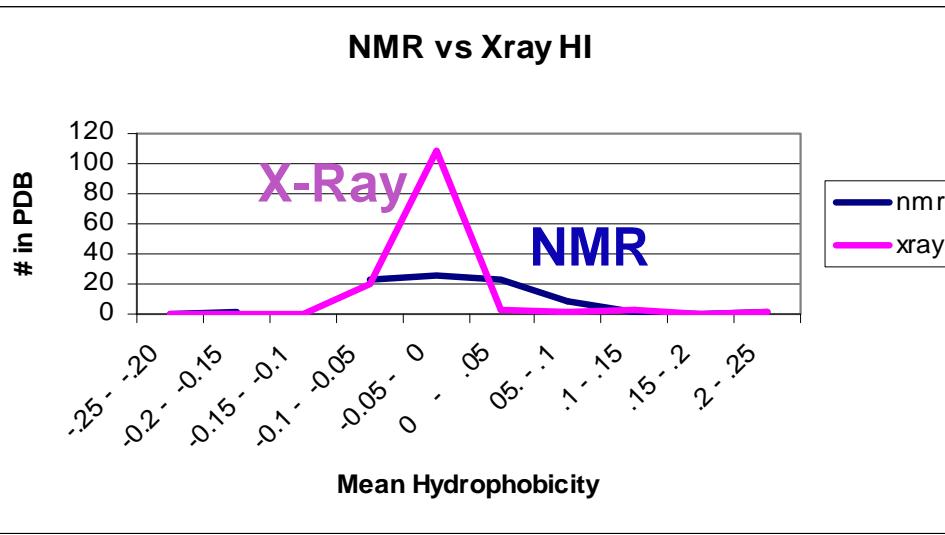
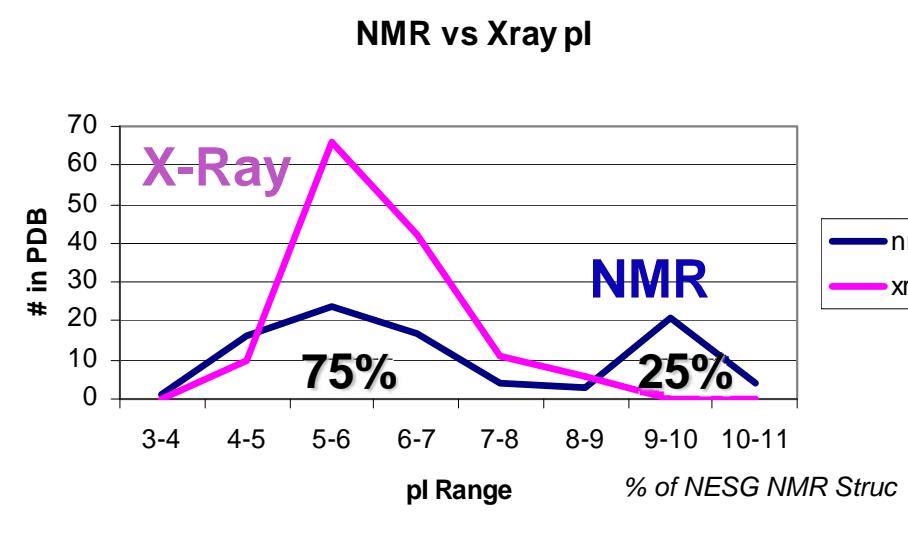
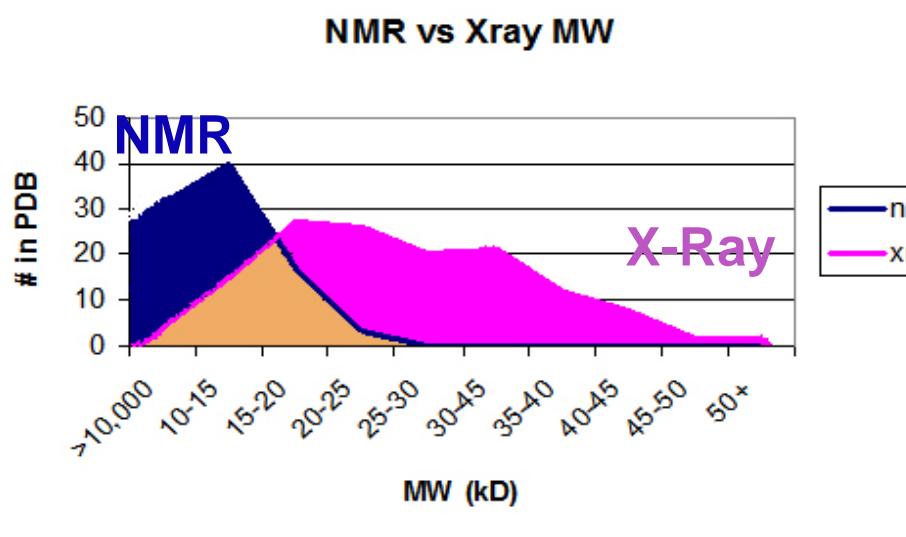
<http://www.iucr.org/cww-top/edu.index.html>

*Crystallography history (fun reading)*

From the Structures of simple Salts to Those of Sophisticated Viruses, by M. G. Rossmann, Acta Crystal. A54, 1998, 716-728

Fifty Years of X-ray Diffraction, Edited by P. P. Ewald, 1962.

# Biophysical Features of 230 NESG Structures in PDB



# Other possible topics

- Time-resolved X-ray crystallography
- Direct method to solve phase problem
- Small angle X-ray scattering
- X-ray powder diffraction to solve protein structures
- Difference between NMR and X-ray crystallography
- Neutron diffraction
- Drug design
- Virus/supercomplex crystallography