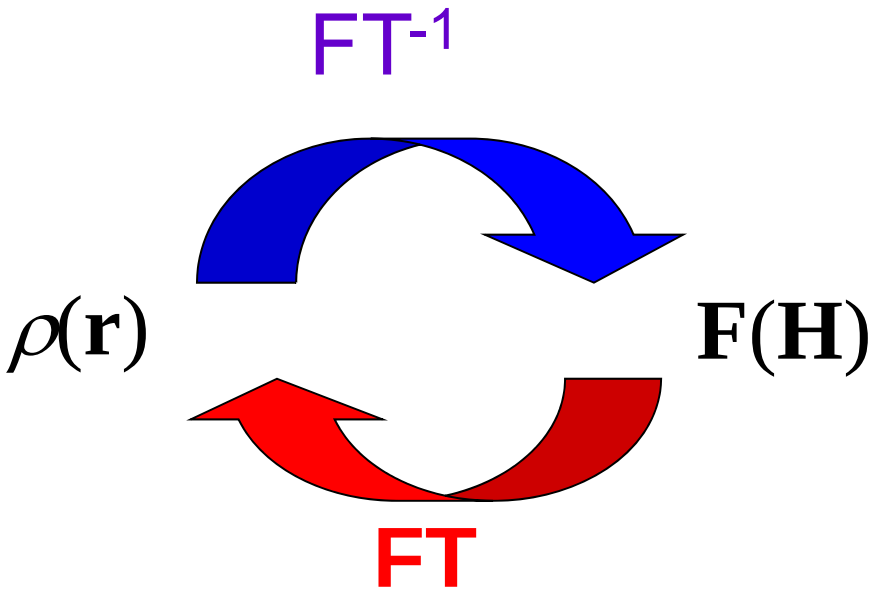
connection between the structure factor and the electron density



¡ phase problem !

In each reciprocal lattice point ***H*** we measure a reflection with the intensity ***I(H)***

