

# Direct methods of crystal structure determination

Peter Main

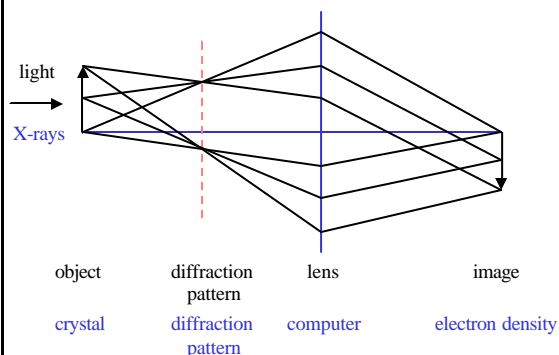


## Direct methods

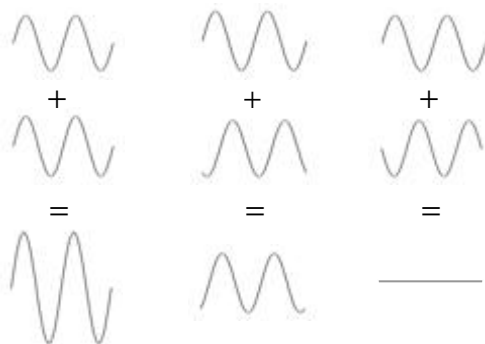
The name *direct methods of structure determination* refers to the determination of electron density, atomic coordinates or phases by mathematical means from a single set of measured X-ray intensities.

Commonly, it is phase determination that goes under this title, and that is the topic addressed here.

## Forming an image



## Addition of waves



## A contribution to $\rho(\mathbf{x})$

Electron density equation:

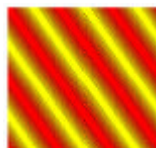
$$\rho(\mathbf{x}) = \frac{1}{V} \sum_{\mathbf{h}} F(\mathbf{h}) \exp(-2\pi i \mathbf{h} \cdot \mathbf{x})$$

Two related terms in the summation:

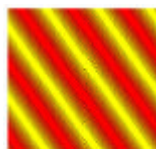
$$\begin{aligned} & F(\mathbf{h}) \exp(-2\pi i \mathbf{h} \cdot \mathbf{x}) + F(\bar{\mathbf{h}}) \exp(2\pi i \mathbf{h} \cdot \mathbf{x}) \\ &= |F(\mathbf{h})| \{ \exp(-2\pi i \mathbf{h} \cdot \mathbf{x} + i f(\mathbf{h})) + \exp(2\pi i \mathbf{h} \cdot \mathbf{x} - i f(\mathbf{h})) \} \\ &= |F(\mathbf{h})| 2 \cos(2\pi \mathbf{h} \cdot \mathbf{x} - f(\mathbf{h})) \end{aligned}$$

The contribution to the electron density of a reflection with its Friedel pair is a cosine wave of density.

## Electron density waves

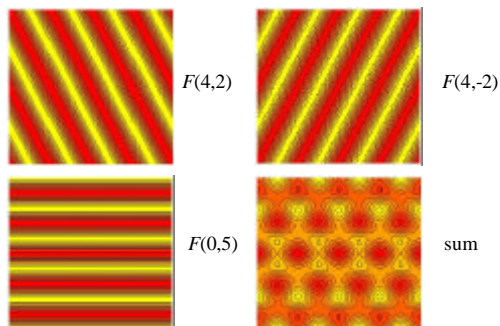


Contribution to the electron density of  $|F(3,2)|$  with a phase of  $0^\circ$ .

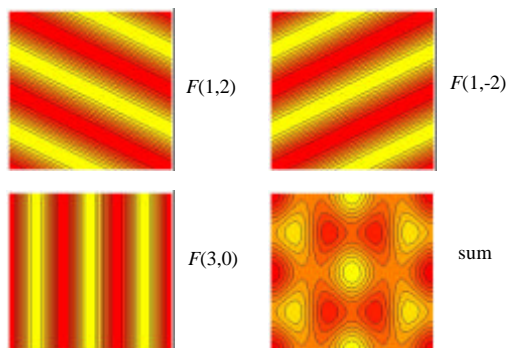


Contribution to the electron density of  $|F(3,2)|$  with a phase of  $90^\circ$ .

