Bi 204 Methods:

Seeing atomic Structure:
Calibrating Molecular Interactions

Bob Stroud
2019

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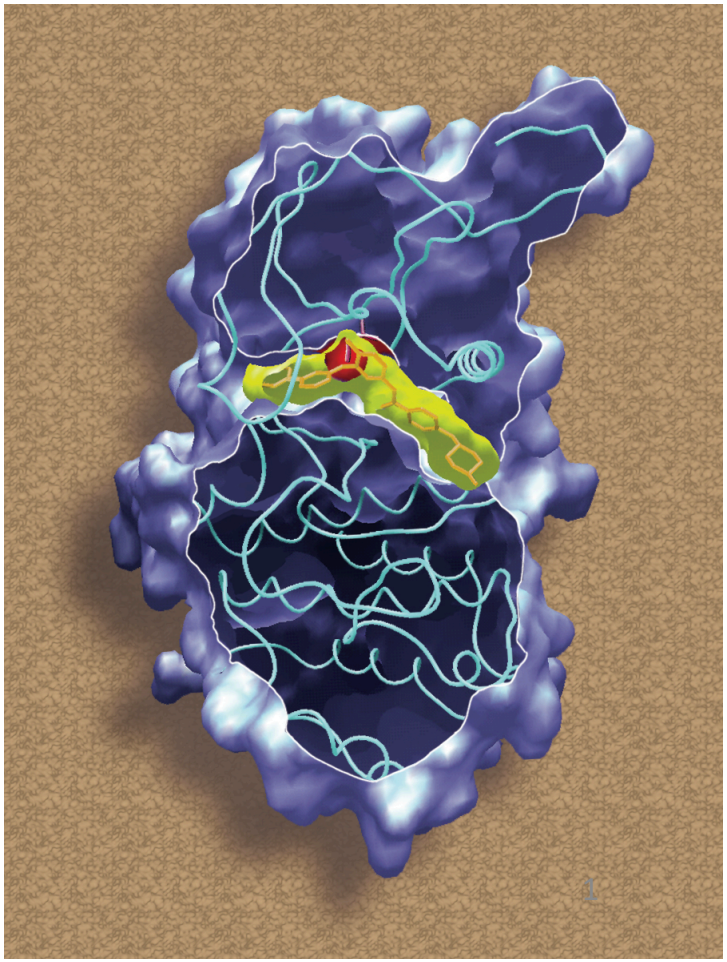
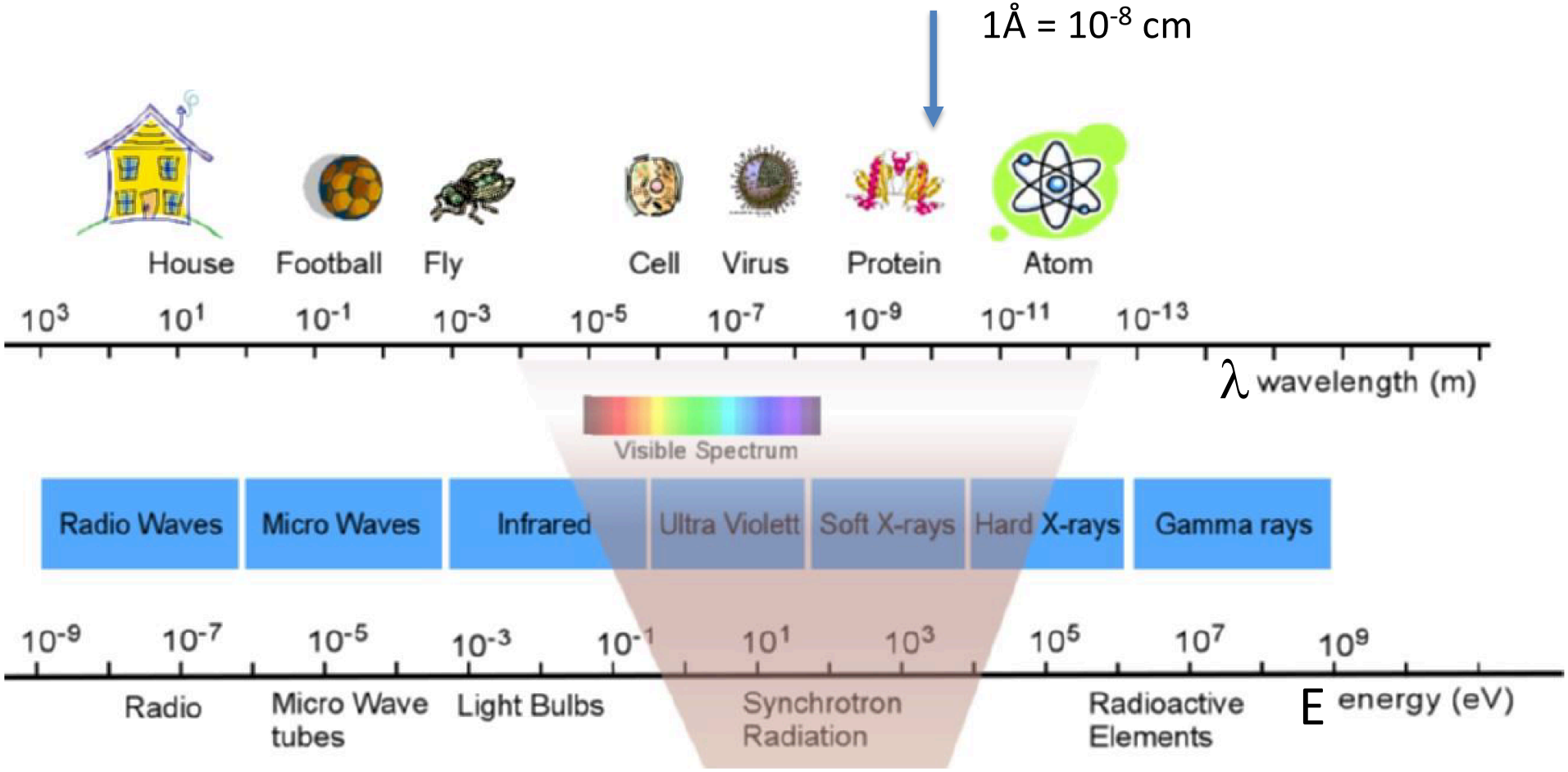


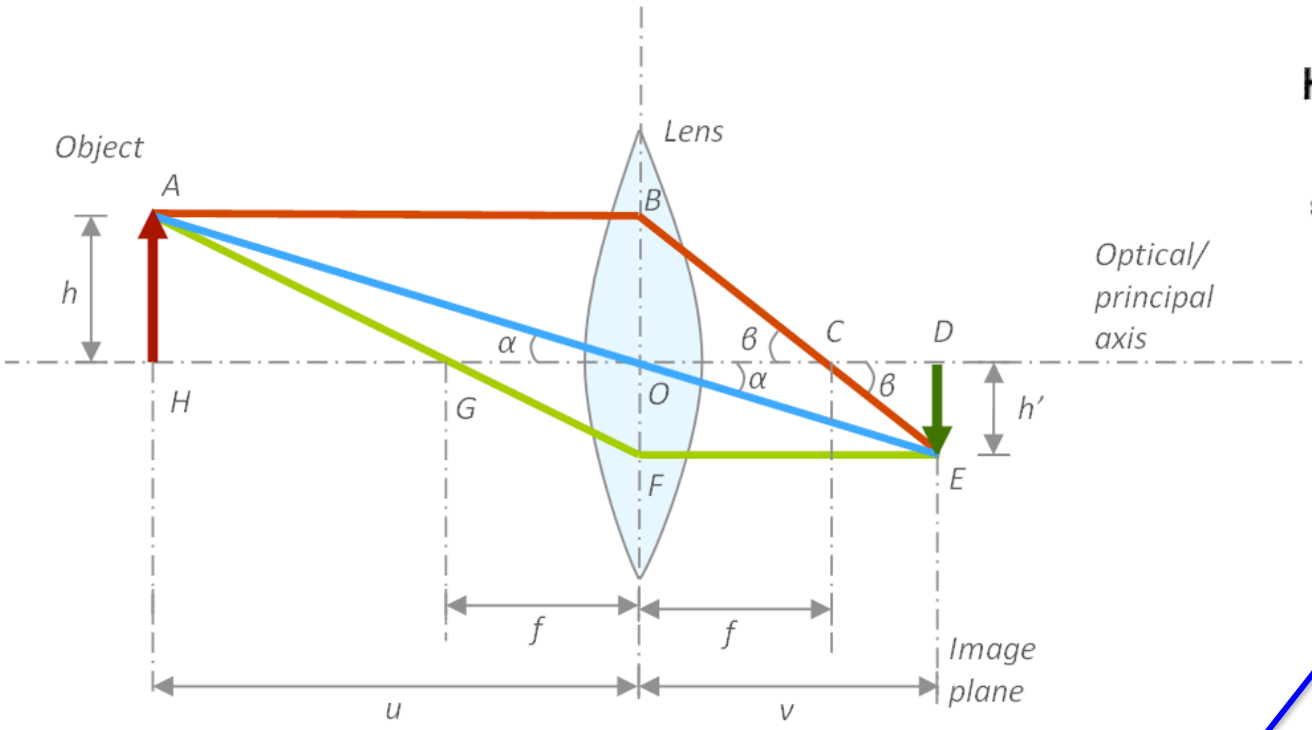
A ‘Ligand’ the cancer drug imatinib (Gleevec) bound to the tyrosine kinase Abl.

The Electromagnetic Spectrum



$E = h\nu = hc/\lambda$

Optical image formation, - without lenses



Functional Mimicry of a Protein Hormone by a Peptide Agonist: The EPO Receptor Complex at 2.8 Å

Oded Livnah, Enrico A. Stura, Dana L. Johnson, Steven A. Middleton, Linda S. Mulcahy, Nicholas C. Wrighton, William J. Dower, Linda K. Jolliffe, Ian A. Wilson*

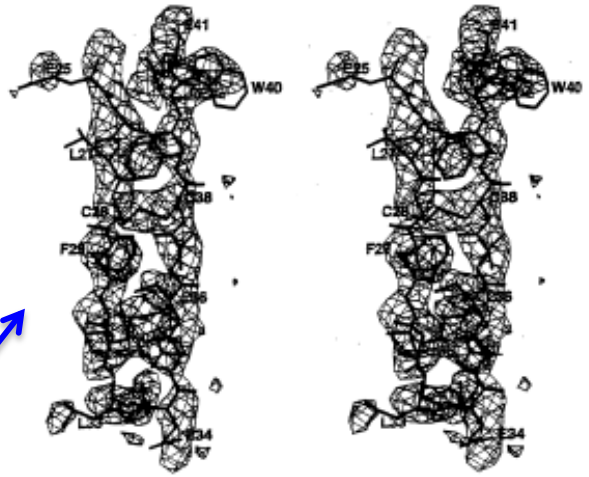
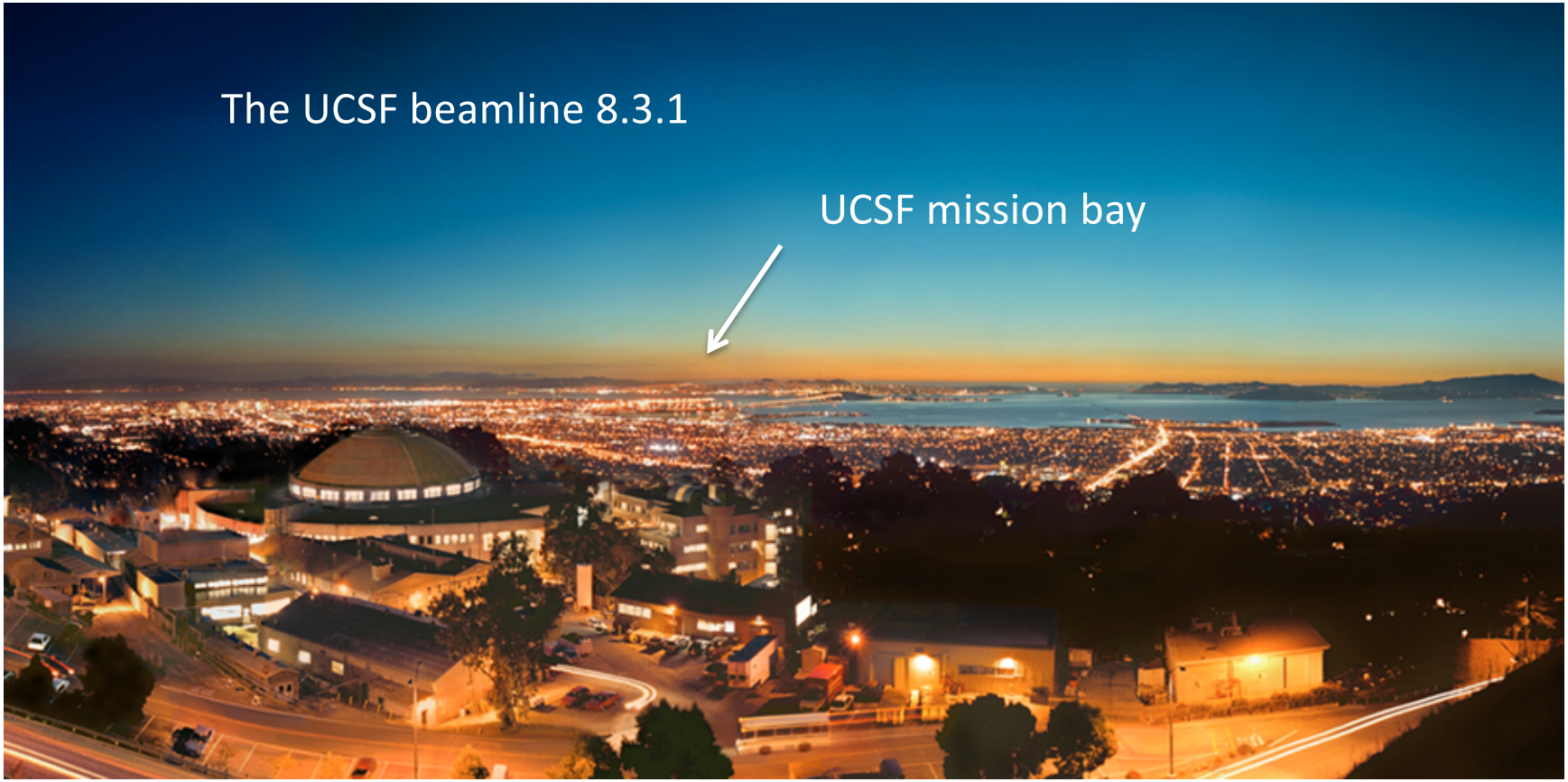
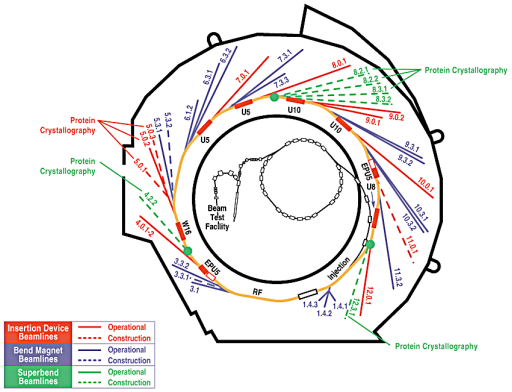
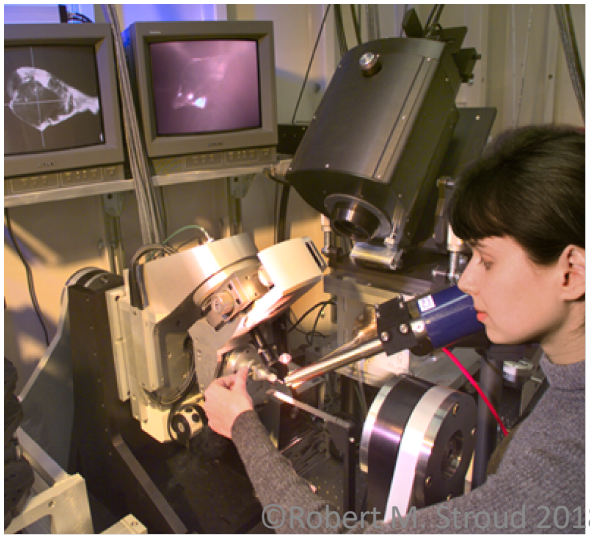


Fig. 1. Stereoview of the initial experimental solvent-flattened MIR electron density map at 25.0 to 3.1 Å resolution, contoured at 1.3 σ for residues 25 to 41 with superimposed coordinates from the final refined structure. This segment covers β strands A and B in D1 and shows one of the two characteristic disulfide bridges [Cys^{25}–Cys^{36}] in the first domain of the cytokine receptor superfamily.

Type of light	wavelength	what we see?	character	speed
Light	5000Å	dielectric	em. waves-good lenses++	speed of light
X-rays	1 to 3 Å	electron density; f ~ n_e	em. waves-NO lenses	speed of light
neutrons	1 to 5 Å	nuclei	particles NO lenses	slow speed thermal neutrons
electrons	0.01 - 0.1 Å	electric fields	particles Poor lenses.	eV~0.5mv^2.







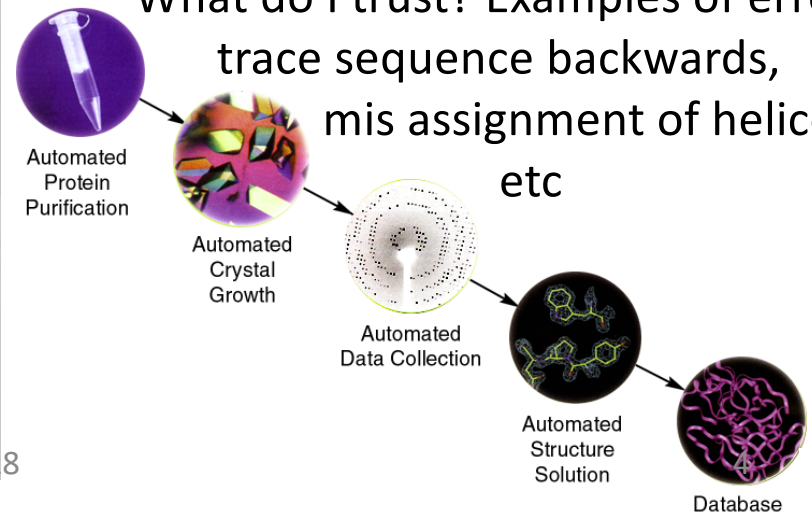
If automated- why are there errors?

What do I trust? Examples of errors

trace sequence backwards,

mis assignment of helices

etc

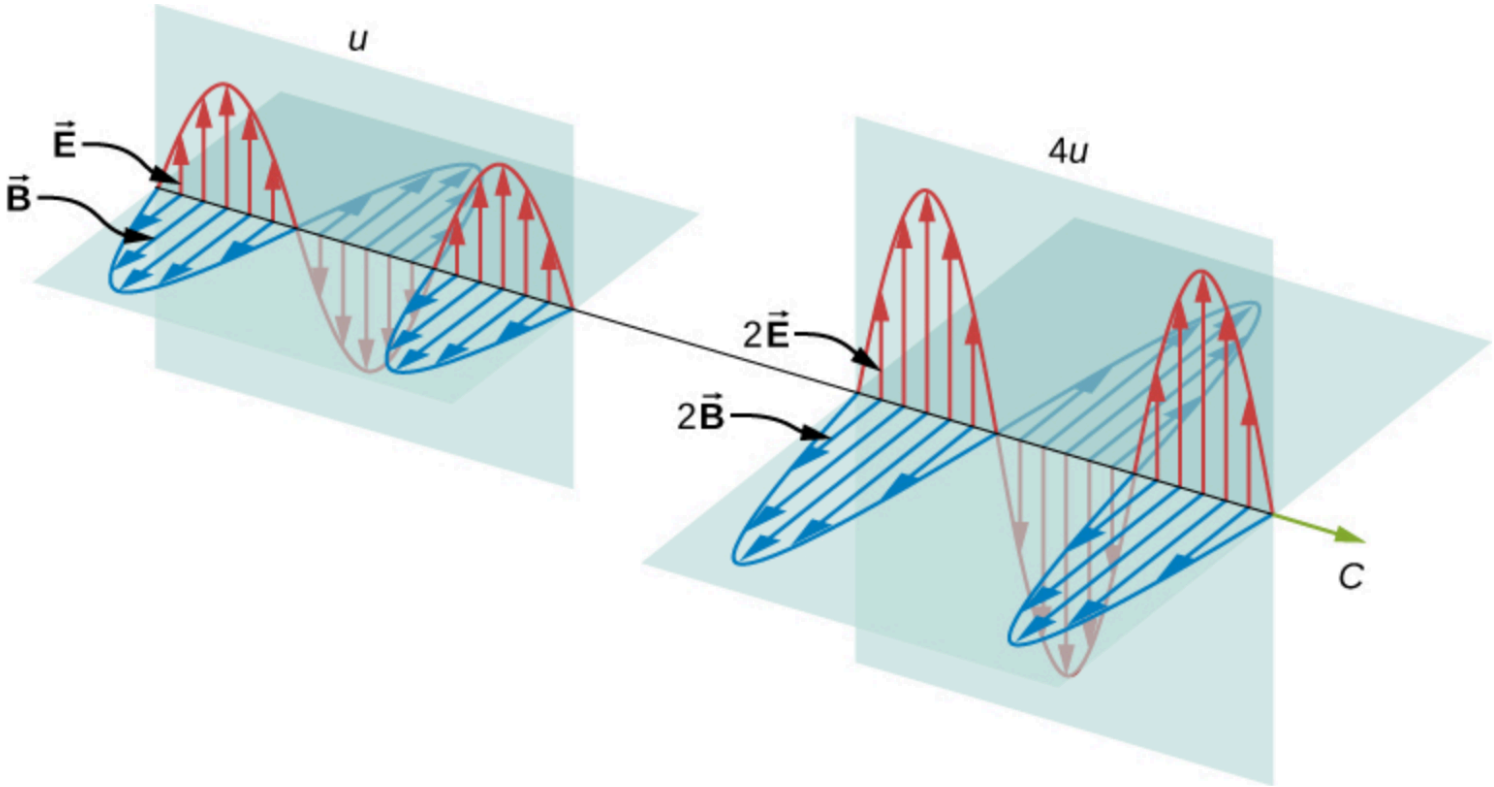


For X-rays, No Lenses……

Intensity (we can observe) = |Amplitude|² (We cannot observe), but can calculate as $= \sqrt{\text{Intensity}}$

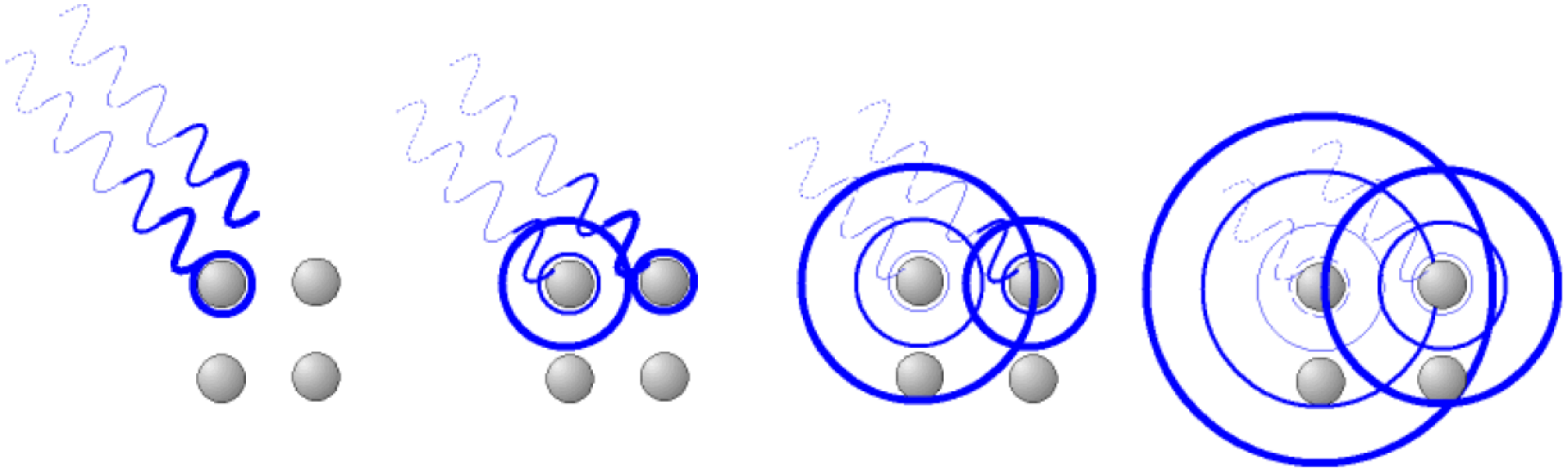
I= ***E*** x ***B*** in the c direction

$I = E_0 B_0 / 2\mu_0$ ~ Amplitude^2



Elastic scattering for structure determination

X-ray scattering



X-rays are scattered at the electrons of the atomic shell. During the scattering process the electron is starts oscilating. It becomes a dipol and a spherical wave is sent out. The wavelength and energy of the scattered wave does not change (elastic scattering).