$$I(\mathbf{H}) \propto |F(\mathbf{H})|^2$$

but

$$\mathbf{F}(\mathbf{H}) = |\mathbf{F}(\mathbf{H})| \exp(i\varphi)$$

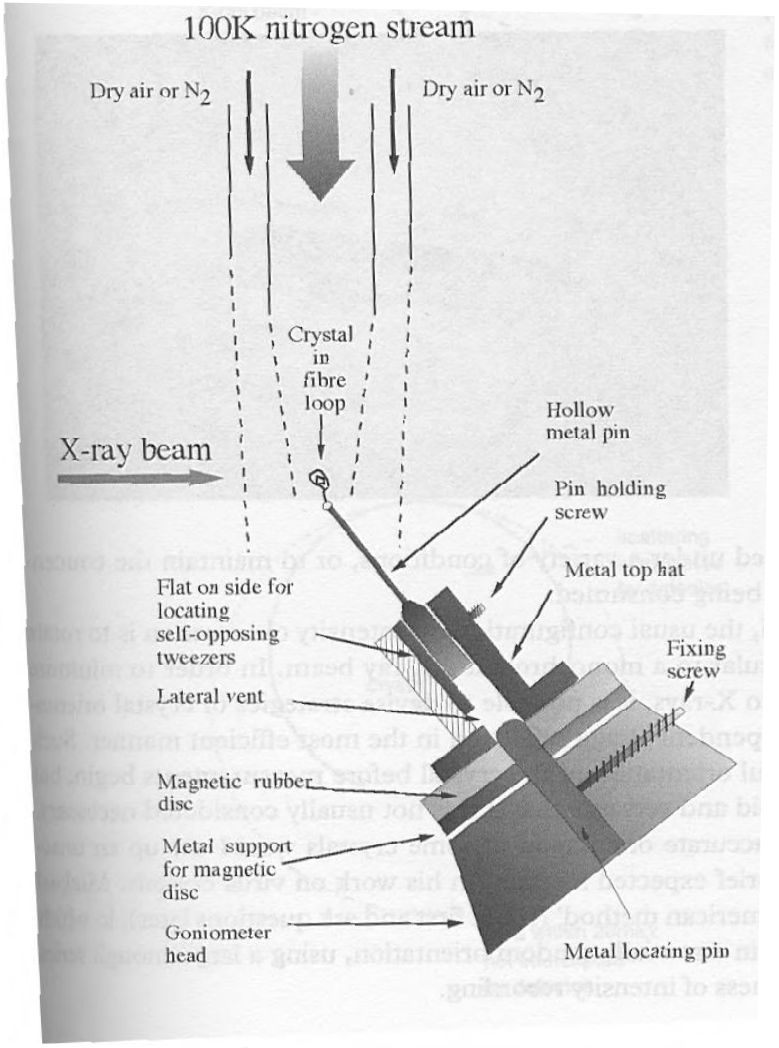
we loose the phase at the measurement

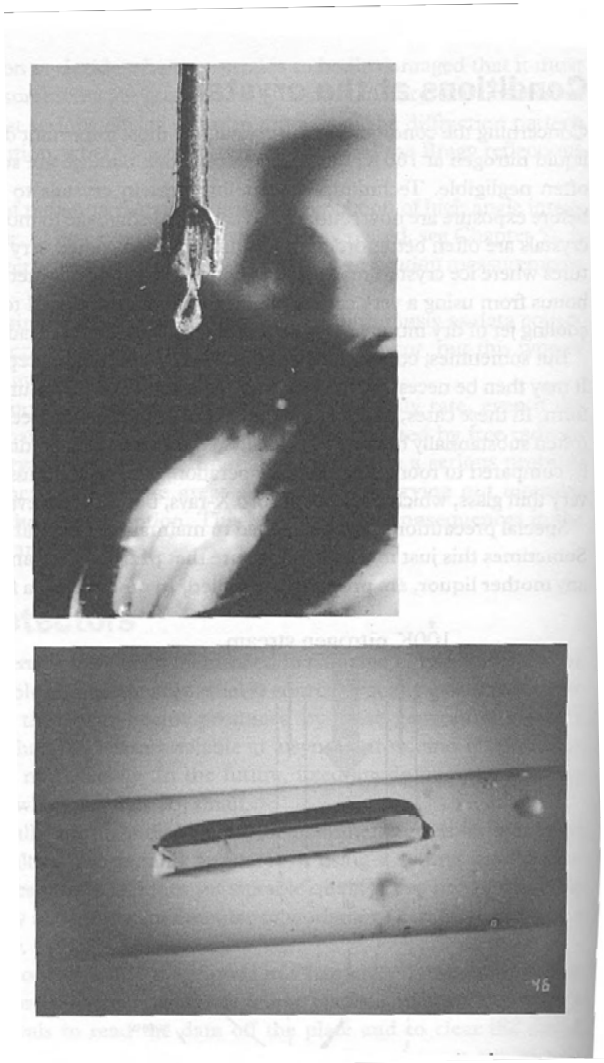
(the cost of not having an X-ray lense)

considerations before data collection

- synchrotron or ’home’
  - normally you end up in a synchrotron
- mounting
  - cryo cooled or room temperature
- data quality
  - how good data do you need before collecting data
- exposure time
  - how much radiation can your crystal take

Typical mount





mounting the crystal

cryo cooling – low temperature

- ✓ reduced radiation damage
- ✓ No capillary necessary
- ✓ can be stored for months

- ✘ You need to find suitable cryo protection
- ✘ risc for ice rings

capillary – room temperature

- ✓ closer to physiological conditions
- ✓ easier to control pH

- ✘ The crystals ”burn up”
- ✘ difficult to mount

typical data collection

- the detector is placed, so that the diffraction reaches the edge
- the exposure time is fixed to a certain time, Δt, between 2 and 30 seconds (depending on the radiation strength and the crystal quality)
- During the time Δt the crystal is turned the angle, $\Delta\varphi$, between ½ and 2 degrees.
- repeat until the wanted part of reciprocal space has been covered – usually 180°

data processing

- you have
  - a collection of diffraction images
- you want
  - a list of *h k l Intensity(hkl)*