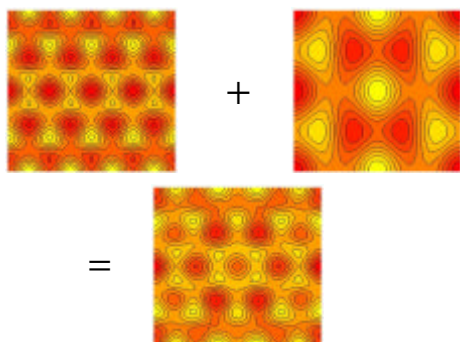
## Adding waves to form an image



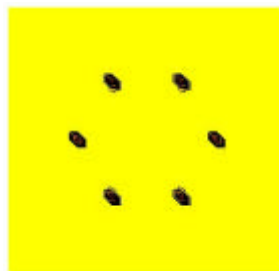
## More waves



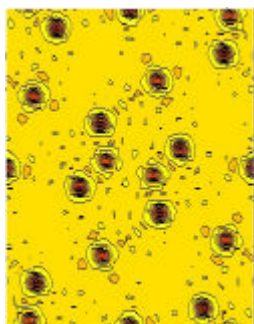
## Adding the sums



## The final sum



## Fourier synthesis



Ammonium oxalate monohydrate,  
 $(\text{NH}_4^+)_2\text{C}_2\text{O}_4^{2-} \cdot \text{H}_2\text{O}$

$a = 8.02 \text{ \AA}$ ,  $b = 10.31 \text{ \AA}$ ,  $c = 3.74 \text{ \AA}$ .

Space group  $\text{P2}_1\text{2}_1\text{2}$

Symmetry of projection is  $\text{pgg}$

## Convolution theorem

Don't worry about the maths – it is the pattern that is important:

$$\begin{array}{ccccc} f(x) & \otimes & g(x) & = & c(x) \\ \uparrow \text{F.T.} & & \uparrow \text{F.T.} & & \uparrow \text{F.T.} \\ F(S) & \times & G(S) & = & C(S) \end{array}$$

You are already familiar with this pattern in X-ray crystallography:

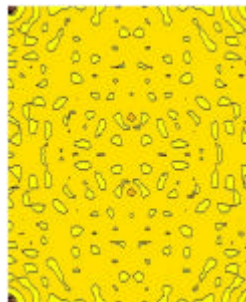
$$\begin{array}{ccccc} \text{unit cell} & \otimes & \text{crystallattice} & = & \text{crystal} \\ \uparrow \text{F.T.} & & \uparrow \text{F.T.} & & \uparrow \text{F.T.} \\ \text{X-ray scattering} & \times & \text{reciprocal} & = & \text{X-ray diffraction} \\ \text{from unit cell} & & \text{lattice} & & \text{pattern} \end{array}$$

This conveniently allows calculations to be performed on a single unit cell rather than on the whole crystal.

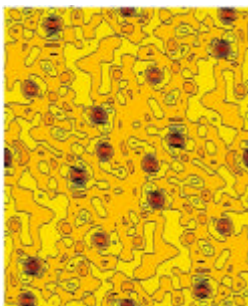
## The importance of phases

$$\begin{array}{ccccc}
 |F(\mathbf{h})| & \times & e^{i\phi(\mathbf{h})} & = & F(\mathbf{h}) \\
 \updownarrow \text{F.T.} & & \updownarrow \text{F.T.} & & \updownarrow \text{F.T.} \\
 \text{magnitude} & \otimes & \text{phase} & = & ?(\mathbf{x}) \\
 \text{synthesis} & & \text{synthesis} & & \\
 \uparrow & & \uparrow & & \\
 \text{similar to Patterson} & & \text{must contain} & & \\
 \text{function (large} & & \text{peaks at} & & \\
 \text{origin peak)} & & \text{atomic sites} & & 
 \end{array}$$