We observe Intensity, (can't observe Amplitude directly)

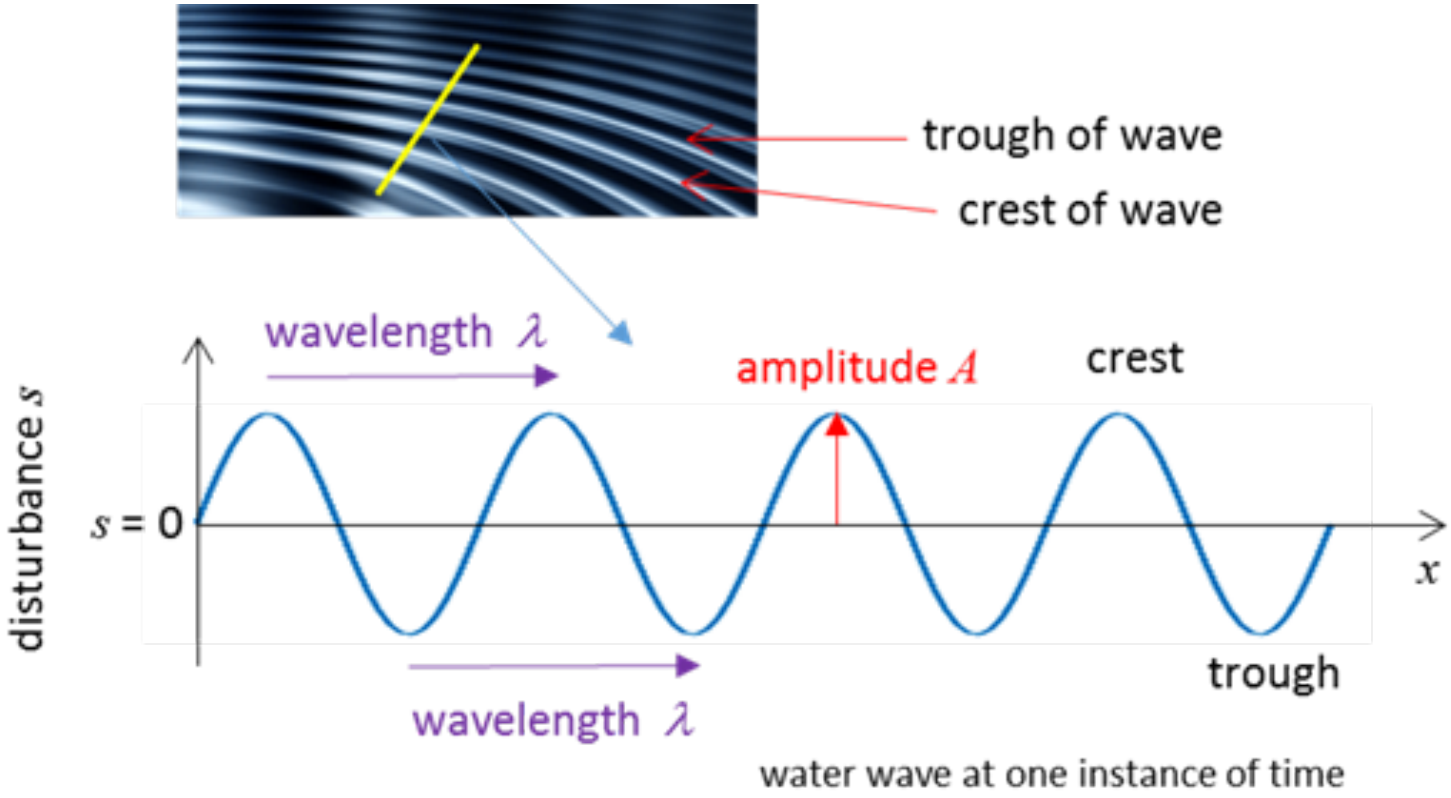
Intensity = Power/ unit area = Energy/sec . unit area

For a vibrating particle, Energy = ½ mv^2 ~ ½ m $(ds/dt)^2$

Eg. If $s = a_0 \sin(\omega t)$ so $ds/dt = a_0 \cos(\omega t)$

Integral $(ds/dt)^2$ over time $= a_0^2/2$

Energy ~ ½ m $(a_0)^2$ per $1/\omega$ time **Intensity ~ $(a_0)^2$**



water wave at one instance of time

Hence Amplitude = $|F|$

$|F|^2$ = $\underline{\mathbf{F}}.\underline{\mathbf{F}}^*$ = Intensity

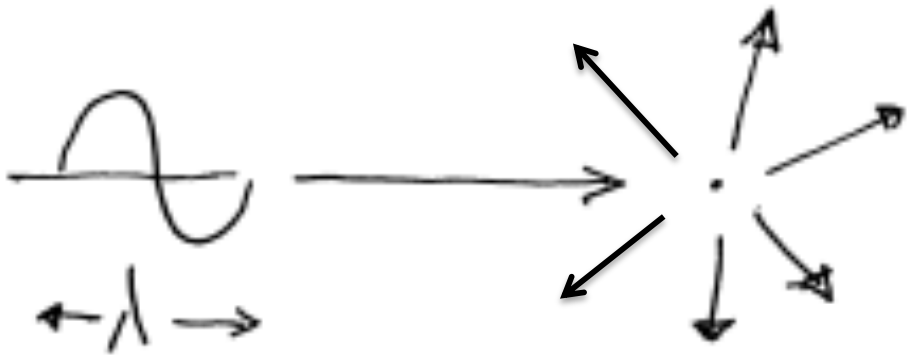
Measure Intensity, each spot position (h,k,l)

Take $\sqrt{\text{Intensity}}$ = $|F|_{h,k,l}$

Then Need relative phase of each….

THE CENTRAL AXIOM

Elastic Scattering from a point is equal in every direction



Scattering from a point is equal in all directions.

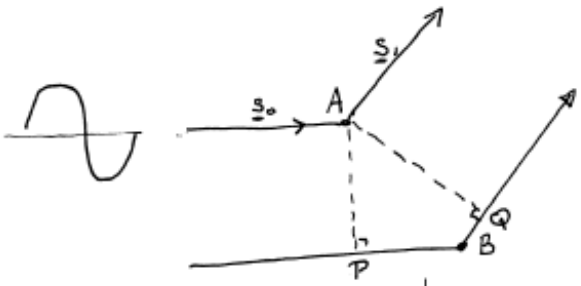
Scattering from multiple points? Add wave amplitudes with phase change

Adding up the scattering of Atoms:
Amplitudes, 'interference' of waves

Scattering by matter -(interference)
of a single wavelength X-ray

Scattering from a point is equal in all directions.

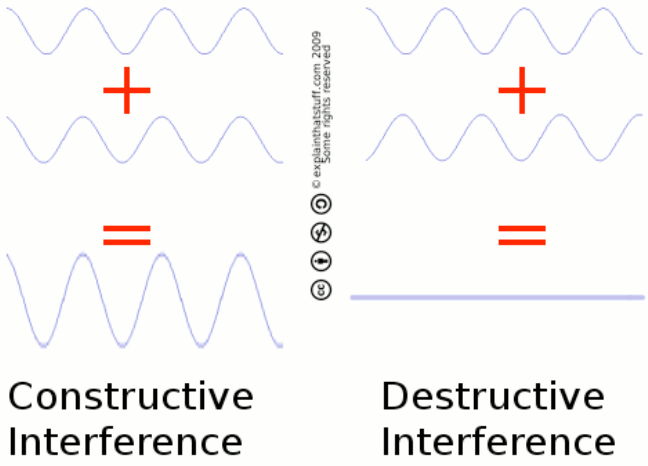
add a second point, scattering in some direction $\underline{s}_1$



The second wave, scattered by B travels further by the distance PB + BQ.
Its scattered wave lags in phase by

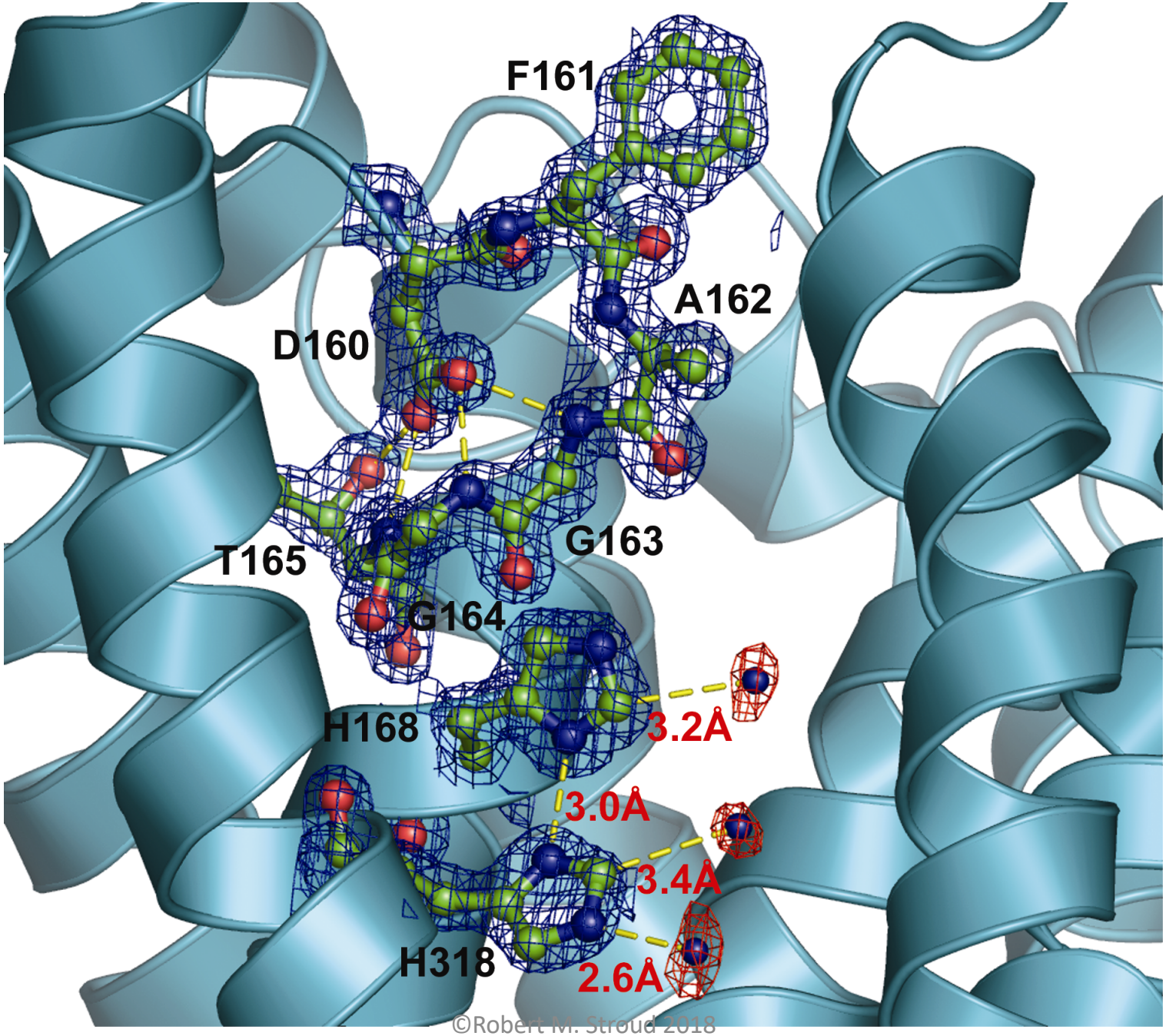
$= \frac{2\pi}{\lambda}([P-B] + [B-Q])$

$= \frac{2\pi}{\lambda}(\Phi)$ where Φ = path length extra for B versus the reference A.

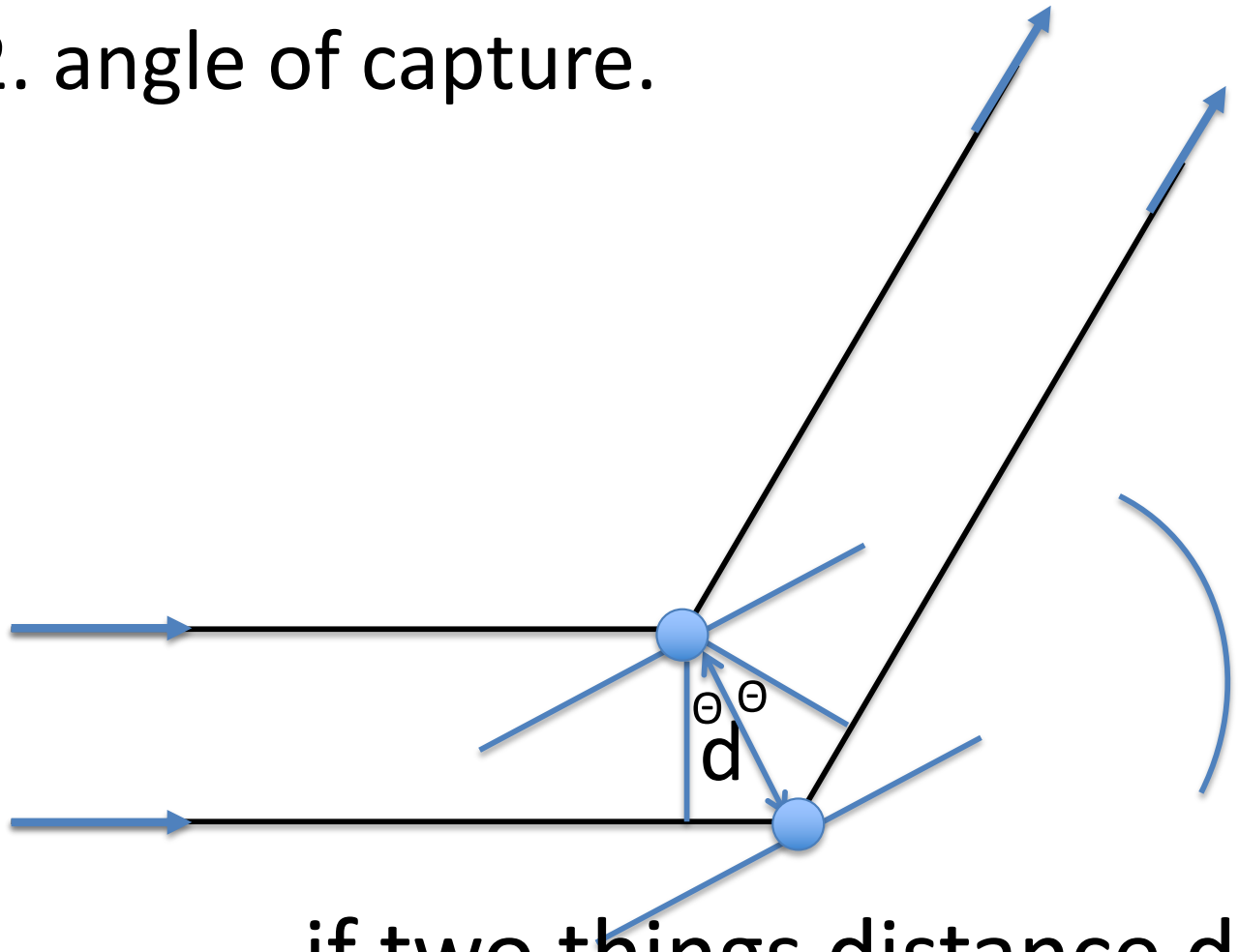


Waves add out of phase by 2π[extra path/λ]

RESOLUTION ? NH3 sites and role of D160 at 1.35Å Resolution

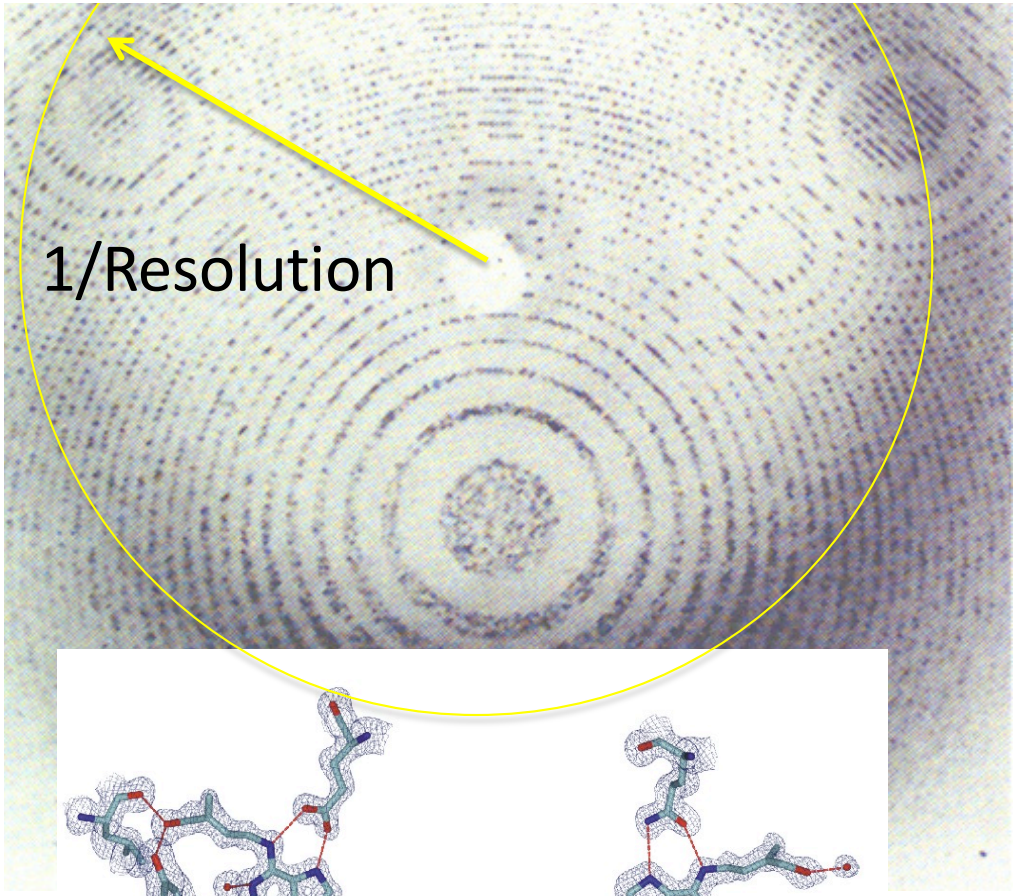


Resolution depends on 1. Wavelength of 'light', and 2. angle of capture.