- From the position of the spots the possible unit cells are calculated
  - the most likely is the one with highest symmetry and the smallest distortion

example on unit cell determination

INDEXING FROM DENZO

Lattice	Metric tensor distortion index	Best cell (symmetrized) / Best cell (without symmetry restrains)					
primitive cubic	18.98%	90.44	98.03	98.05	119.89	89.95	89.96
		95.58	95.58	95.58	90.00	90.00	90.00
I centred cubic	30.49%	133.33	133.34	98.21	68.44	68.46	79.05
		122.75	122.75	122.75	90.00	90.00	90.00
F centred cubic	40.61%	192.30	133.61	133.40	85.28	71.43	108.58
		155.59	155.59	155.59	90.00	90.00	90.00
primitive rhombohedral	25.77%	90.44	98.05	98.03	119.89	90.04	90.05
		95.58	95.58	95.58	100.10	100.10	100.10
		133.44	133.44	133.40	90.00	90.00	120.00
primitive hexagonal	0.07%	98.05	98.03	90.44	90.04	90.05	119.89
		98.04	98.04	90.44	90.00	90.00	120.00
primitive tetragonal	17.48%	98.03	98.05	90.44	90.05	89.96	60.11
		98.04	98.04	90.44	90.00	90.00	90.00
I centred tetragonal	16.74%	133.45	133.40	98.03	68.40	68.55	79.16
		133.43	133.43	98.03	90.00	90.00	90.00
primitive orthorhombic	17.48%	90.44	98.03	98.05	119.89	89.95	89.96
		90.44	98.03	98.05	90.00	90.00	90.00
C centred orthorhombic	0.03%	98.21	169.72	90.44	90.01	89.91	90.02
		98.21	169.72	90.44	90.00	90.00	90.00
I centred orthorhombic	16.74%	98.03	133.40	133.45	100.84	111.45	68.40
		98.03	133.40	133.45	90.00	90.00	90.00
F centred orthorhombic	11.88%	98.03	170.02	205.68	90.02	118.43	90.10
		98.03	170.02	205.68	90.00	90.00	90.00
primitive monoclinic	0.04%	98.03	90.44	98.05	90.05	119.89	90.04
		98.03	90.44	98.05	90.00	119.89	90.00
C centred monoclinic	0.01%	98.21	169.72	90.44	89.99	90.09	90.02
		98.21	169.72	90.44	90.00	90.09	90.00

data processing

- after determination of the unit cell all reflections may be indexed (given *hkl)*
  - *there is a unique connection between the direct and the reciprocal lattice*
- Then the reflections are integrated (add all counts in a reflection – subtract the background)
- the list *h k l Intensity(hkl)* is made

determination of the crystal symmetry – merging reflections

calculate an internal R-factor

$$R_{sym} = \frac{\sum_N \left[\sum_n \left(I_i(\mathbf{h}) - \bar{I}(\mathbf{h}) \right) / n \right]}{\sum_N \bar{I}(\mathbf{h})}$$

the smaller R_{sym} - the better

it is always somewhat larger when more reflections are averaged

NB: several R-factors appear in crystallography. This one is a measure of data quality and has nothing to do with the one from REFMAC5

data collection for *tobacco* peroxidase

Beamline	I911-5, MAX II, Sweden
Detector	MARResearch ccd
Wavelength (Å)	0.9074
Temperature (K)	100
Space group	$C222_1$
Unit-cell parameters (Å)	a = 99.81, b = 123.47, c=59.06
Resolution range (Å)	20 – 2.0 (2.2 – 2.0)
No. of reflections	83299 (23829)
No. of unique reflections	21620 (6125)
Mosaicity (°)	0.35
Redundancy	3.85 (3.89)
Completeness (%)	86.2 (99.5)
$I/\sigma(I)$	9.84 (3.90)
R_{int} (%)	12.6 (36.1)