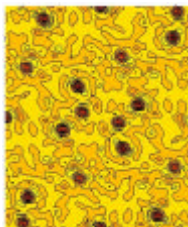
## Magnitude synthesis



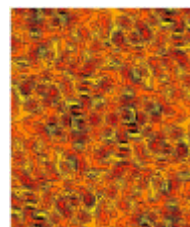
## Phase synthesis



## Random magnitudes and phases

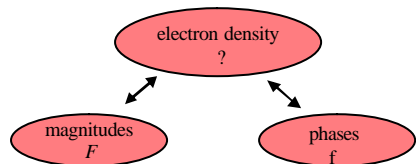


Correct phases  
Random magnitudes



Random phases  
Correct magnitudes

## The connection between magnitudes and phases

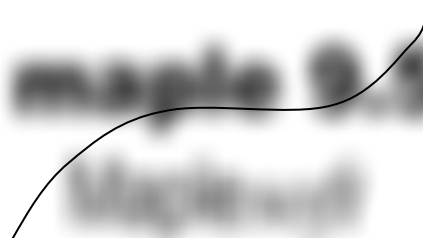


2

completely unknown  
completely known  
partially known  
partially known

F and f

no link between F and f  
F and f can both be calculated  
given F, f can be calculated  
given f, F can be calculated



## Constraints on the electron density

- |  |                                 |
|--|---------------------------------|
| 1. Discrete atoms  | Use $E$ 's instead of $F$ 's    |
| 2. $\rho(\mathbf{x}) \geq 0$   | Inequality relationships        |
| 3. Random atomic distribution  | Phase probability relationships |
| 4. $\int_V \rho^3(\mathbf{x}) dV = \max$   | Tangent formula                 |
| 5. Equal atoms   | Sayre's equation                |
| 6. Equal molecules   | Non-crystallographic symmetry   |
| 7. $-\int_V \rho(\mathbf{x}) \ln \left( \frac{\rho(\mathbf{x})}{\bar{\rho}(\mathbf{x})} \right) dV = \max$ | Maximum entropy                 |
| 8. $\rho(\mathbf{x}) = \text{constant or known distribution}$  | Density modification            |

## Normalised structure factors

$$E(\mathbf{h}) \times \text{atomic scattering factor} = F(\mathbf{h})$$

$$\uparrow \text{F.T.} \quad \quad \quad \uparrow \text{F.T.} \quad \quad \quad \uparrow \text{F.T.}$$

$$\text{point atom structure} \otimes \text{real atom} = \rho(\mathbf{x})$$

$$|E(\mathbf{h})|^2 = \frac{|F(\mathbf{h})|^2}{e_h \sum_i f_i^2}$$

$$\langle |E(\mathbf{h})|^2 \rangle = 1$$

For  $N$  equal atoms in the cell:  $E(\mathbf{0}) = N^{1/2}$

## Karle – Hauptman inequalities

The electron density is the sum of a Fourier series. If the sum is everywhere positive, the constraints on the Fourier coefficients can be expressed as:

$$\begin{vmatrix} E(\mathbf{0}) & E(\mathbf{h}_1) & E(\mathbf{h}_2) & \dots & E(\mathbf{h}_n) \\ E(\mathbf{h}_1) & E(\mathbf{0}) & E(\mathbf{h}_1 + \mathbf{h}_2) & \dots & E(\mathbf{h}_1 + \mathbf{h}_n) \\ \dots & \dots & \dots & \dots & \dots \\ E(\mathbf{h}_n) & E(\mathbf{h}_n + \mathbf{h}_1) & E(\mathbf{h}_n + \mathbf{h}_2) & \dots & E(\mathbf{0}) \end{vmatrix} \geq 0$$

- The determinant can be of any order.
- Note that the  $E$ 's in any row or column define the whole determinant.
- The phases must make the value of the determinant positive.

## Inequality example

For a centrosymmetric structure, all phases are either 0 or  $\pi$ .