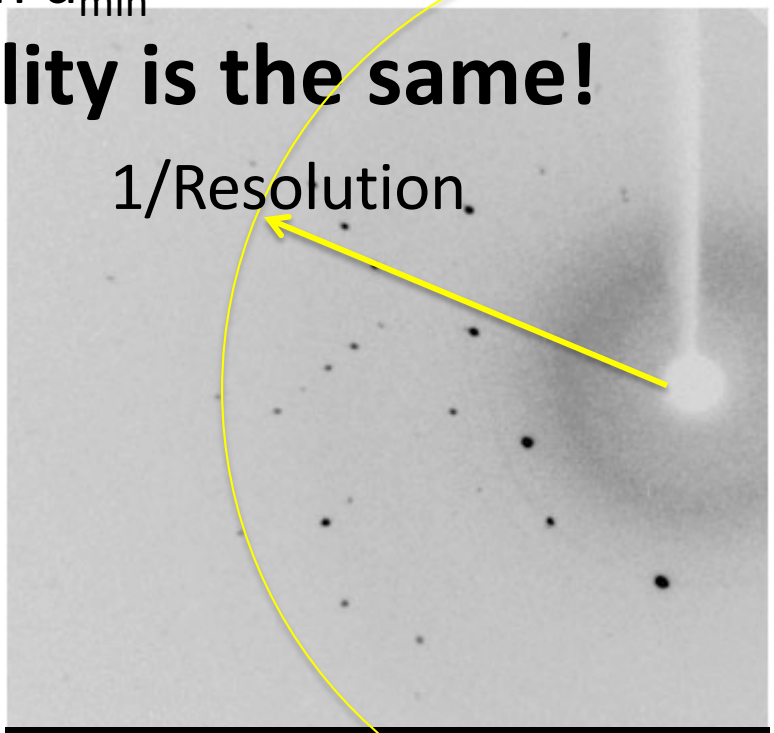
if two things distance d apart. scattered waves reinforce when

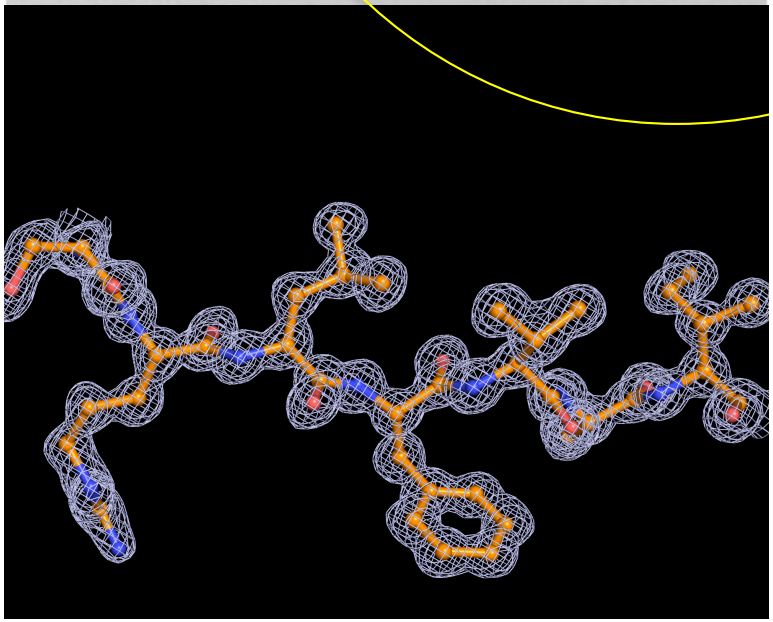
$2\ d \sin(\Theta) = \lambda$



Data/Parameter is the same for all molecular sizes at the same resolution d_{min}

ie. quality is the same!

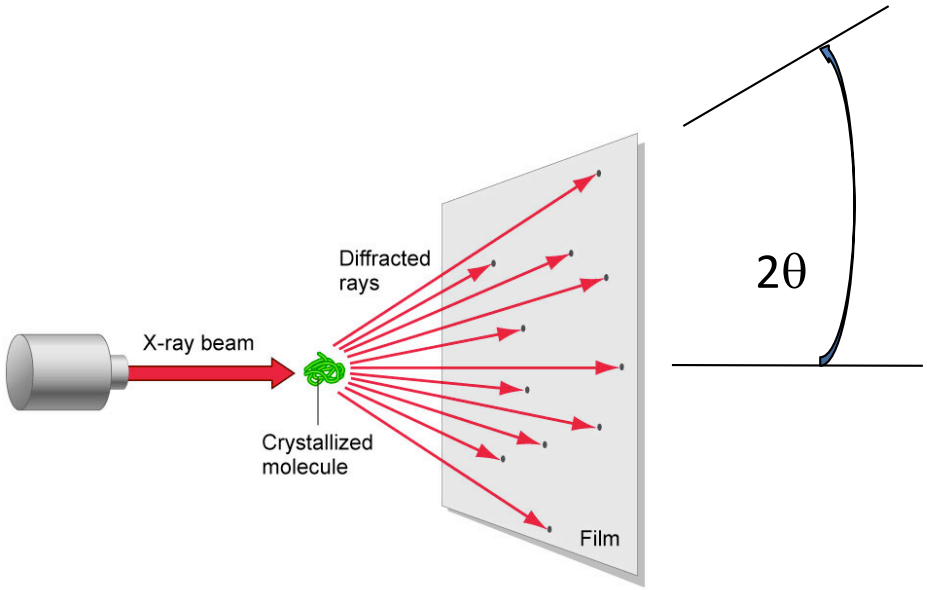


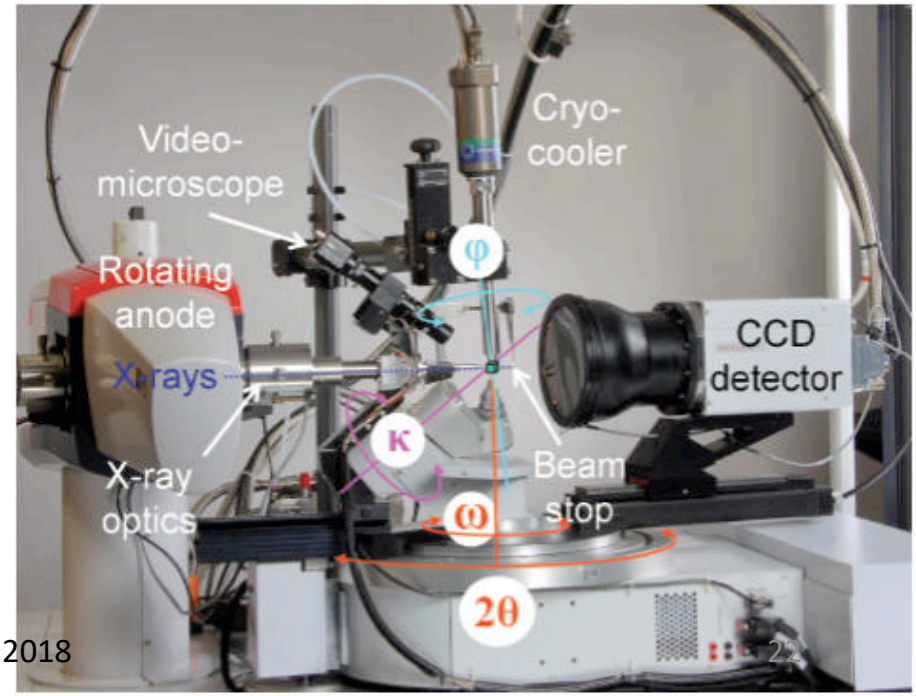


Determining Atomic Structure

- X-ray crystallography = optics λ ~ 1.5Å (no lenses)
- Bond lengths ~1.4Å
- Electrons scatter X-rays; X-rays 'see electrons'
- Resolution –Best is $\lambda/2$ Typical is 1 to 3 Å
- Accuracy of atom center positions ±1/10 Resolution

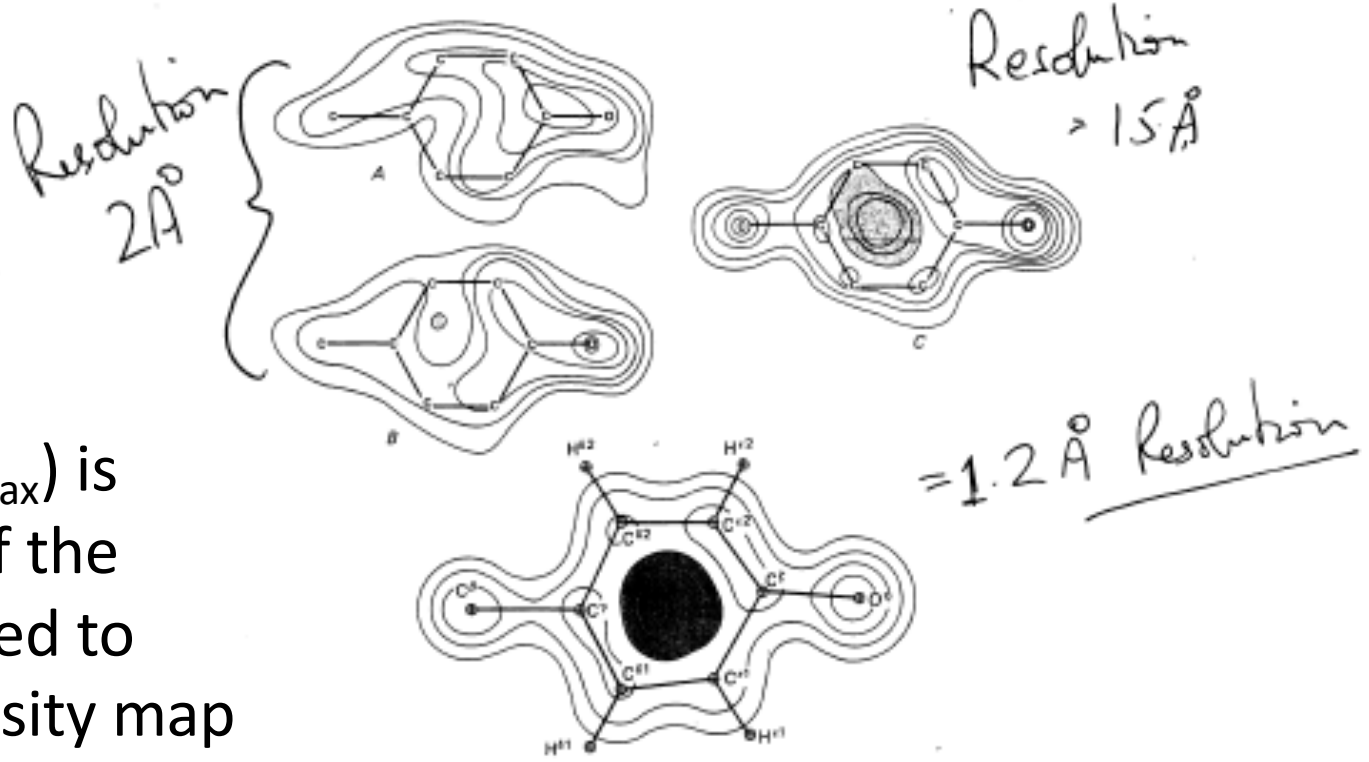
$$d_{\min} = \frac{\lambda}{2 \sin \theta_{\max}}.$$





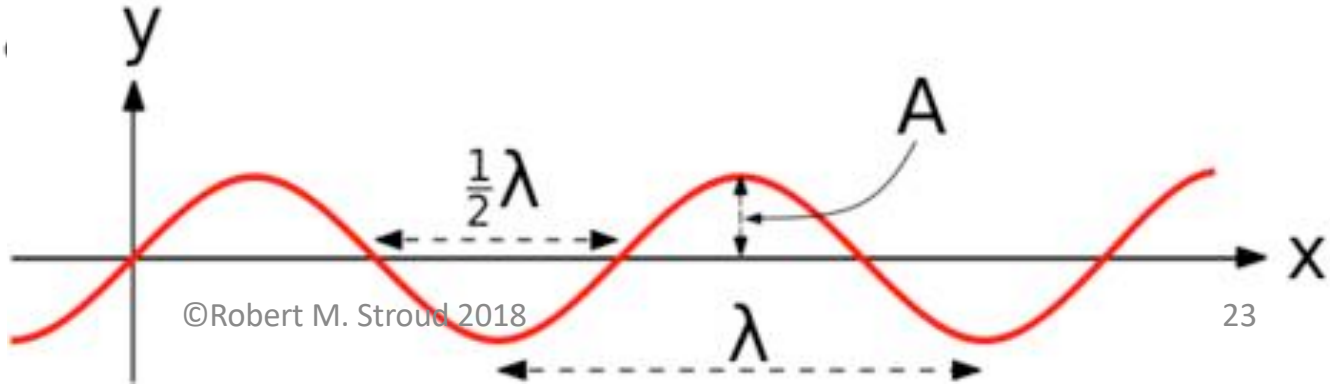
Resolution $d_{min} = \lambda / 2 \sin(\theta_{max})$

differs from Rayleigh criterion



$d_{min} = \lambda / 2 \sin(\theta_{max})$ is the wavelength of the shortest wave used to construct the density map

FIGURE



The Rayleigh Criterion

- The Rayleigh criterion is the generally accepted criterion for the minimum resolvable detail - the imaging process is said to be diffraction-limited when the first diffraction minimum of the image of one source point coincides with the maximum of another.

Single slit perpendicular to beam

$d_{min} = \lambda / \sin(2\theta_{max})$

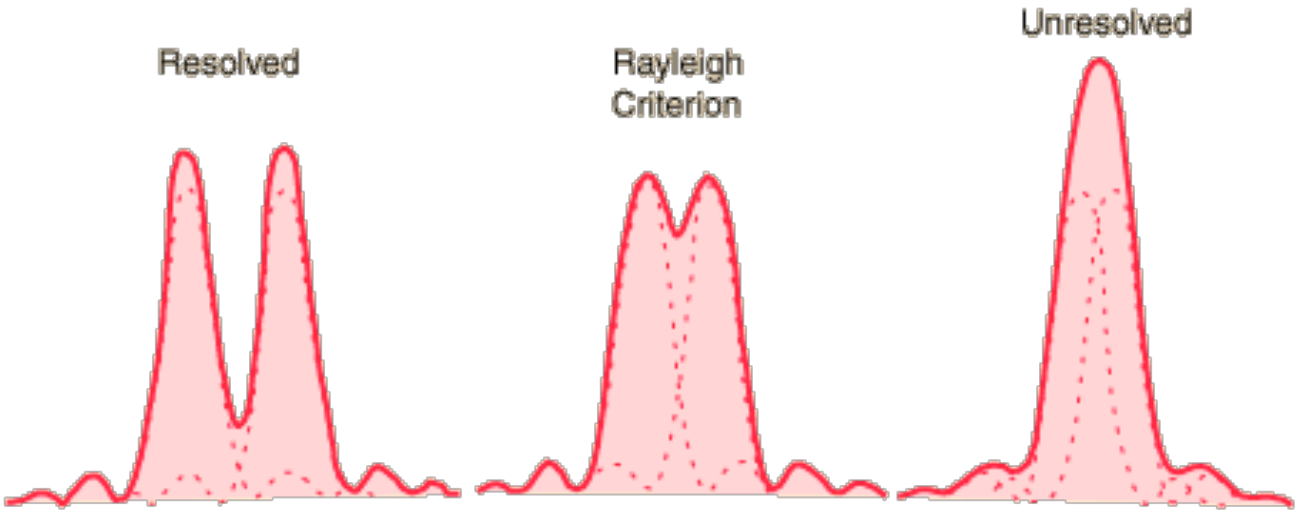
Circular hole

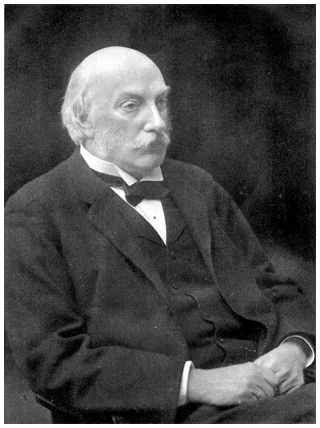
$d_{min} = 1.22\ \lambda / \sin(2\theta_{max})$

X-ray/EM/Neutrons

In 3 Dimensions:

$d_{min} = \lambda / 2 \sin(\theta_{max})$

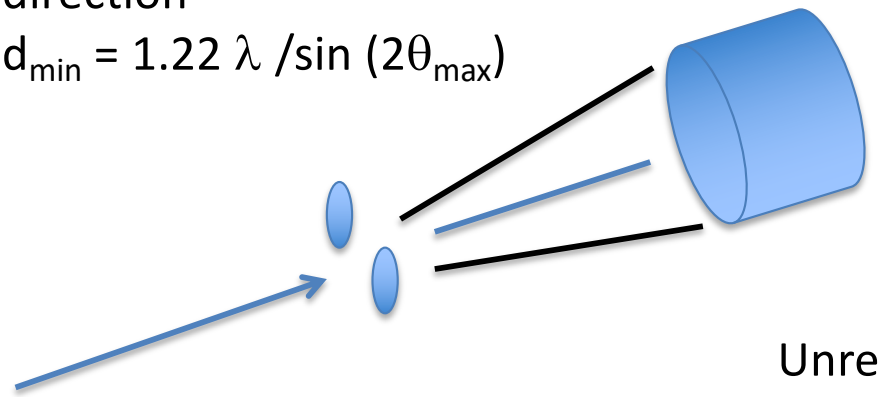




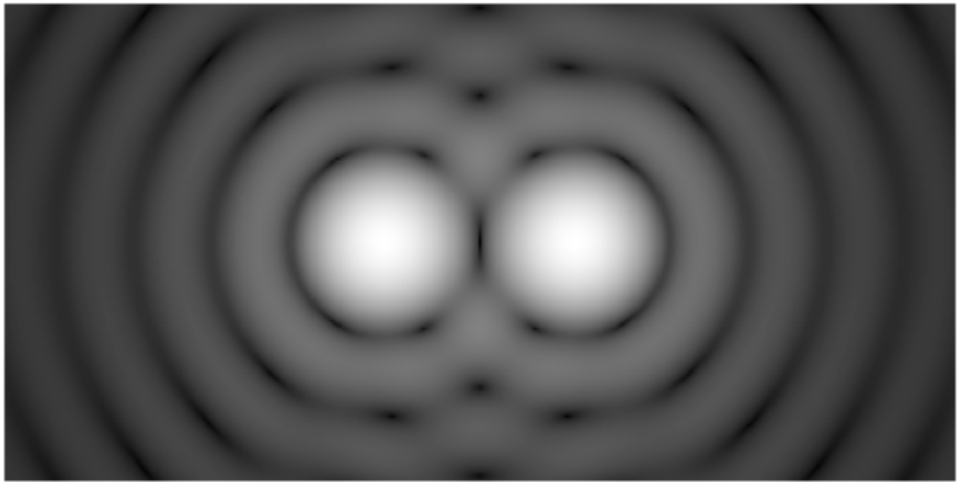
Lord Rayleigh
U.Cambridge
Nobel 1904

Diffraction from Circular hole, forward direction

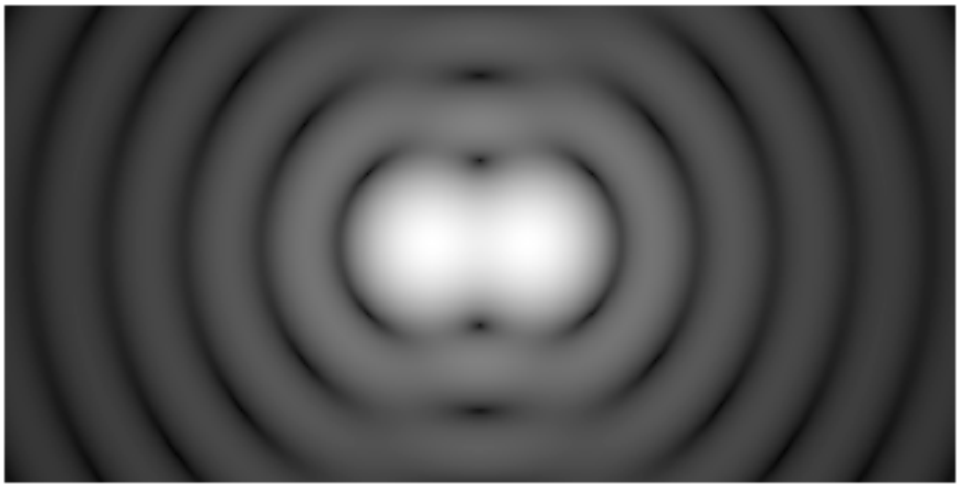
$d_{min} = 1.22\ \lambda\ /\sin(2\theta_{max})$



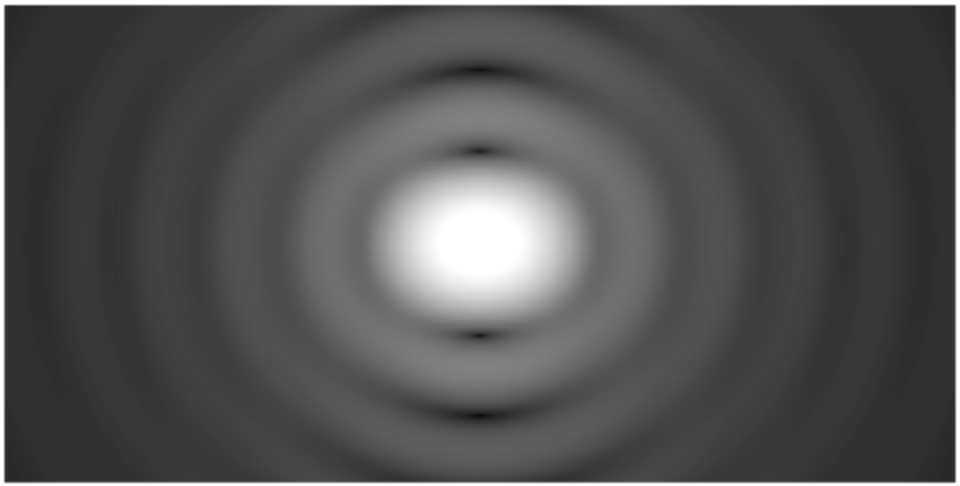
Crystal



Rayleigh



Unresolved

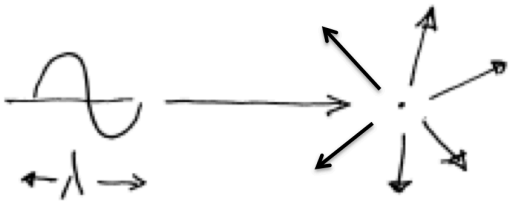


#2 Dot Product,

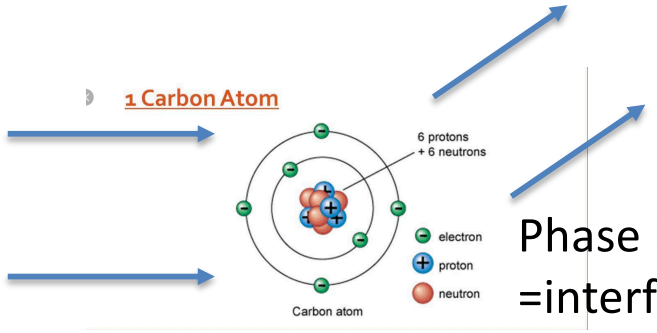
Whole Protein Scattering

Lattice of Proteins

Form factor: finite size means fall off of scattering f(**s**)

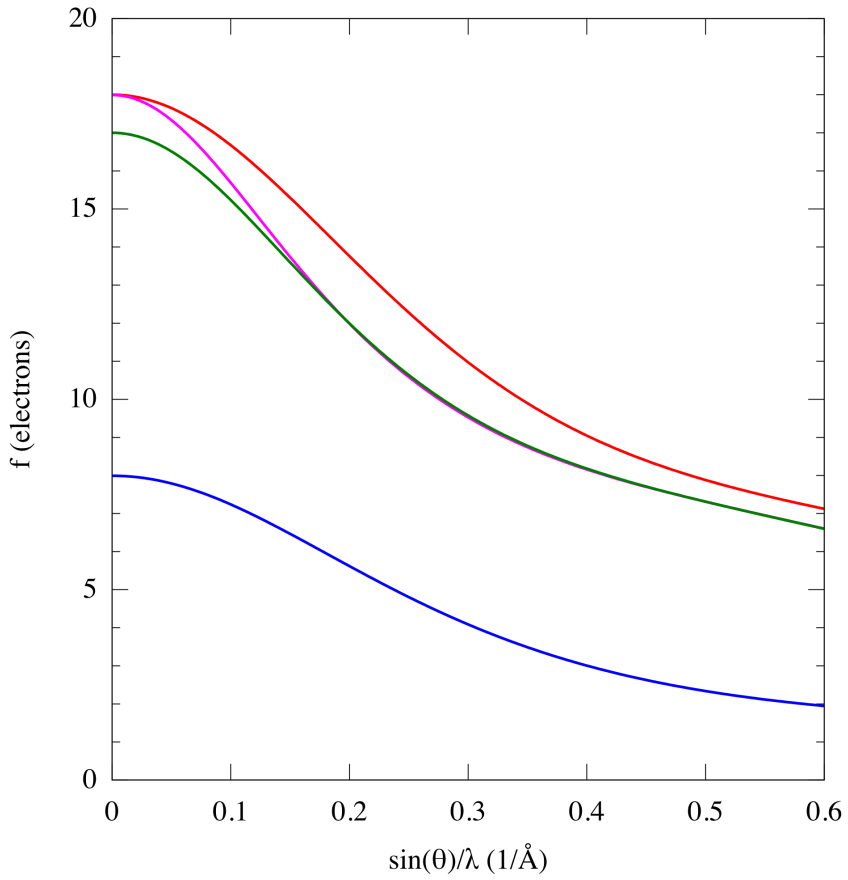


Scattering from a point is equal in all directions.