In this case,  $E(\mathbf{h}) = E(\bar{\mathbf{h}})$  and the phase becomes a sign (+ or -).

The order 3 Karle-Hauptman inequality  $\begin{vmatrix} E(\mathbf{0}) & E(\mathbf{h}) & E(2\mathbf{h}) \\ E(\bar{\mathbf{h}}) & E(\mathbf{0}) & E(\mathbf{h}) \\ E(2\mathbf{h}) & E(\bar{\mathbf{h}}) & E(\mathbf{0}) \end{vmatrix} \geq 0$

gives:  $|E(\mathbf{h})|^2 \leq \frac{1}{2} E(\mathbf{0}) [E(\mathbf{0}) + E(2\mathbf{h})]$

where only the sign of  $E(2\mathbf{h})$  is unknown.

With large enough magnitudes, this can prove that the sign of  $E(2\mathbf{h})$  must be +.

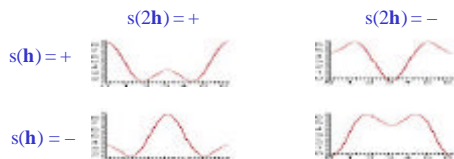
## Inequality example continued

$$|E(\mathbf{h})|^2 \leq \frac{1}{2} E(\mathbf{0}) [E(\mathbf{0}) + E(2\mathbf{h})]$$

With  $E(\mathbf{0}) = 4.0$ ,  $E(\mathbf{h}) = 2.5$  and  $E(2\mathbf{h}) = 2.0$ :

$$2.5^2 = 6.25 \leq \frac{4}{2} [4 + 2] = 12 \text{ or } 4$$

so the sign of  $E(2\mathbf{h})$  must be positive.



## Another example

Still assuming a centrosymmetric structure, the order 3 inequality

$$\begin{vmatrix} E(\mathbf{0}) & E(\mathbf{h}) & E(\mathbf{k}) \\ E(\bar{\mathbf{h}}) & E(\mathbf{0}) & E(\bar{\mathbf{h}} + \mathbf{k}) \\ E(\bar{\mathbf{k}}) & E(\bar{\mathbf{k}} + \mathbf{h}) & E(\mathbf{0}) \end{vmatrix} \geq 0$$

Gives  $E(\mathbf{0}) [E^2(\mathbf{0}) - E^2(\mathbf{h}) - E^2(\mathbf{k}) - E^2(\mathbf{h} - \mathbf{k})] + 2E(\mathbf{h})E(\bar{\mathbf{h}} + \mathbf{k})E(\bar{\mathbf{k}}) \geq 0$

and for large enough magnitudes, this can prove that

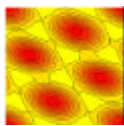
$$E(\mathbf{h}) E(\bar{\mathbf{h}} + \mathbf{k}) E(\bar{\mathbf{k}}) \text{ is positive}$$

In terms of signs, this becomes  $s(\mathbf{h}) s(\bar{\mathbf{h}} + \mathbf{k}) s(\bar{\mathbf{k}}) = +$

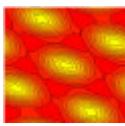
Therefore, knowing two of the signs determines the third.

## The sign of $E(\mathbf{h})E(\bar{\mathbf{h}}+\mathbf{k})E(\bar{\mathbf{k}})$

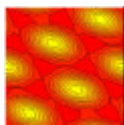
Electron density maps calculated from three reflections.



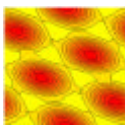
signs: + + +



signs: + + -



signs: + - +



signs: + - -

The contouring shows high density as red and low or negative density as yellow.

When the product of the three signs is +, peaks of high density are produced.

When the product of the three signs is -, peaks of negative density are produced.

## An inequality relationship for a noncentrosymmetric structure

$$\begin{vmatrix} E(0) & E(\mathbf{h}) & E(\mathbf{k}) \\ E(\bar{\mathbf{h}}) & E(0) & E(\bar{\mathbf{h}}+\mathbf{k}) \\ E(\bar{\mathbf{k}}) & E(\bar{\mathbf{k}}+\mathbf{h}) & E(0) \end{vmatrix} \geq 0$$

$$E(0) \left[ E^2(\mathbf{h}) - |E(\mathbf{h})|^2 - |E(\mathbf{k})|^2 - |E(\mathbf{h}-\mathbf{k})|^2 \right] + 2 \left[ E(\mathbf{h})E(\bar{\mathbf{h}}+\mathbf{k})E(\bar{\mathbf{k}}) \right] \cos(f(\mathbf{h}) + f(\bar{\mathbf{h}}+\mathbf{k}) + f(\bar{\mathbf{k}})) \geq 0$$

For large enough magnitudes, this can prove that

$$\cos(f(\mathbf{h}) + f(\bar{\mathbf{h}}+\mathbf{k}) + f(\bar{\mathbf{k}})) \text{ is close to } 1$$

i.e. that  $f(\mathbf{h}) + f(\bar{\mathbf{h}}+\mathbf{k}) + f(\bar{\mathbf{k}})$  is close to 0.

Knowing two of the phases then gives an estimate for the third.

## Limitations of inequalities

Inequalities only have an effect over those regions of the cell for which  $\varphi(\mathbf{x}) < 0$ .

If the total volume these regions is a small fraction of the unit cell volume, very few useful inequality relationships will be found.

If there is no negative density, the inequalities will give no phase information at all.

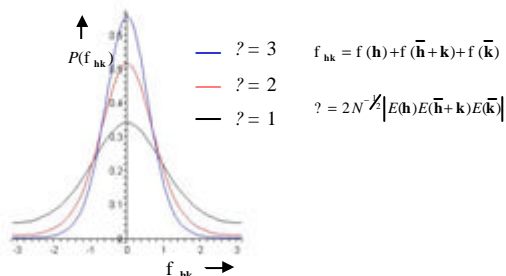
This becomes more likely as the size of the structure increases, since  $E(0)$  increases in value but  $E(\mathbf{h})$ , in general, will remain the same.

Inequalities are only useful for small structures.

## Extension to probabilities

Negative values of the determinant are forbidden.

High values are most likely.



## The tangent formula

The tangent formula is the main formula for phase determination in small molecule crystallography.

It is most conveniently expressed as:

$$f(\mathbf{h}) \approx \text{phase of } \left\{ \sum_{\mathbf{k}} E(\mathbf{k}) E(\mathbf{h}-\mathbf{k}) \right\}$$

It is based on the electron density constraints:

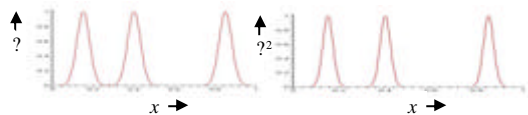
1. Discrete atoms (use of  $E$ 's).
2.  $\int_V \varphi^2(\mathbf{x}) dV = \text{maximum (over the whole cell)}$ .

A single term in the tangent formula gives  $f(\mathbf{h}) \approx f(\mathbf{k}) + f(\mathbf{h}-\mathbf{k})$

The complete formula combines the probabilities of many such relationships.

## Sayre's equation

In a structure composed of equal atoms, the squared electron density is the same as the original with a change in atomic shape.



$$F(\mathbf{h}) = \frac{1}{V} \frac{f}{g} \sum_{\mathbf{k}} F(\mathbf{k}) F(\mathbf{h}-\mathbf{k})$$

$f$  = atomic scattering factor

$g$  = scattering factor of squared atom

## Structure determination

1. Estimate  $E$ 's from  $F$ 's scaling and statistics
2. Set up phase relationships terms in tangent formula
3. Find starting reflections convergence procedure
4. Assign values to unknown phases random numbers
5. Determine and refine phases tangent formula
6. Choose best phase sets figures of merit
7. Calculate map Fourier transform
8. Interpretation peak search and stereochemistry

## Calculation of $E$ 's

$$|E(\mathbf{h})|^2 = \frac{|F(\mathbf{h})|^2}{e_h \langle I \rangle}$$

$e_h$  accounts for the effect of symmetry on  $|F(\mathbf{h})|^2$ .