Phase lag =interference

Atoms have finite size of Electron density, hence electron Density away from the center Scatters waves out of phase with Density on the other side. These Cancel each other out at higher Angles (or $\sin(\theta)/\lambda$)



X-ray atomic form factors of oxygen (blue), chlorine (green), Cl^- (magenta), and K^+ (red); smaller charge distributions have a wider form factor.

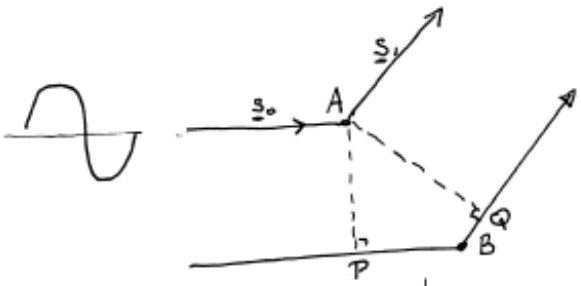
Scattering from multiple points? Add wave amplitudes with phase change

Adding up the scattering of Atoms:
Amplitudes, 'interference' of waves

Scattering by matter -(interference)
of a single wavelength X-ray

Scattering from a point is equal in all directions.

add a second point, scattering in some direction $\underline{s}_1$

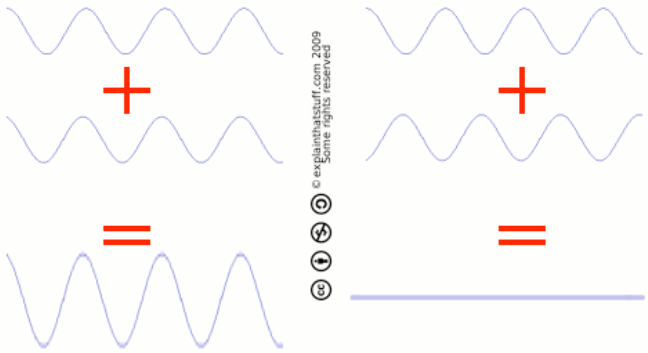


The second wave, scattered by B travels further by the distance PB + BQ.
Its scattered wave lags in phase by

$$= \frac{2\pi}{\lambda}\left([P-B] + [B-Q]\right)$$

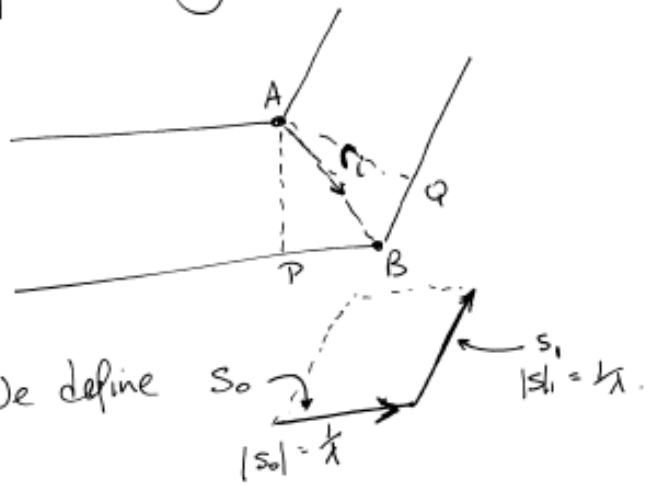
$$= \frac{2\pi}{\lambda}\left(\Phi\right)$$

where Φ = path length extra for B versus the reference A.



Waves add out of phase by 2π[extra path/λ]

The phase lag can also be simplified

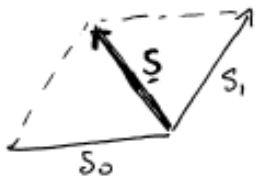


the PB = projection of r_i on S_0
BQ = projection of r_i on S_1

$$\text{so } \frac{\Phi}{\lambda} = \frac{PB + BQ}{\lambda} = -\underline{r}_i \cdot \underline{S}_0 + \underline{r}_i \cdot \underline{S}_1$$

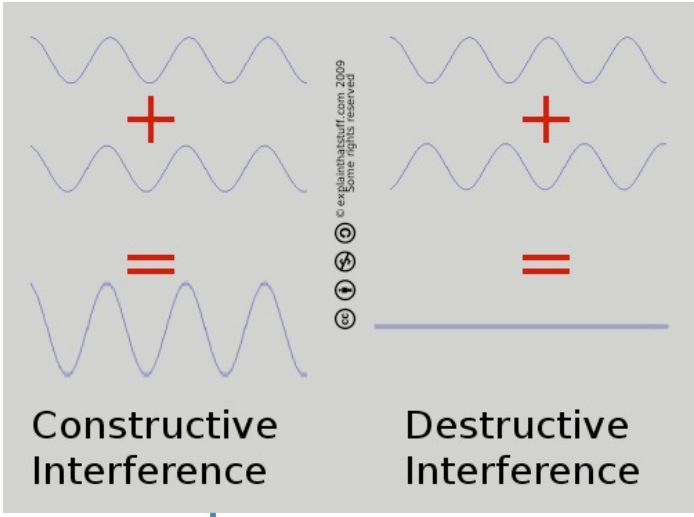
$$= \underline{r}_i \cdot (\underline{S}_1 - \underline{S}_0)$$

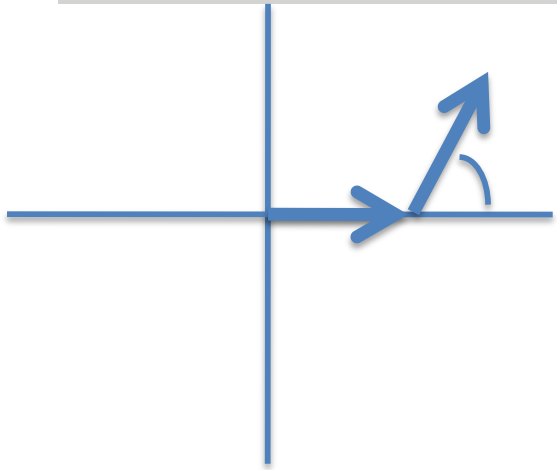
So we define $\underline{S} = S_1 - S_0$



$$\text{Then } \frac{\Phi}{\lambda} = \underline{r} \cdot \underline{S}$$

Adding up the scattering of Atoms: 'interference' of waves





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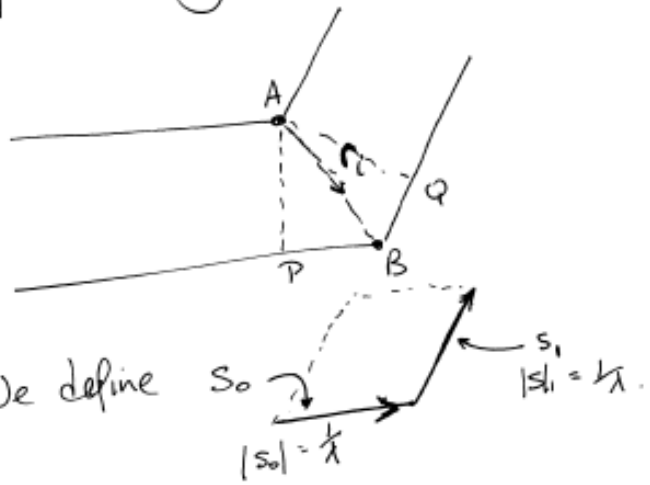

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$$\text{so } \frac{\Phi}{\lambda} = \frac{PB + BQ}{\lambda} = -\underline{r}_i \cdot \underline{S}_0 + \underline{r}_i \cdot \underline{S}_1$$

$$= \underline{r}_i \cdot (\underline{S}_1 - \underline{S}_0)$$

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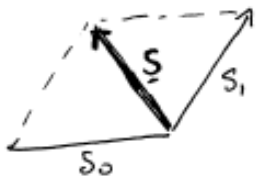
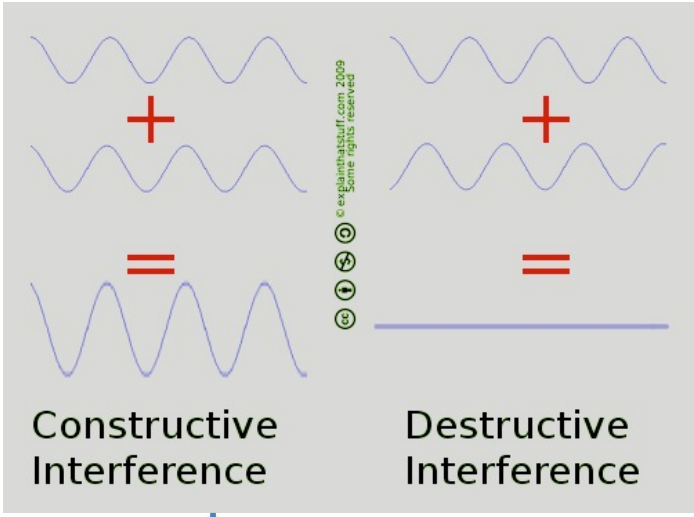
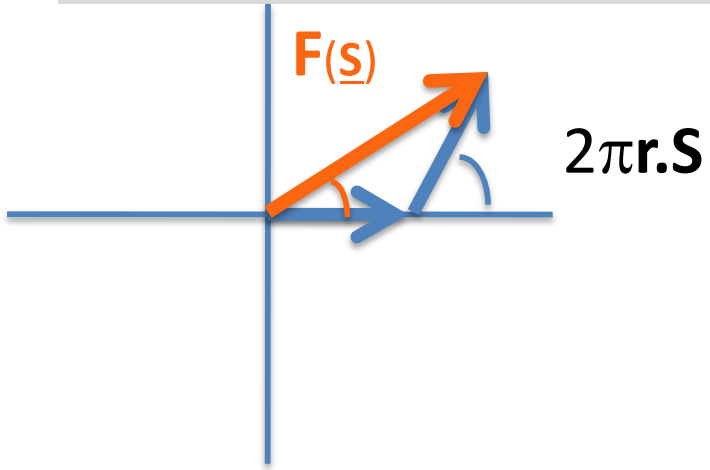


$$\text{Then } \frac{\Phi}{\lambda} = \underline{r} \cdot \underline{S}$$

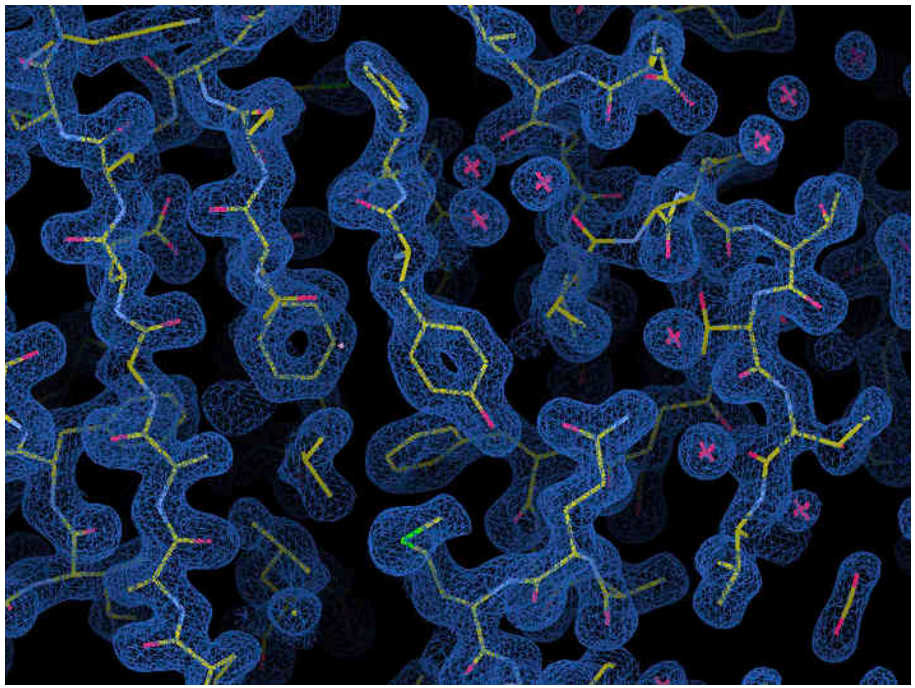
Adding up the scattering of Atoms: 'interference' of waves

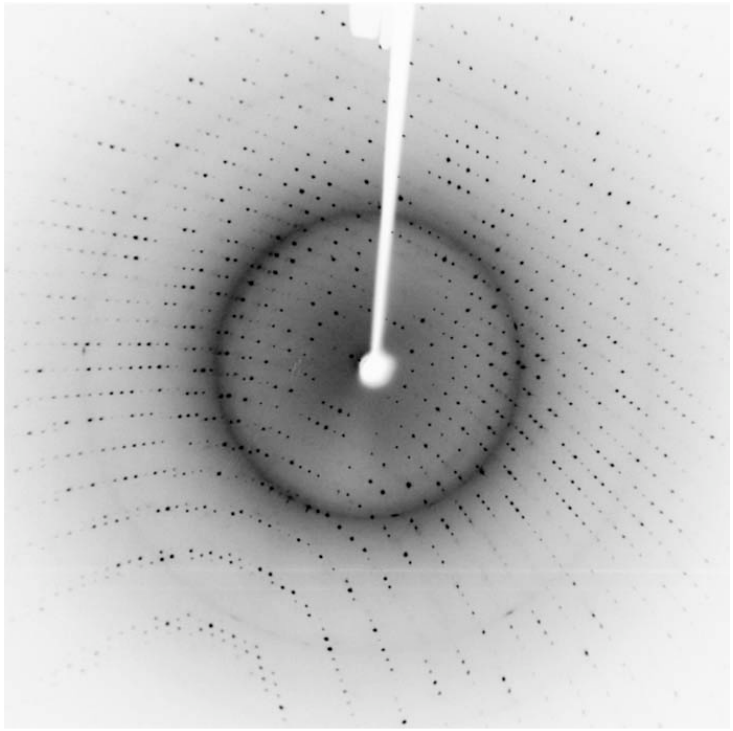




What do we want?

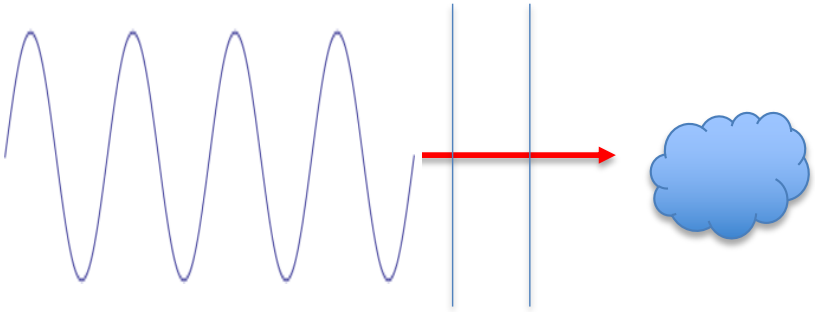
"Real" space (x,y,z)





$$\rho(x,y,z) = const \cdot \int_{hkl} F(h,k,l) e^{-2\pi i(hx+ky+lz)+i\varphi(h,k,l)} dhdkdl$$

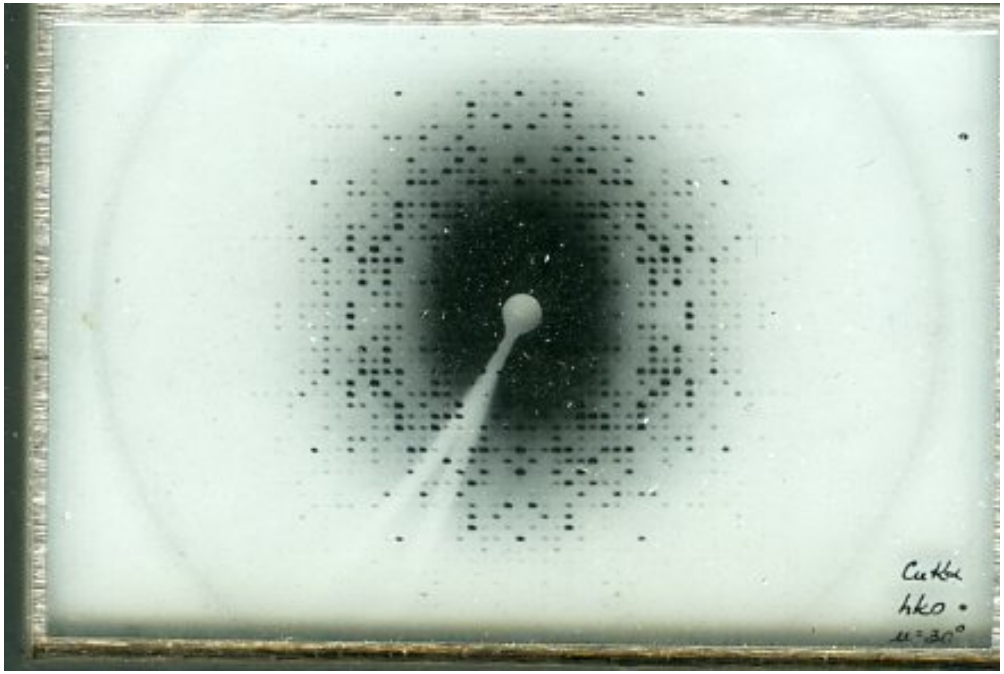
$I = F^2$



Scattering from a molecule is described by

$$F(s) = \sum_i f_i \, e^{(2\pi i \mathbf{r}.\mathbf{s})}$$

The Scattering from one molecule is sampled at the diffraction positions. How so??



Validation? R factors

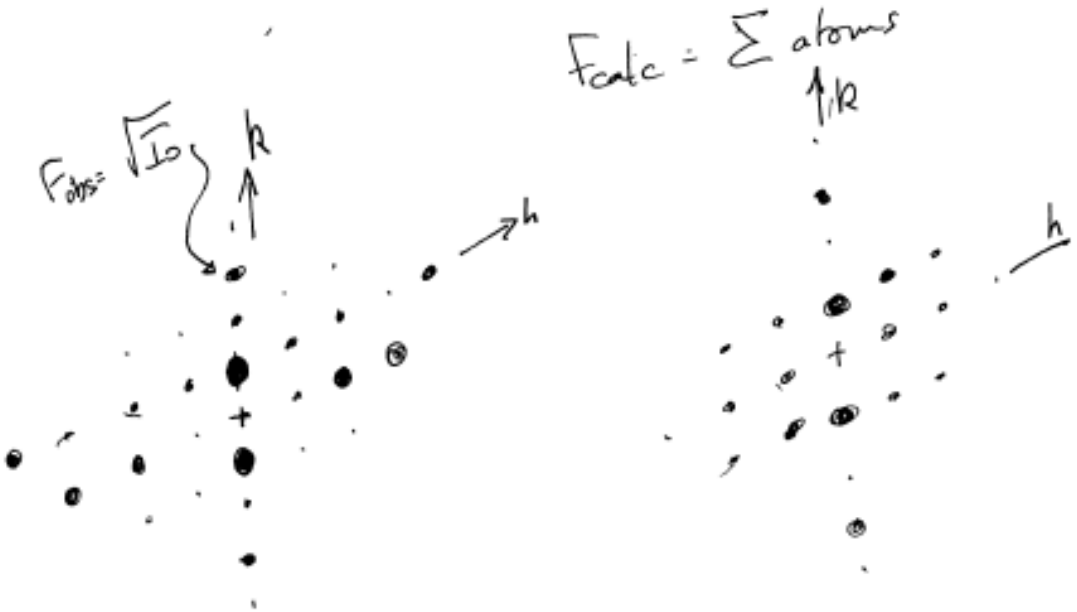
- Use Current structure to calculate Amplitudes
- $F_{(h,k,l)}calc$ and Phase $_{(h,k,l)}calc$
- Compare differences between Observed and Calculated Amplitudes

Bottom Lines: $I_{nke} = |F_{nke}|^2$

Intensity = Amplitude²

- "R factor" = Agreement between Amplitudes calculated, $F_{calc}^{hkl} = \sum_j f_j e^{2\pi i(hx+ky+lz)}$ and Amplitudes observed $\sqrt{I_{obs}}_{hkl} = Fobs_{hkl}$

$$R = \frac{\sum_{hkl} ||Fo| - |Fc||}{\sum_{hkl} |Fo|}$$



How do we judge the Quality of structure?