$\langle I \rangle$  = local average intensity

Obtain  $\langle I \rangle$  from a Wilson plot.

Accumulate intensity statistics

Choose  $E$ 's for phase determination.

$\sim 4 \times$  number of independent atoms + 150

## Wilson plot

A Wilson plot may be used to determine the scale factor,  $A$ , required to put the observed intensities on an absolute scale.

It is related to the overall thermal displacement parameter,  $B$ , by:

$$A|F_o|^2 = |F_c|^2 \exp(-2B \sin^2 q / I^2)$$

where  $|F_o|^2$  is the observed intensity on an arbitrary scale and  $|F_c|^2$  is the calculated intensity on an absolute scale.

Average over all reflections in a narrow range of  $\sin^2 q / I^2$  and use Wilson's result that  $\langle |F_c|^2 \rangle = \sum_{i=1}^N f_i^2$  for randomly positioned atoms:

$$A \overline{|F_o|^2} = \sum f_i^2 \exp(-2B \sin^2 q / I^2)$$

## Wilson plot continued

An algebraic rearrangement of the previous equation gives:

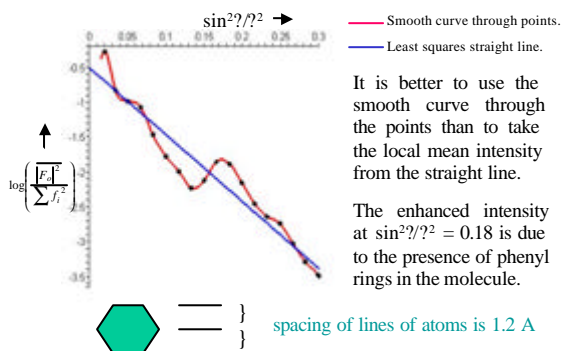
$$\frac{\overline{|F_o|^2}}{\sum f_i^2} = \frac{1}{A} \exp(-2B \sin^2 q / I^2)$$

and taking logs:  $\log \left( \frac{\overline{|F_o|^2}}{\sum f_i^2} \right) = -\log(A) - 2B \frac{\sin^2 q}{I^2}$

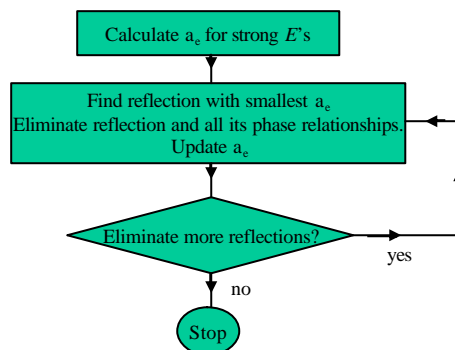
A plot of  $\log \left( \frac{\overline{|F_o|^2}}{\sum f_i^2} \right)$  against  $\frac{\sin^2 q}{I^2}$  gives a straight line:

slope =  $-2B$       intercept =  $-\log(A)$

## Wilson plot



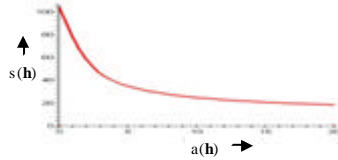
## Convergence procedure



## Variance of determined phases

$$a(\mathbf{h}) = 2N^{-1/2} \left| E(\mathbf{h}) \left| \sum_{\mathbf{k}} E(\mathbf{k}) E(\mathbf{h}-\mathbf{k}) \right| \right|$$

$$s^{-2}(\mathbf{h}) = \frac{p^2}{3} + 4 \sum_{\mathbf{k}} \frac{(-1)^{\mathbf{k}} I_1(\mathbf{h})}{n^2 I_0(a(\mathbf{h}))}$$



Statistical estimation of  $a(\mathbf{h})$ :

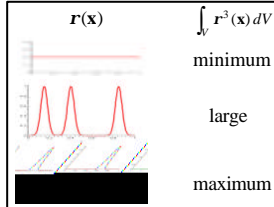
$$a^2(\mathbf{h}) = \sum_{\mathbf{k}} \frac{I_1(\mathbf{h})}{I_0(\mathbf{h})} + \sum_{\mathbf{k}} \frac{I_1(\mathbf{h})}{I_0(\mathbf{h})} \frac{I_1(\mathbf{h})}{I_0(\mathbf{h})}$$

$$I_1(\mathbf{h}) = 2N^{-1/2} \left| E(\mathbf{h}) E(\mathbf{k}) E(\mathbf{h}-\mathbf{k}) \right|$$

## Maximisation of $\int_V r^3(\mathbf{x}) dV$

The tangent formula maximises the value of  $\int_V r^3(\mathbf{x}) dV$  over the whole cell, making it very powerful.

It is also closely related to Sayre's equation which squares the electron density. This means that heavy atoms will appear even heavier in the squared density, which will distort the image.



Additional constraints are needed to avoid the distorted image that occurs at the maximum.

## Negative quartets

Consider the Karle – Hauptman determinant:

$$\begin{vmatrix} E(0) & E(\mathbf{h}) & E(\mathbf{h}+\mathbf{k}) & E(\mathbf{h}+\mathbf{k}+\mathbf{l}) \\ E(\mathbf{h}) & E(0) & E(\mathbf{k}) & E(\mathbf{k}+\mathbf{l}) \\ E(\mathbf{h}-\mathbf{k}) & E(\mathbf{k}) & E(0) & E(\mathbf{l}) \\ E(\mathbf{h}-\mathbf{k}-\mathbf{l}) & E(\mathbf{k}-\mathbf{l}) & E(\mathbf{l}) & E(0) \end{vmatrix} \geq 0$$

If  $|E(\mathbf{h}+\mathbf{k})| = |E(\mathbf{k}+\mathbf{l})| = 0$ , this simplifies to:

terms in intensities only  $-2E_4 \cos(f_4) \geq 0$

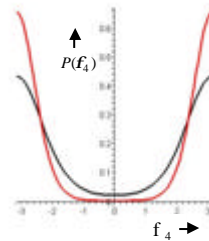
where  $E_4 = |E(\mathbf{h})E(\mathbf{k})E(\mathbf{l})E(\mathbf{h}-\mathbf{k}-\mathbf{l})|$

$$f_4 = f(\mathbf{h}) + f(\mathbf{k}) + f(\mathbf{l}) + f(\mathbf{h}-\mathbf{k}-\mathbf{l})$$

To maximise the determinant value,  $\cos(f_4) = -1$

$$\text{i.e. } f(\mathbf{h}) + f(\mathbf{k}) + f(\mathbf{l}) + f(\mathbf{h}-\mathbf{k}-\mathbf{l}) = \pi$$

## Negative quartet probability distribution



—  $B = 1.5$   
—  $B = 3.0$

$$B = 2N^{-1} |E(\mathbf{h})E(\mathbf{k})E(\mathbf{l})E(\mathbf{h}-\mathbf{k}-\mathbf{l})|$$

$$f_4 = f(\mathbf{h}) + f(\mathbf{k}) + f(\mathbf{l}) + f(\mathbf{h}-\mathbf{k}-\mathbf{l})$$

The probability distribution assumes that  $|E(\mathbf{h}+\mathbf{k})| = |E(\mathbf{k}+\mathbf{l})| = |E(\mathbf{l}+\mathbf{h})| = 0$

## Tangent formula with negative quartets

$f(\mathbf{h})$  = phase of  $\{a(\mathbf{h}) - g(\mathbf{h})\}$

$$a(\mathbf{h}) = 2N^{-1/2} \left| E(\mathbf{h}) \sum_{\mathbf{k}} E(\mathbf{k}) E(\mathbf{h}-\mathbf{k}) \right|$$

$$g(\mathbf{h}) = 2N^{-1} \left| E(\mathbf{h}) \sum_{\mathbf{k}, \mathbf{l}} E(\mathbf{k}) E(\mathbf{l}) E(\mathbf{h}-\mathbf{k}-\mathbf{l}) \right|$$