2. Overall quality criteria: agreement of observations with diffraction calculated from the interpreted structure.

3. Since we refine the structure To match the I_{hkl} overfitting ?

Define R_{free} for a 'hold-out ' set of observations.

4. OK? R < 20%, R free< 25%

5. But the experimental errors in measuring Fo are ~ 3%. inadequate models of solvent, atom motion, anharmonicisity

6 Accuracy ~ 0.5*res*R

“R” factors

R_{cryst} (or just “R”)

observed vs calculated data (Fs)

R_{free}

cross-check with “random” subset of data

should be < 0.3 and < R_{cryst} + 0.1

R_{sym} = R_{merge} (self-consistency of data: Is)

“R” factors

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

completely random: **0.59**

starting MR solution: **0.4-0.55**

something still wrong?: **> 0.3**

correct chain trace: **< 0.2**

small molecule: **~ 0.05**

“R” factors

R_{cryst} (or just “R”)

observed vs calculated data (Fs)

R_{free}

cross-check with “random” subset of data

should be < 0.3 and < R_{cryst} + 0.1

R_{merge}

$$R_{merge} = \frac{\sum \left| I_{obs} - \langle I \rangle \right|}{\sum I_{obs}}$$

← blows up as $I_{obs} \rightarrow 0$

completely random: **0.59**

weak data (high angle): **0.7- ∞**

wrong symmetry choice?: **~0.2-0.55**

small or disordered crystal: **~0.1-0.2**

typical: **~ 0.05**

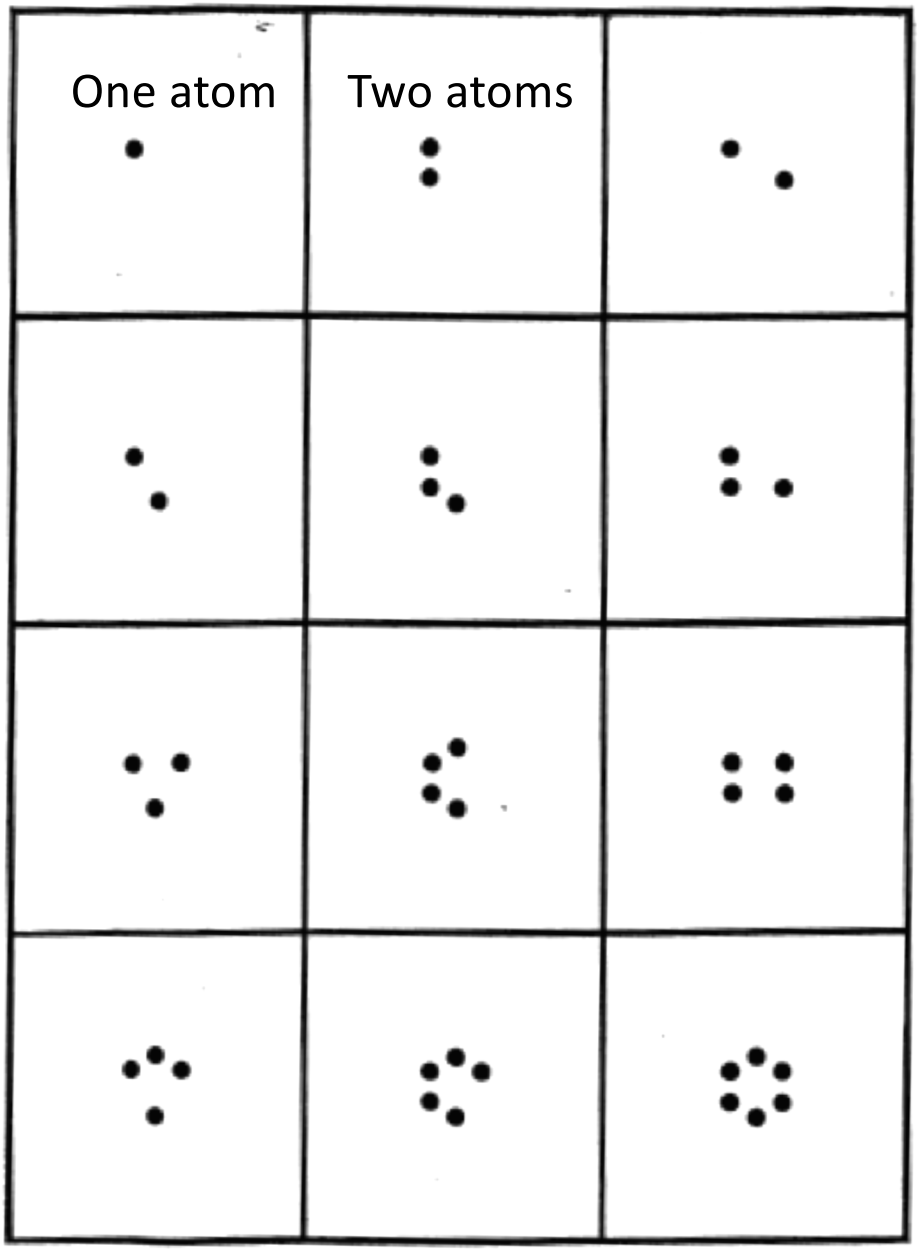
Repetition in crystal==sampling in diffraction

Plate 2 Superimposed fringes

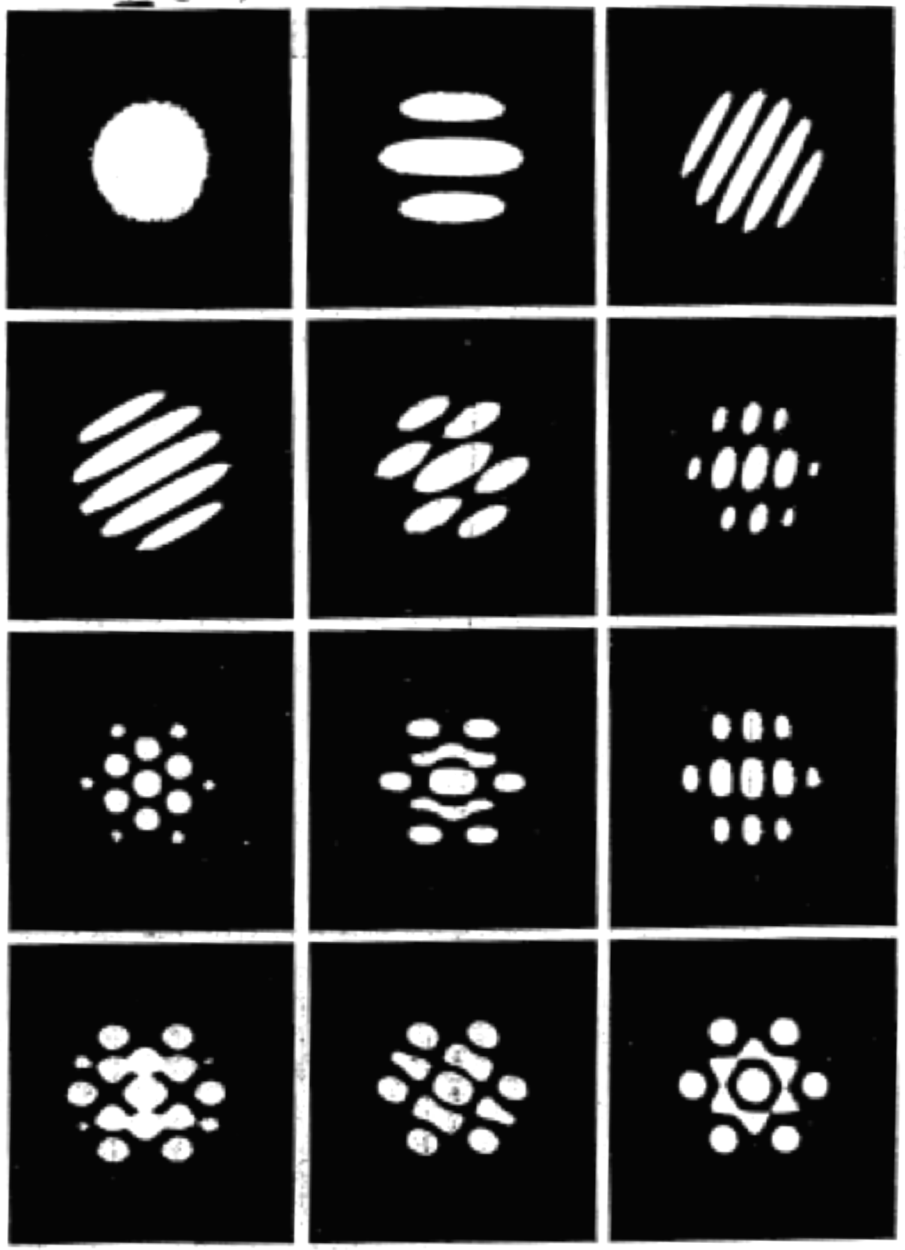
The sequence shows how the diffraction pattern of a simple object is built up by superposition of sets of fringes.

Plate 2

$\rho(r)$



$F(s)$

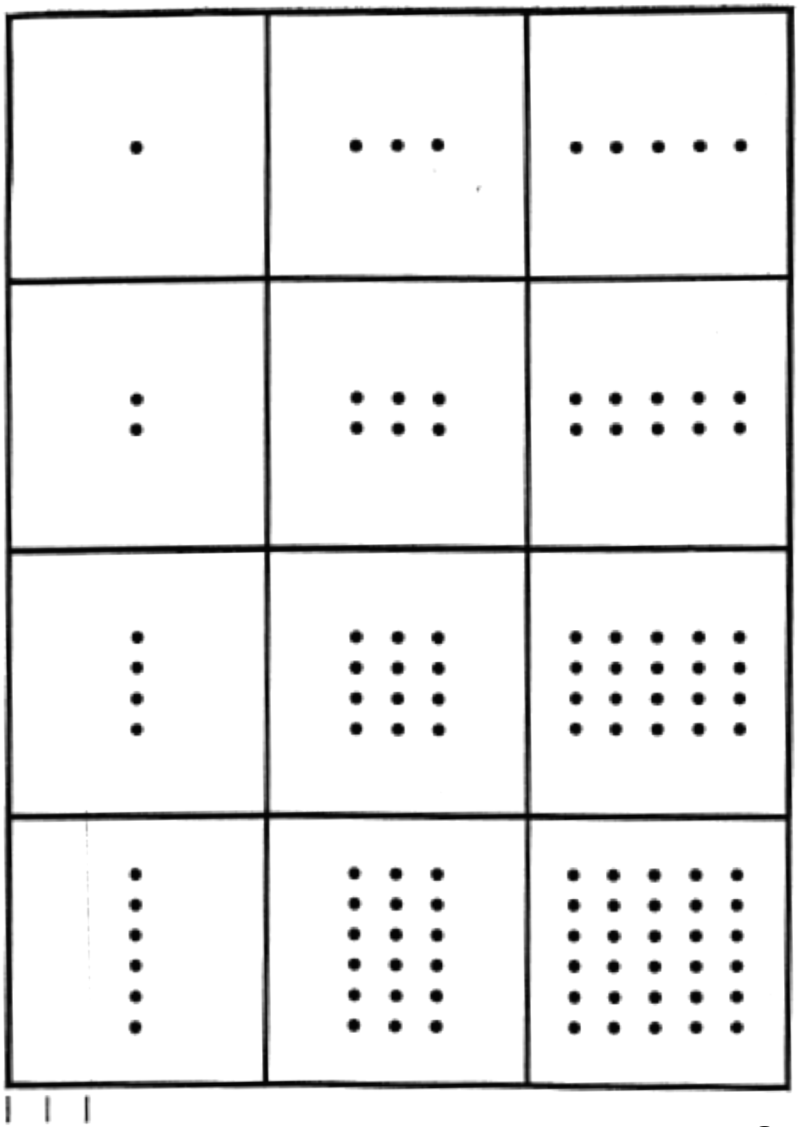


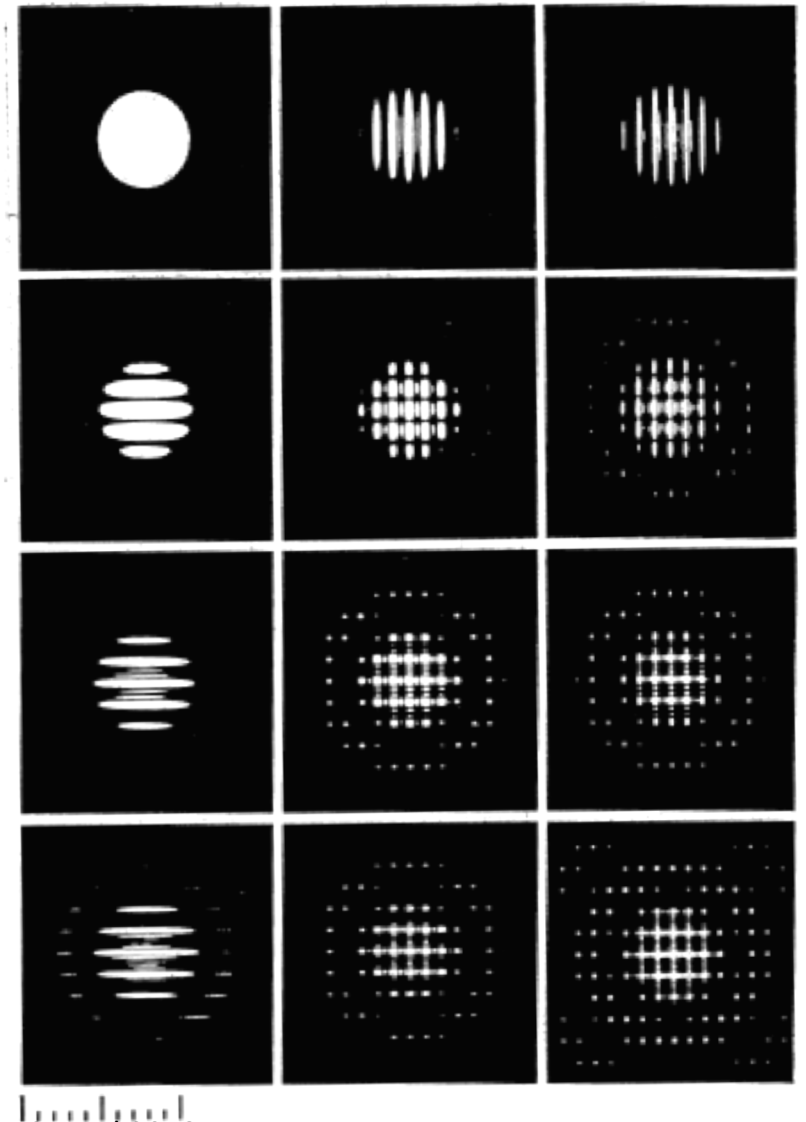
Diffraction due to repeats, is the same as the object, sampled by (1/repeat)

Plate 10 Development of a lattice

A single aperture is repeated to build up rows and columns which are combined to produce the lattice of 12. Subsidiary diffraction maxima due to the small number of apertures can be seen clearly.

Plate 10

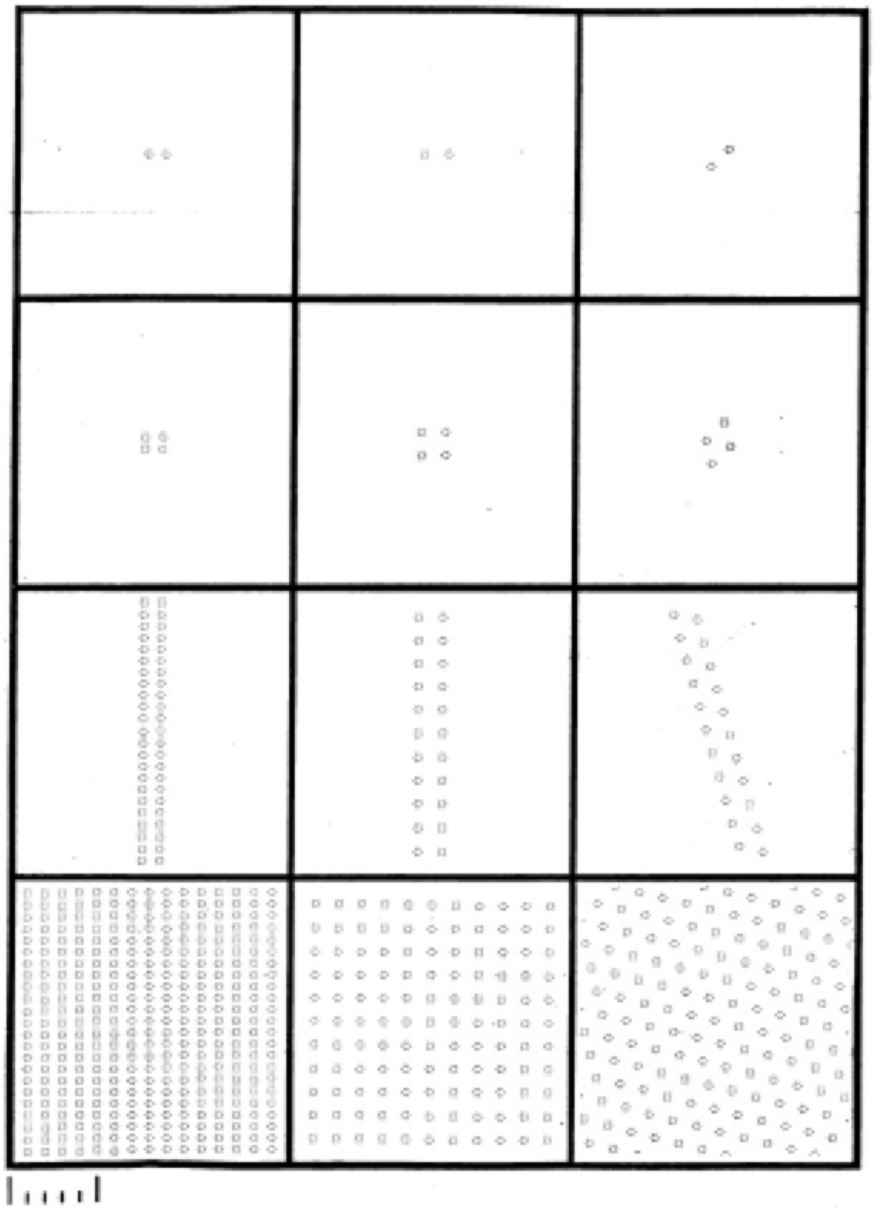




Build a crystal

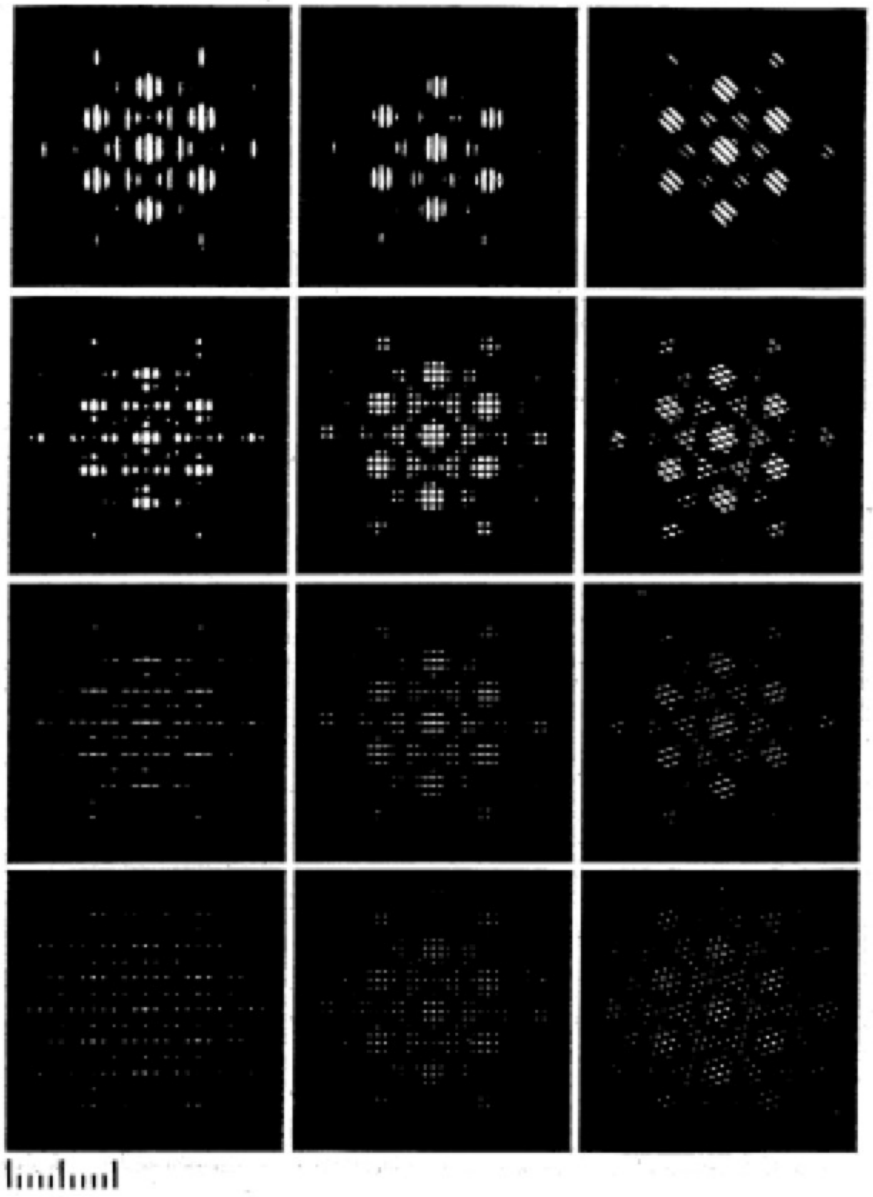
Plate 11

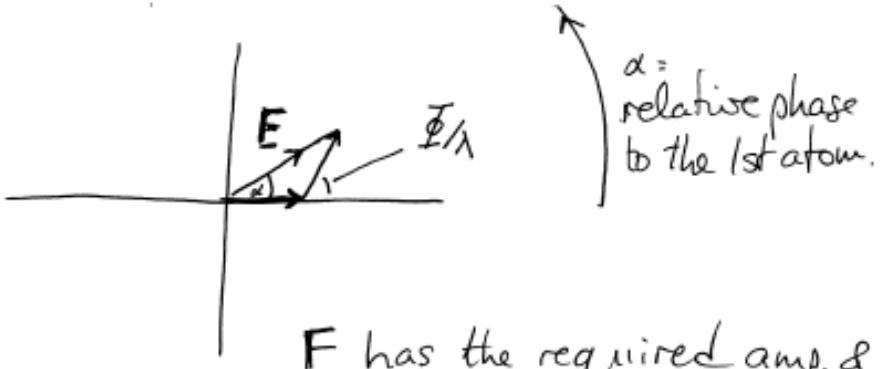
Object



Scattering

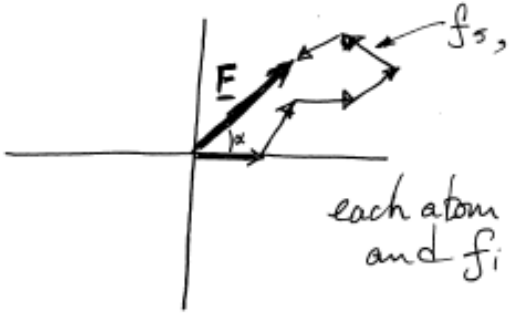
Plate 11





F has the required amp. & phase.