Terms in  $g(\mathbf{h})$  all have  $|E(\mathbf{h}+\mathbf{k})| = |E(\mathbf{k}+\mathbf{l})| = |E(\mathbf{l}+\mathbf{h})| = 0$

$g$  is a scale factor to balance the effect of  $g(\mathbf{h})$  relative to  $a(\mathbf{h})$

## Figures of merit

$$R_a = \frac{\sum_{\mathbf{h}} |a(\mathbf{h}) - g(\mathbf{h})|}{\sum_{\mathbf{h}} a(\mathbf{h})}$$

Measures agreement between actual and expected  $a(\mathbf{h})$ .

$R_a$  is small for the correct phases.

$$NQAL = \frac{\left| \sum_{\mathbf{h}} a(\mathbf{h}) g(\mathbf{h}) \right|}{\sum_{\mathbf{h}} |a(\mathbf{h})| |g(\mathbf{h})|}$$

Measures consistency of phases indicated by  $a(\mathbf{h})$  and  $g(\mathbf{h})$ .

$NQAL$  is  $-1$  for correct phases,  $\sim 0$  for random phases

$$NQEST = \frac{\sum_{\mathbf{h}, \mathbf{k}, \mathbf{l}} E_4 \cos(f_4)}{\sum_{\mathbf{h}, \mathbf{k}, \mathbf{l}} E_4}$$

$$E_4 = |E(\mathbf{h})E(\mathbf{k})E(\mathbf{l})E(\mathbf{h}-\mathbf{k}-\mathbf{l})|$$

$$f_4 = f(\mathbf{h}) + f(\mathbf{k}) + f(\mathbf{l}) + f(\mathbf{h}-\mathbf{k}-\mathbf{l})$$

Measures mean value of  $-ve$  quartets  
=  $-1$  for correct phases,  $0$  for random.

## Improvement of E-maps

Use Sayre's equation with the complete set of data to determine and refine the phases of all reflections.

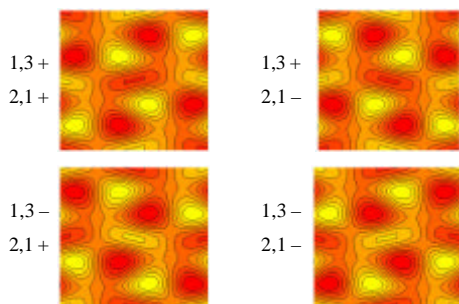
- gives a much better map than that obtained from the small number of phases determined by the tangent formula.
- very few spurious peaks.
- very few missing atoms.
- density peaks correspond more closely to the atomic positions.

## Worth noting

1. For solving the structure, the extent of the data is more important than its accuracy. Poor data to high resolution is better than accurate data to low resolution.
2. Low symmetry structures may need more reflections in the phasing procedure than high symmetry structures.
3. An uninterpretable map may still contain correct fragments of the structure. Use plausible peaks to calculate starting phases and try again.

## Origin definition

Illustrating the effect of changing the signs of origin-defining reflections.



## Symbolic addition

Symbolic addition is a technique of phase determination that was originally carried out by hand.

For a centrosymmetric structure, the phases are all 0 or  $\pi$ , giving rise to the signs + or – of the structure factors.

The main sign determining formula is  $s(\mathbf{h}) = s(\mathbf{k}) s(\mathbf{h} - \mathbf{k})$

Note that the sum of the diffraction indices on the right gives the diffraction index of the sign on the left hand side.

Examples:

$$\begin{array}{rcl} -5 & 7 & + \\ \hline 14 & 5 & + \\ 9 & 12 & + \end{array} \quad \begin{array}{rcl} 14 & 5 & + \\ \hline -9 & 12 & - \\ 5 & 17 & - \end{array}$$

## E-map using 55 unique reflections