If we use this method we can add $i = 1$ to n different atoms; each amplitude f_i



each atom has $f_i \cos\alpha_i$ along x and $f_i \sin\alpha_i$ along y

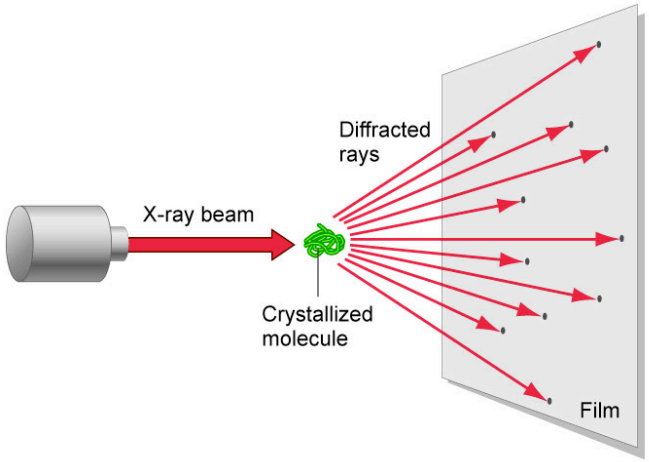
If we put 'units' on the axes, we can add up the 'x' and 'y' components to write the sum over "x", the sum over "y", — hence calculate $\underline{F}$ as a wave of amplitude

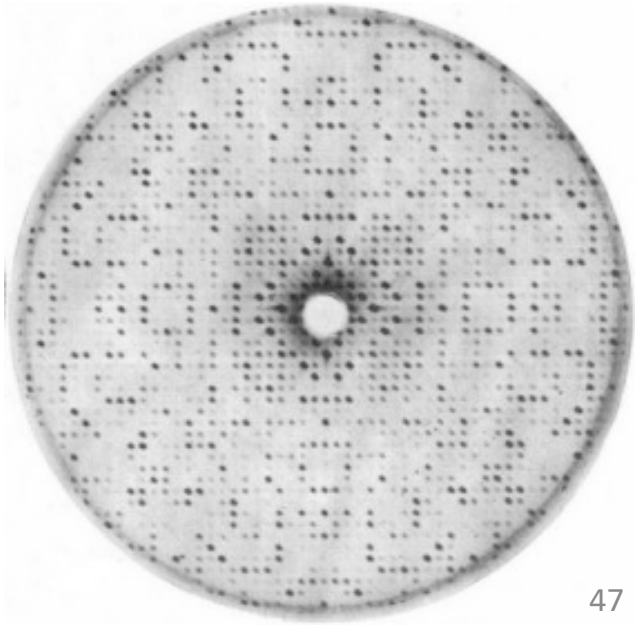
$$|\underline{F}| = \sqrt{\left(\sum_x f_i \cos\alpha_i\right)^2 + \left(\sum_y f_i \sin\alpha_i\right)^2}$$

and $$\alpha = \tan^{-1}\left(\frac{\sum_y f_i \sin\alpha_i}{\sum_x f_i \cos\alpha_i}\right)$$

Many atoms add by the same rules.

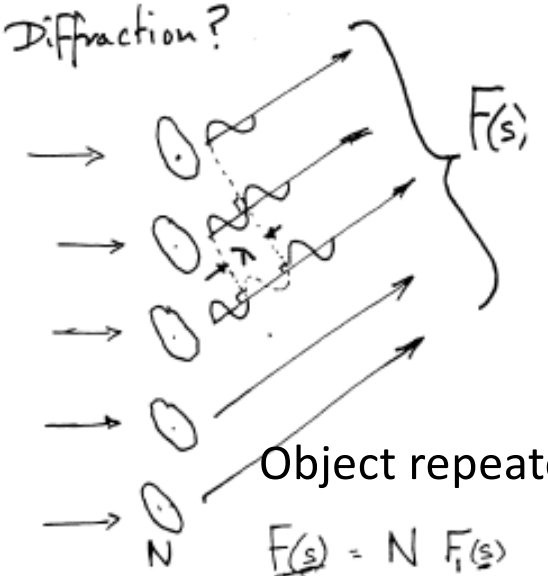
Different in every direction.





(8)

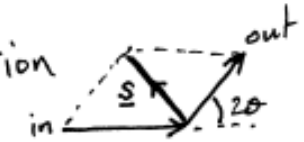
Why Diffraction?



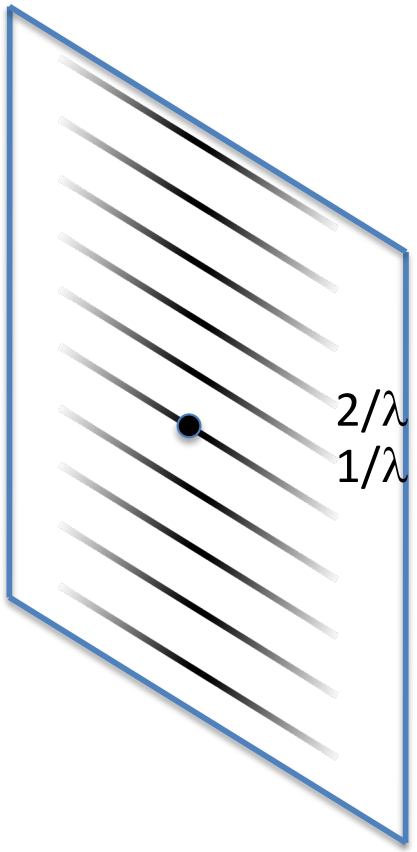
Object repeated

$F_{Tot}(\underline{s}) = N\, F_1(\underline{s})$

$\underline{s}$ refers to a scattering direction

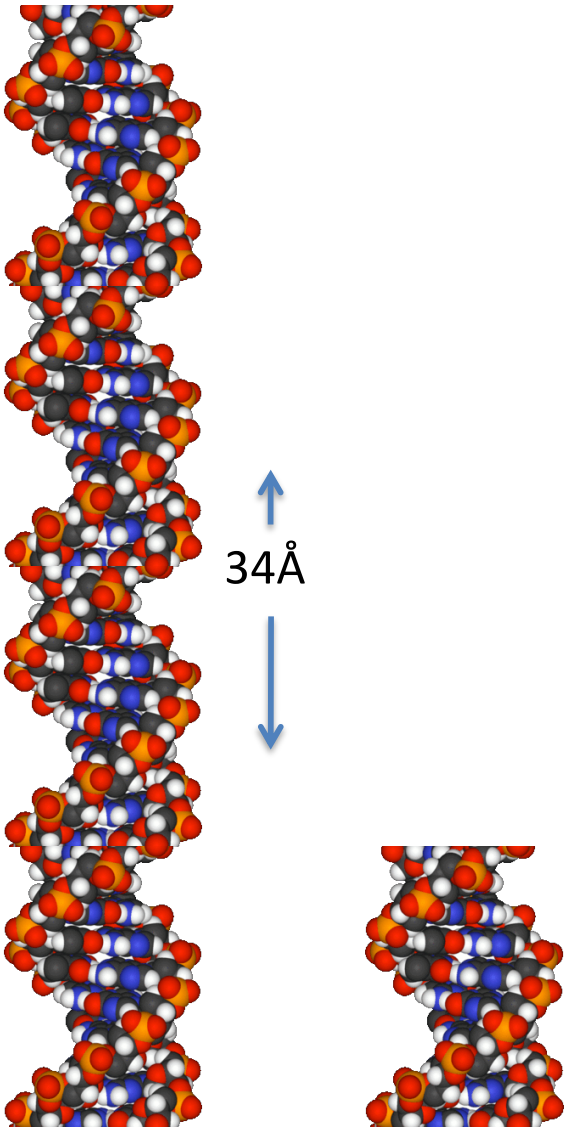


so $|\underline{s}| = \frac{2\sin\theta}{\lambda}$



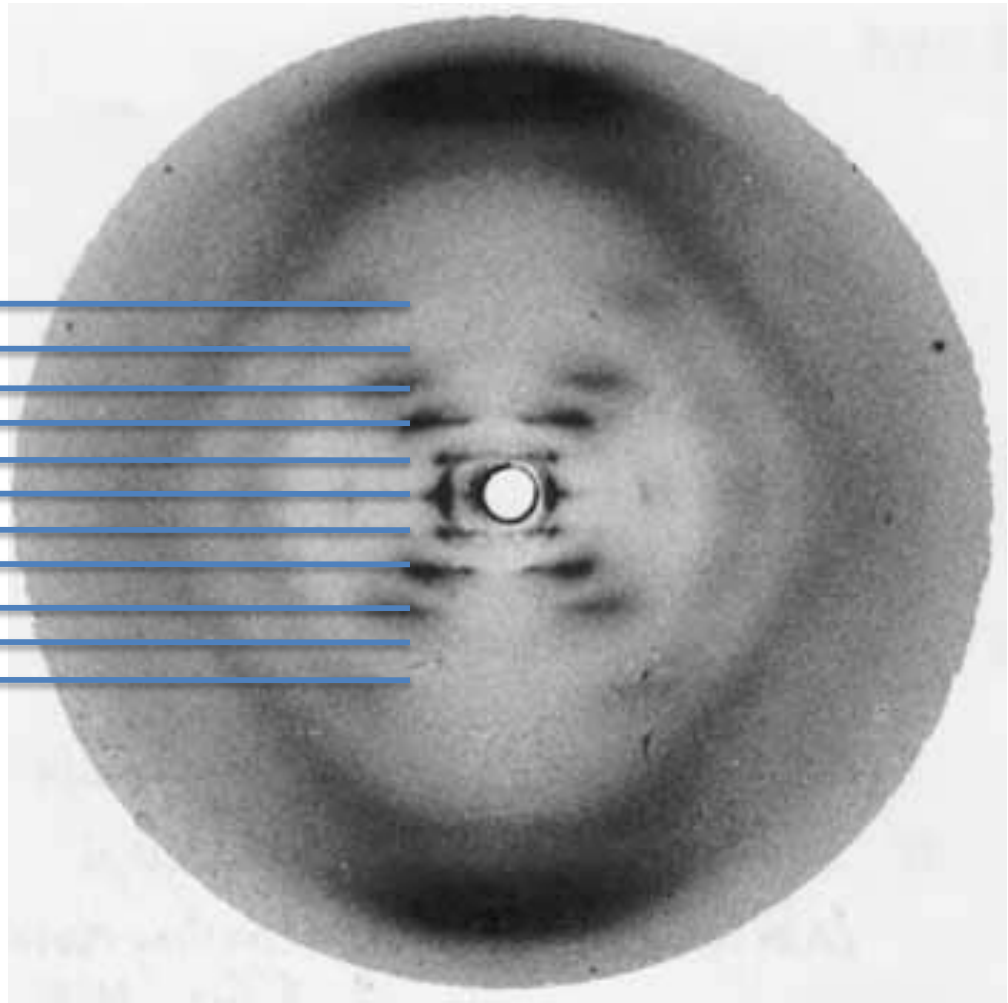
Consequences of being a crystal?

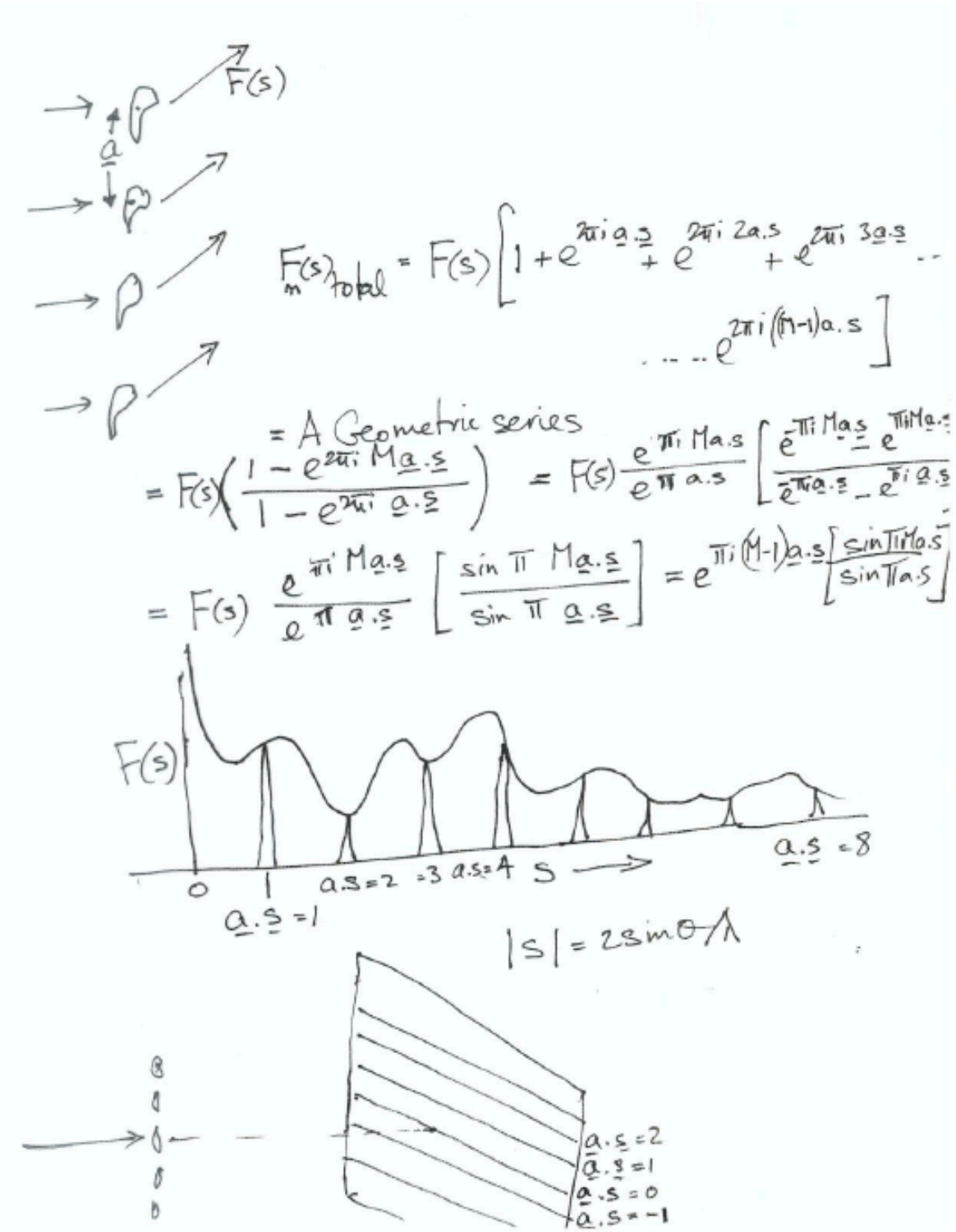
- Repetition = sampling of $F_{(S)}$



34Å

1/34Å^{-1}

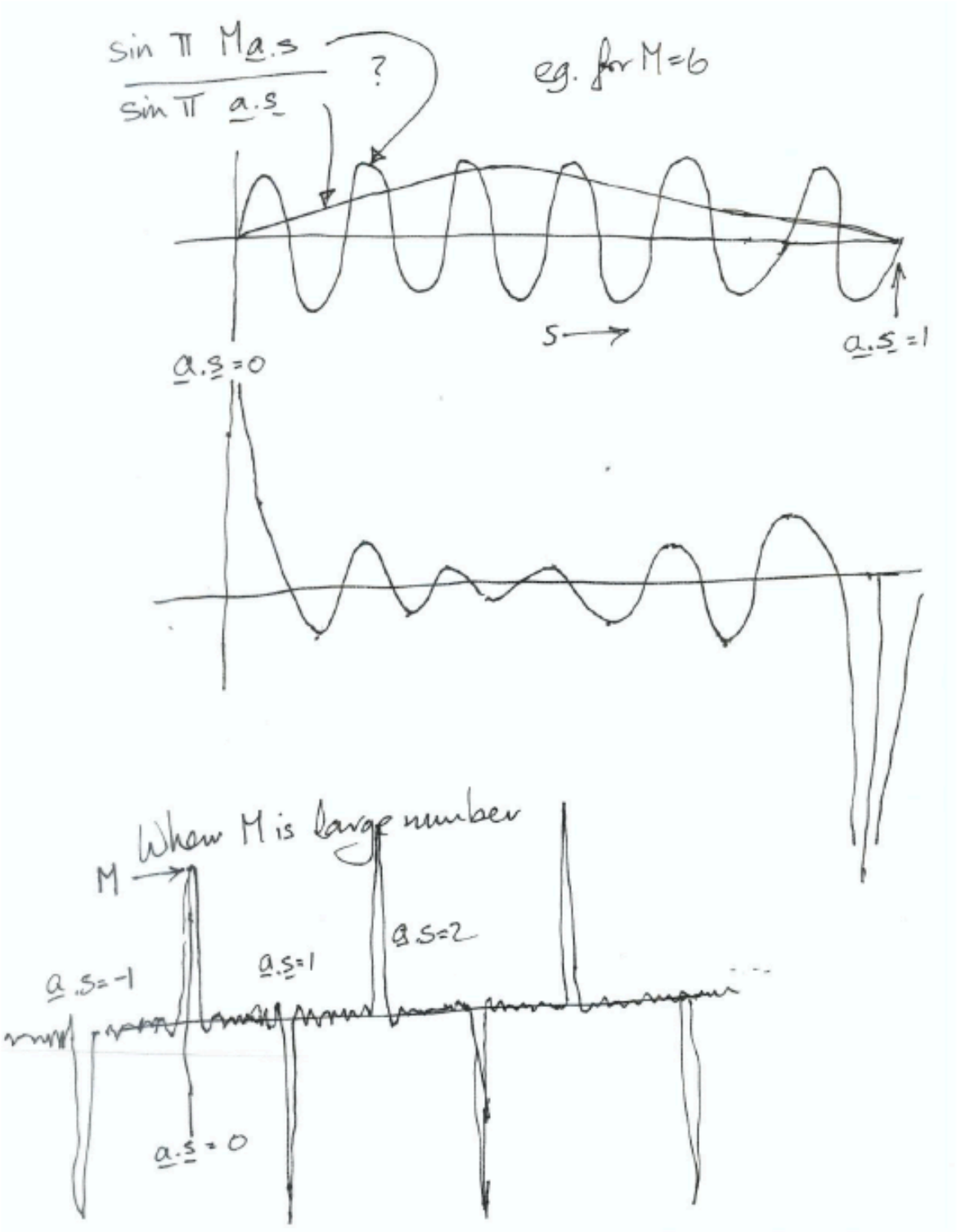




Build up a crystal from Molecules…

First 1 dimension,
a direction

When Phase shift is 2π they will add Amplitude F(**s**)


sin π M a.s
sin π a.s
?
eg. for M=6
s
a.s =0
a.s =1
When M is large number
M
a.s=2
a.s=1
a.s=-1
a.s = 0

6 The reciprocal lattice

For a crystal, the function $G(\underline{S})$ can only be observed at all, ie for any rotation of the scattering object; - $G(S)$ only exists for

$$\underline{a}.\underline{S} = h$$
$$\underline{b}.\underline{S} = k$$
$$\underline{c}.\underline{S} = \ell$$

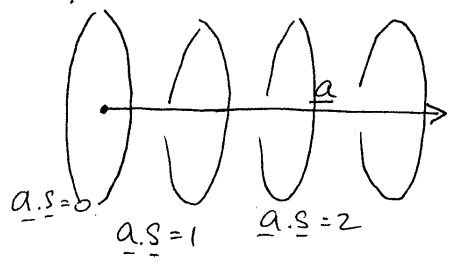
as a consequence of summing all unit cells. $\left(\frac{\sin \pi Ma.s}{\sin \pi a.s}\right)$ etc

These 3 intersecting sets of planes describe a lattice of points: The first two planesets

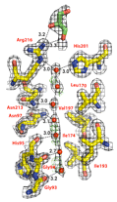
$$a.S = h$$
$$b.S = k$$

describe a set of lines and the third set of planes cut these lines. at positions where $c.S = \ell$.

The planes $\underline{a}.\underline{S}$ are perpendicular to $\underline{a}$



This is all there is? YES!!



FT

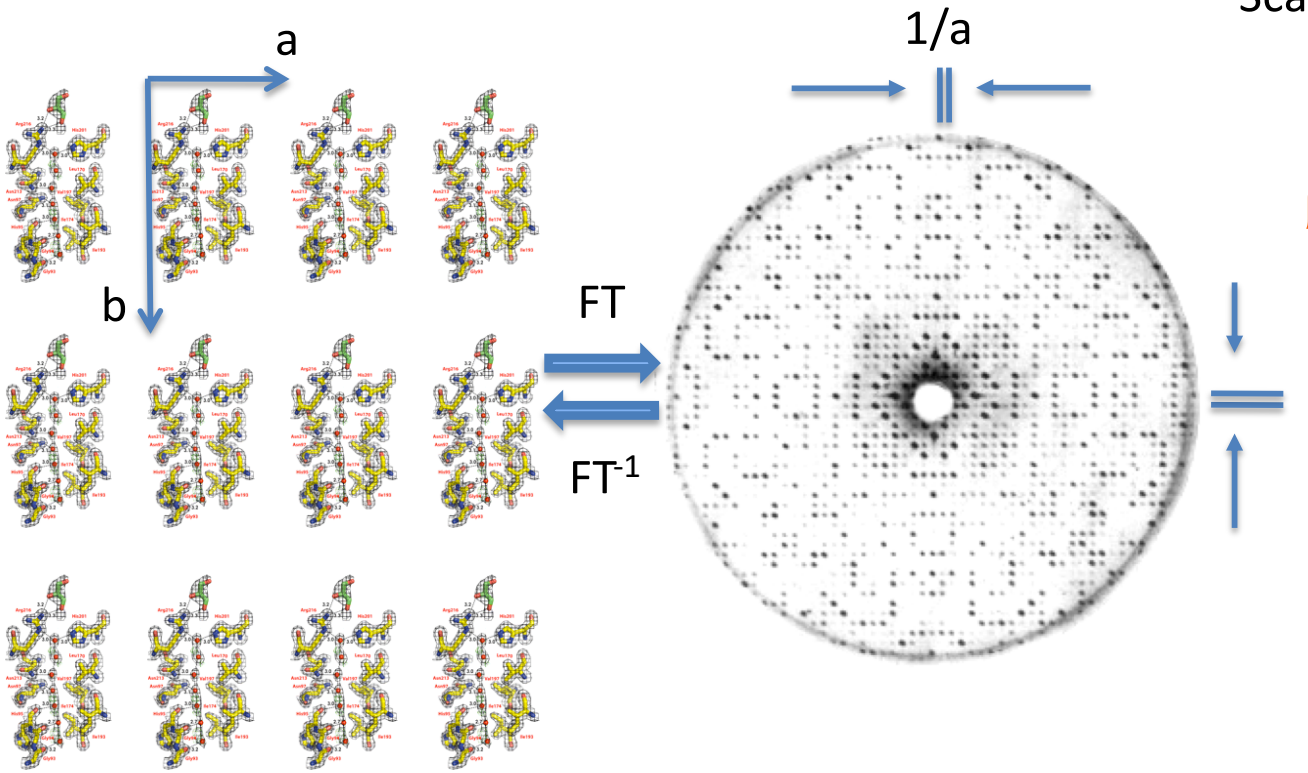
FT^{-1}

Scattering pattern is the Fourier transform (FT) of the structure: Amplitude and phase of waves is a sum of waves from each atom

$$F(\underline{S}) = \sum_j f_j \, e^{(2\pi i \mathbf{r}_j.\mathbf{S})}$$

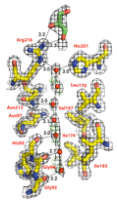
Observe $I(\underline{S}) = F(\underline{S}).F^*(\underline{S})$

Structure is the 'inverse' Fourier transform of the Scattering pattern $F(\underline{S})$



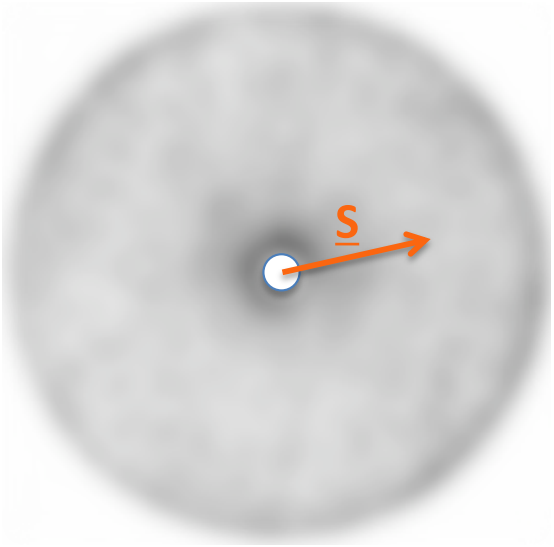
$$\rho(\underline{r}) = \sum F(\underline{S}) \, e^{(-2\pi i \mathbf{r}.\mathbf{S})}$$

This is all there is?



FT

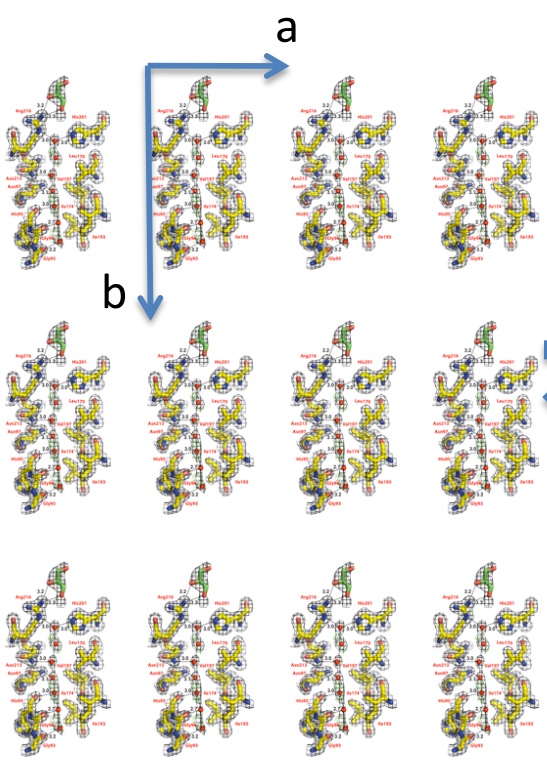
FT^{-1}



Scattering pattern is the Fourier transform of the structure

$$\underline{F}(\underline{S}) = \Sigma_j f_j e^{(2\pi i \mathbf{rj.S})}$$

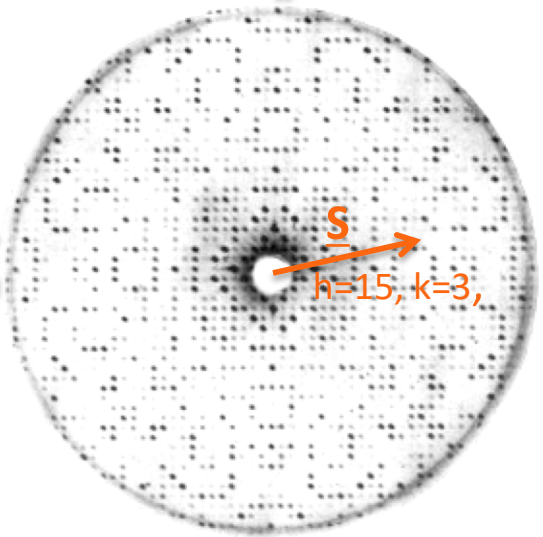
Structure is the 'inverse' Fourier transform of the Scattering pattern



FT

FT^{-1}

1/a



1/b

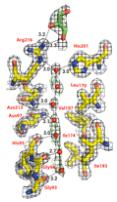
$$\rho(\mathbf{r}) = \Sigma \underline{F}(\underline{S}) e^{(-2\pi i \mathbf{r.S})}$$

$$\underline{F}(h,k,l) = \Sigma_j f_j e^{(2\pi i\ (hx+ky+lz))}$$

$$\rho(x,y,z) = \Sigma \underline{F}(h,k,l) e^{(-2\pi i \mathbf{r.S})}$$

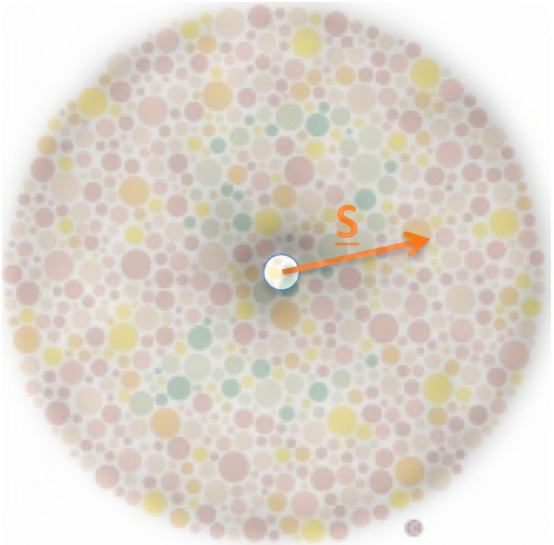
This is all there is?

PHASES-as colors !



FT

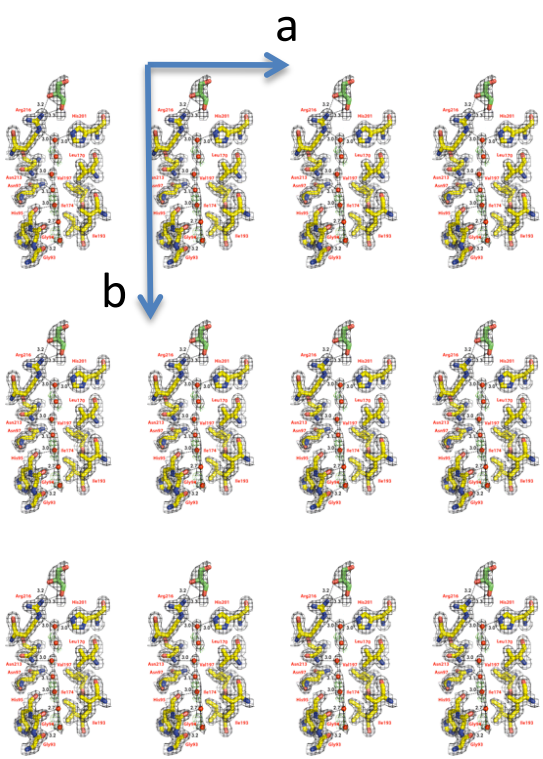
FT^{-1}



Scattering pattern is the Fourier transform of the structure

$$\underline{F}(\underline{S}) = \Sigma_j f_j e^{(2\pi i \mathbf{rj.S})}$$

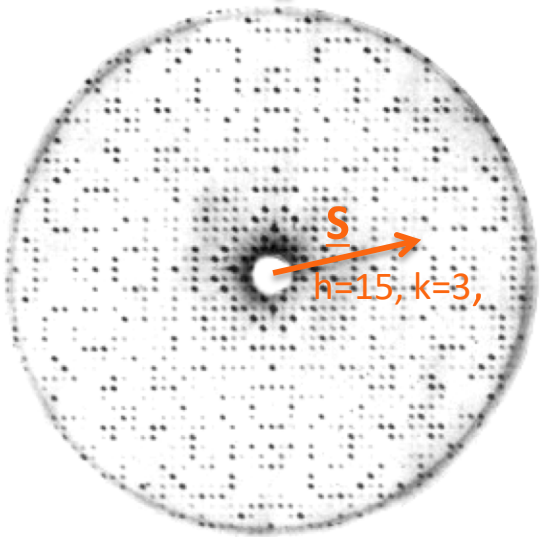
Structure is the 'inverse' Fourier transform of the Scattering pattern



FT

FT^{-1}

1/a



1/b

$$\rho(\underline{r}) = \Sigma \underline{F}(\underline{S}) e^{(-2\pi i \mathbf{r.S})}$$

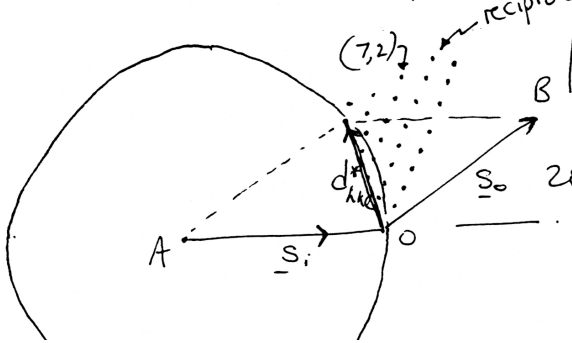
$$\underline{F}(h,k,l) = \Sigma_j f_j e^{(2\pi i\ (hx+ky+lz))}$$

$$\rho(x,y,z) = \Sigma \underline{F}(h,k,l) e^{(-2\pi i \mathbf{r.S})}$$

describe any reciprocal lattice point as

$$\underline{S} = \underline{d}^* = h\underline{a}^* + k\underline{b}^* + l\underline{c}^*$$

This (hkl) lattice point will only be observed when the crystal is turned so that the (hkl) point $\underline{S} = \underline{d}^*_{hkl}$ ~~lies~~ ends in the Ewald sphere.



$$I_{hkl} \propto |F_{hkl}|^2 = G(\underline{S})\,G^*(\underline{S})$$

Relative Information in Intensities versus phases

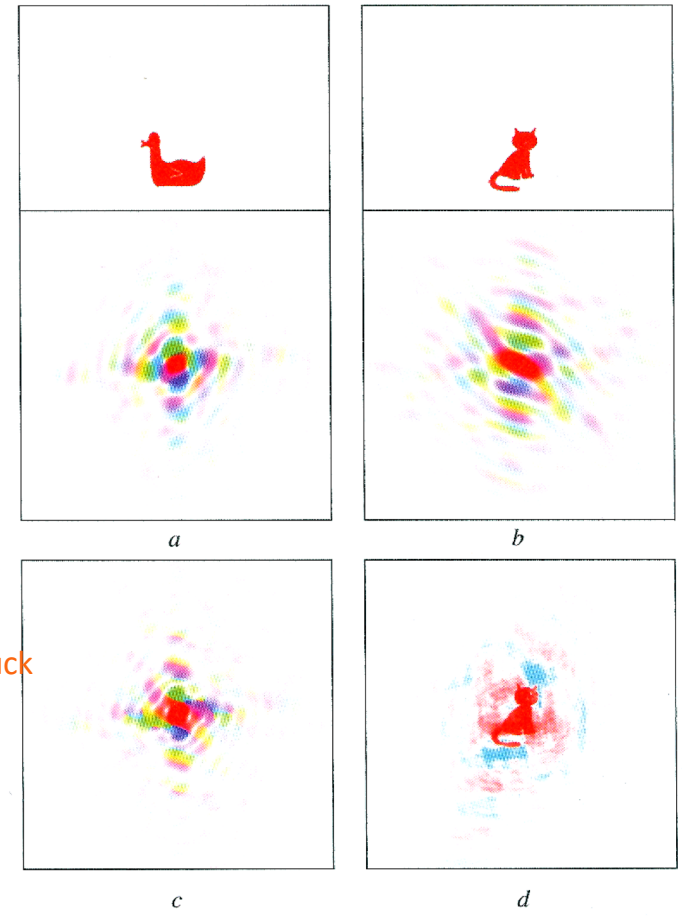
$\rho(\underline{r})$ duck

$\rho(\underline{r})$ cat

$\underline{F}_{(\underline{S})}$ duck → $|F_{(\underline{S})}|$, $\phi_{(\underline{S})}$

$\underline{F}_{(\underline{S})}$ cat

$$\underline{F}_{(\underline{S})} = \Sigma_j f_j e^{(2\pi i \mathbf{rj.S})}$$



$|F_{(\underline{S})}|$ duck

$\phi_{(\underline{S})}$ cat

$$\rho(\underline{r}) = \Sigma \underline{F}_{(\underline{S})} e^{(-2\pi i \mathbf{r.S})}$$

Looks like a .....

Figure 6.1 ► Relative amounts of information contained in reflection intensities and phases. (*a*) and (*b*) Duck and cat, along with their Fourier transforms. (*c*) Intensity (shading) of the duck transform, combined with the phases (colors) of the cat transform. (*d*) Back-transform of (*c*) produces recognizable image of cat, but not duck. Phases contain more information than intensities. Figure generously provided by Dr. Kevin Cowtan.

Relative Information in Intensities versus phases

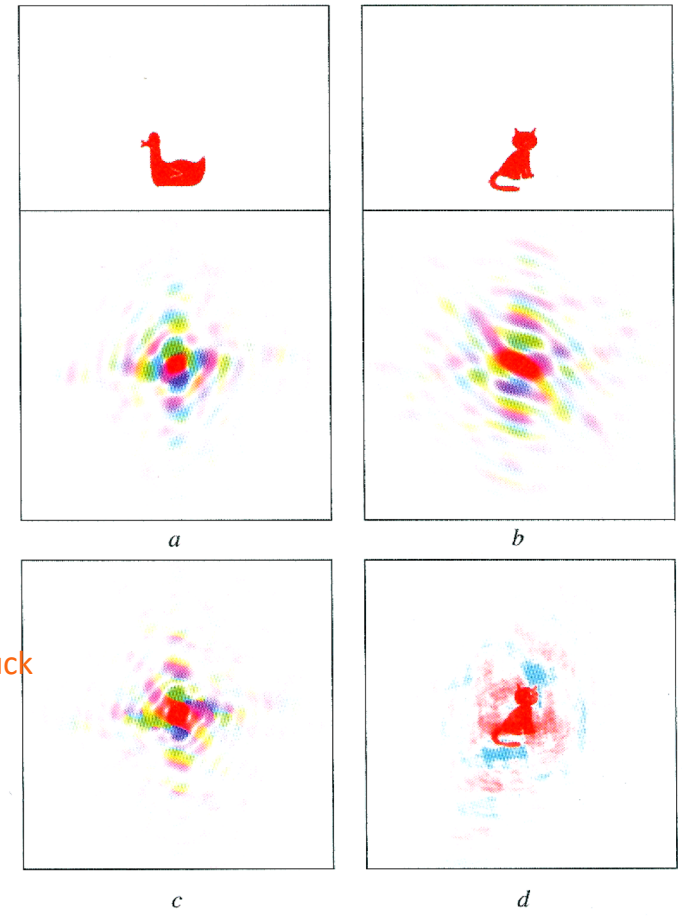
$\rho(\underline{r})$ duck

$\rho(\underline{r})$ cat

$\underline{F}_{(\underline{S})}$ duck → $|F_{(\underline{S})}|$, $\phi_{(\underline{S})}$

$\underline{F}_{(\underline{S})}$ cat

$$\underline{F}_{(\underline{S})} = \Sigma_j f_j e^{(2\pi i \mathbf{rj.S})}$$



$|F_{(\underline{S})}|$ duck

$\phi_{(\underline{S})}$ cat

$$\rho(\underline{r}) = \Sigma \underline{F}_{(\underline{S})} e^{(-2\pi i \mathbf{r.S})}$$

Figure 6.1 ► Relative amounts of information contained in reflection intensities and phases. (*a*) and (*b*) Duck and cat, along with their Fourier transforms. (*c*) Intensity (shading) of the duck transform, combined with the phases (colors) of the cat transform. (*d*) Back-transform of (*c*) produces recognizable image of cat, but not duck. Phases contain more information than intensities. Figure generously provided by Dr. Kevin Cowtan.

Looks like a CAT

PHASES DOMINATE:

-Incorrect phases = incorrect structure

-incorrect model = incorrect structure

-incorrect assumption = incorrect structure

‘Difference maps’

-Define bound ligands

-to find any missing atoms during refinement,

-to find ligands

-define movements of protein or water

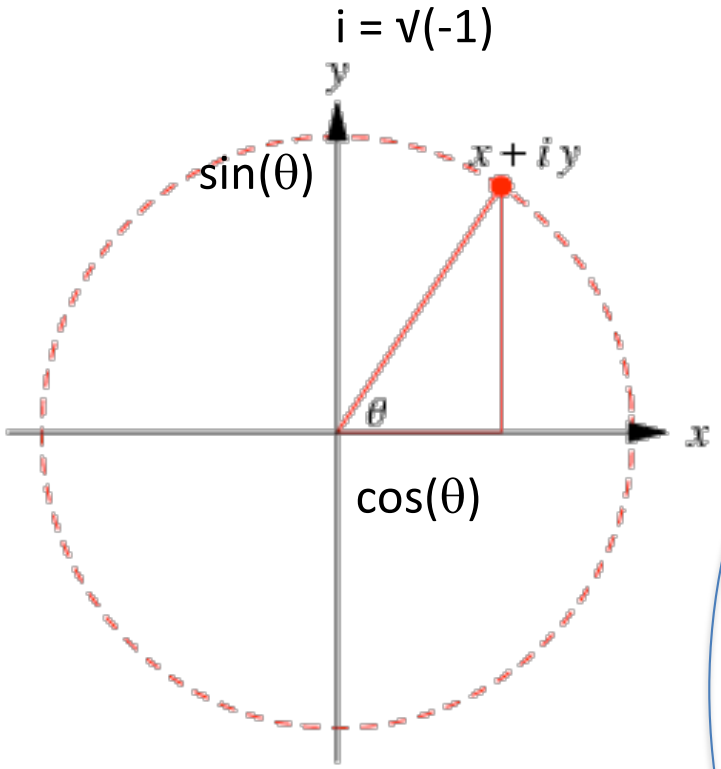
-determine ion positions

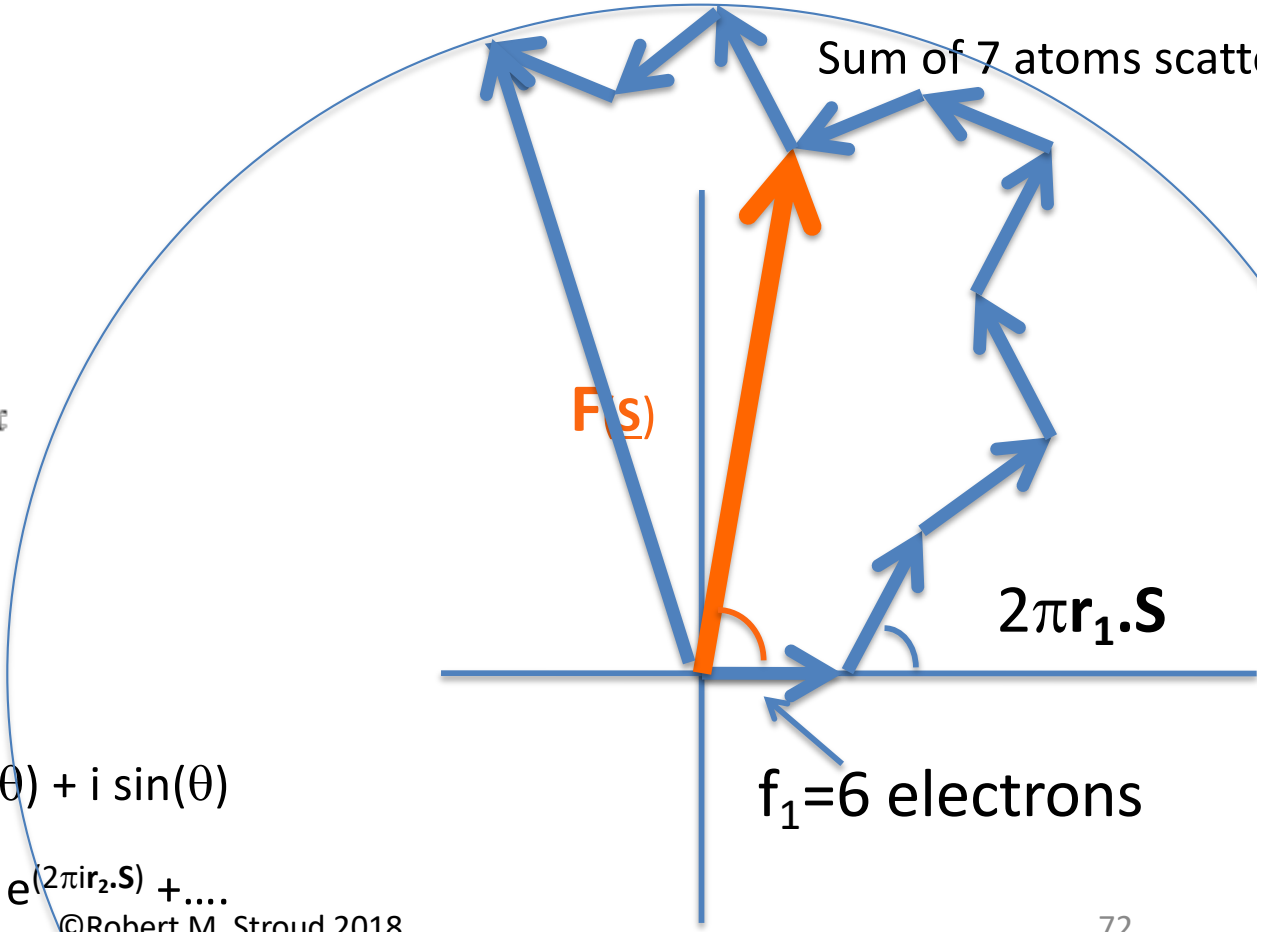
-determine changes in dynamic motion

Suppose we interpret 7 atoms; but 3 remain to be found in density

Result is a wave of
amplitude $|\mathbf{F}(\underline{\mathbf{S}})|$
phase $\Phi(\underline{\mathbf{S}})$

In reality, maybe 3 atoms are missing.
How to see what is missing?





$$e^{(i\theta)} = \cos(\theta) + i \sin(\theta)$$

$$\mathbf{F}(\underline{\mathbf{S}}) = f_1 e^{(2\pi i \mathbf{r}_1.\mathbf{S})} + f_2 e^{(2\pi i \mathbf{r}_2.\mathbf{S})} + \ldots$$

USES: 2. Add a substrate, Grow a new crystal

Measure New $|\mathbf{F}(\underline{\mathbf{S}})|_{obs+substrate}$ Compare with the apo-protein.

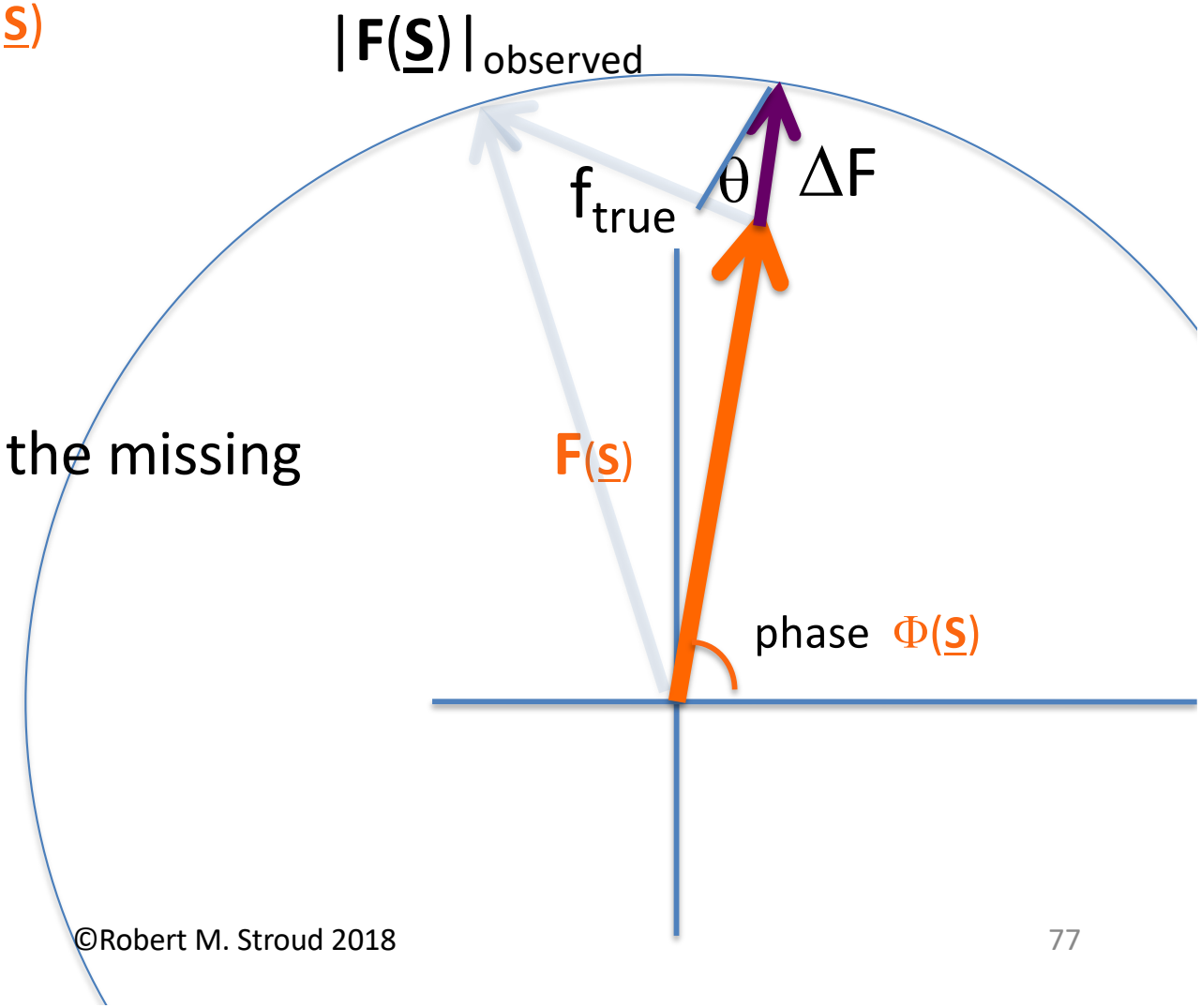
Transform $\Delta F = \left| |\mathbf{F}(\underline{\mathbf{S}})|_{obs+substrate} - |\mathbf{F}(\underline{\mathbf{S}})|_{obs} \right| \Phi(\underline{\mathbf{S}})$

or

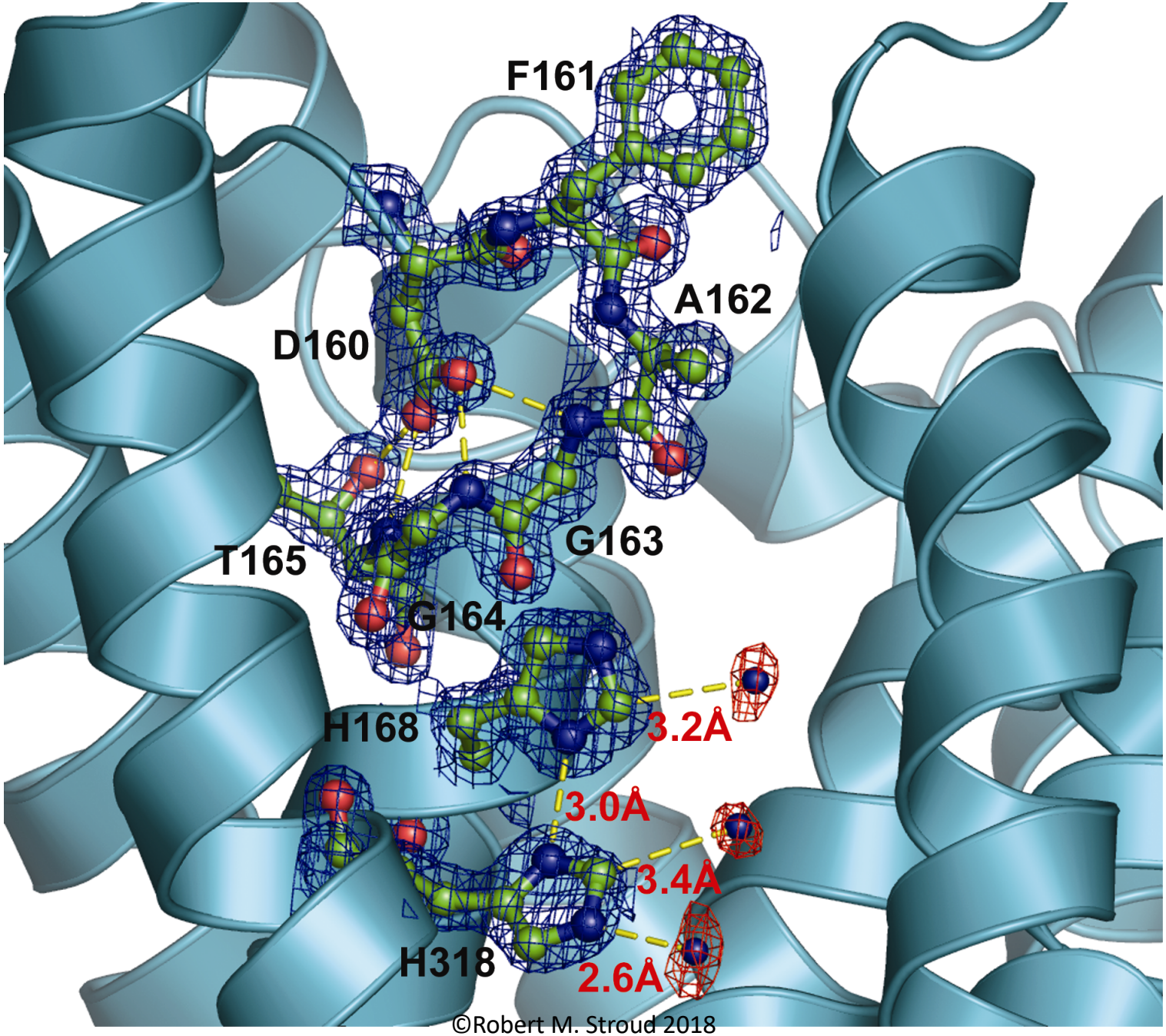
$[2|\mathbf{F}(\underline{\mathbf{S}})|_{obs+substrate} - |\mathbf{F}(\underline{\mathbf{S}})|_{obs}] \Phi(\underline{\mathbf{S}})$

= a '$2F_0$-F_o map'

It is **unbiased** as to where the missing substrate is.



A Dfference map shows 1/3 occupied NH3 sites and the role of D160 at 1.35Å Resolution. Here are 0.3 NH_3 peaks!



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Fo-Fc maps identify everything ordered that is 'missing'

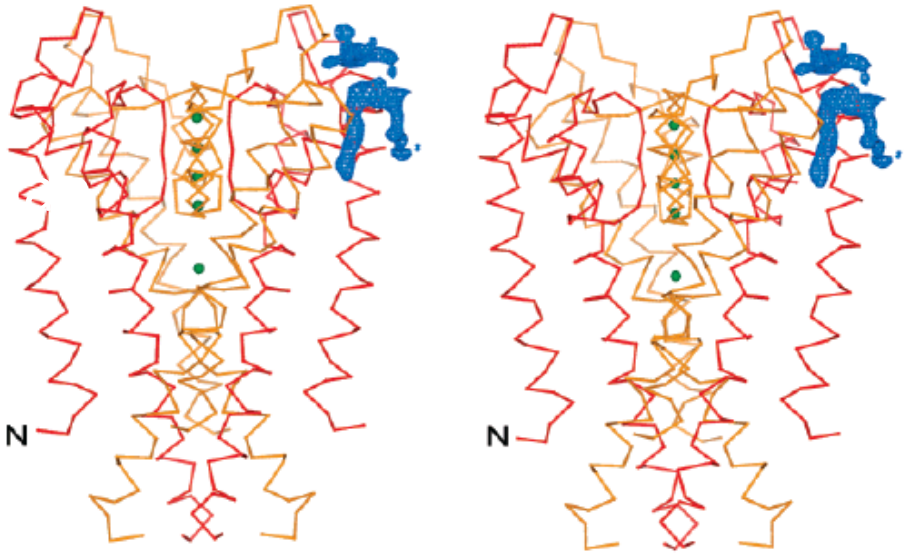

FIGURE 1: Lipid molecules in KcsA crystals. A stereoview of the KcsA structure with electron density corresponding to the lipid molecule. The backbone of KcsA is shown as a red and yellow trace. Green spheres represent potassium ion binding sites. The $F_o - F_c$ map (contoured at 3σ) was calculated using a model that does not contain lipid molecules. For clarity, density corresponding to only one of the lipid molecules is shown. The KcsA monomer consists of an N-terminal outer helix, a central pore helix, and a C-terminal inner helix. This figure was prepared with MOLSCRIPT (*31*) and Raster3D (*32*).

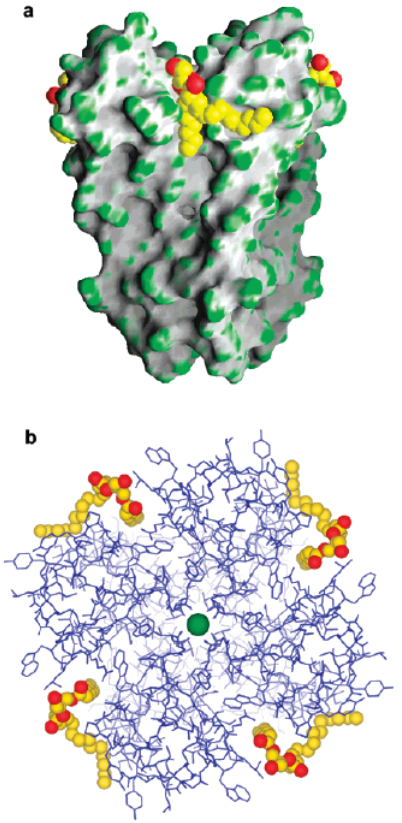

FIGURE 3: Structural analysis of lipid binding to KcsA. (a) Binding surface of the lipid molecule. The surface of KcsA is colored according to curvature (green, convex; gray, concave). The lipid molecule, built as 1,2-diacylglycerol, is shown in CPK representation with oxygen atoms colored red and carbon atoms colored yellow. (b) Lipid-binding site viewed from the extracellular side along the 4-fold axis of KcsA. The channel is colored blue. The green sphere represents the potassium ion. The lipid molecule is in CPK representation colored as in panel a. Panel a was prepared with GRASP (*33*). Panel b was prepared with MOLSCRIPT (*31*) and Raster3D (*32*).

-Eliminate Bias
-Half electron content
-See electrons

The closer you get –the lower the noise.
Can see single electrons.

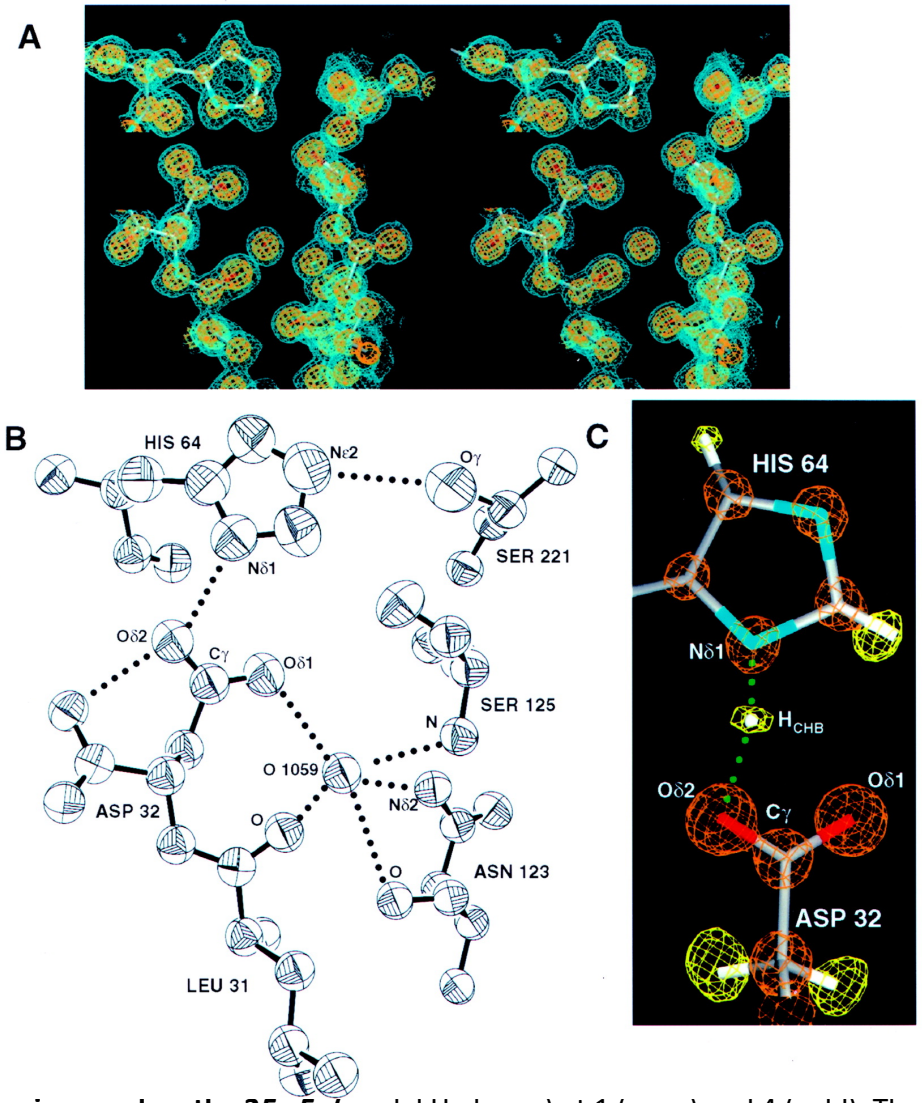

Figure 3 The catalytic triad. **(A) Stereoview displaying Model H superimposed on the 2*Fo Fc* (**model H phases) at 1 (aqua) and 4 (gold). The densities for C and N in His 64 are weaker than in Asp 32. The Asp 32 CO2 bond at 4 is continuous, while the density for the C and O1 are resolved. (B) Schematic of the catalytic residues and hydrogen bonded neighbors with thermal ellipsoid representation countered at 50% probability (*29*). Catalytic triad residues Ser 221 and His 64 show larger thermal motion than the Asp 32. Solvent O1059 appears to be a relatively rigid and integral part of the enzyme structure. (C) Catalytic hydrogen bond (CHB)**. A *Fo Fc* (model H phases) difference map** contoured at +2.5 (yellow) and 2.5 (red) and a 2*Fo Fc* (model H phases) electron density map contoured at 4 (gold). The position of the short hydrogen atom (labeled HCHB) in the CHB is positioned in the positive electron density present between His 64 N1 and Asp 32 O2.

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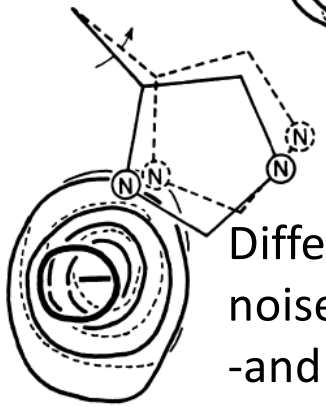
DOI: 10.1021/bi9813983

Seeing small shifts
-down to ±0.1Å



Difference Map
noise ~20% of noise in the parent protein
-and only two peaks!

FIG. 7. The peaks associated with His57 on the difference map. The lower peak is negative density (−) while the other one is positive (+). The latter peak is a composite with a solvent molecule density (see text).

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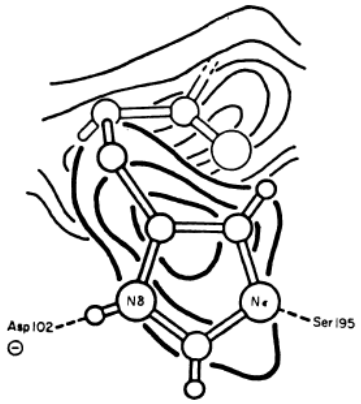

FIG. 8. Electron density for His57 in the DIP-trypsin Fourier map, computed for the plane parallel to the imidazole ring.

TABLE 1

Analysis of Fourier maps

Map	$\langle\lvert F_{obs}\rvert\rangle$ (e)	σ (e)	Calculated† $\langle\Delta\rho^2\rangle^{\frac{1}{2}}$ (e Å^{-3})	Observed‡ r.m.s. error (e Å^{-3})	Observed highest noise (e Å^{-3})	S.D.§	Observed highest peak (e Å^{-3})	S.D.
BA-trypsin—DIP-trypsin	84·7	2·3	0·069	0·059	0·17	2·5	0·75	11
DIP-trypsin	573·0	21·0	0·38	—	—	—	—	—

† ΔF: $\langle\Delta\rho^2\rangle = \frac{1}{2V^2}\sum_{hkl}\Delta F^2\,(2-m^2)$,

F_{DIPT}: $\langle\Delta\rho^2\rangle = \frac{1}{V^2}\sum_{hkl}F^2_{DIPT}\,(1-m^2)$,

(after Henderson & Moffat, 1971).

‡ The observed root mean-square density error is based on a relatively featureless region of the map.

§ S.D., the electron density given as a multiple of the calculated r.m.s. error.