The phase problem:

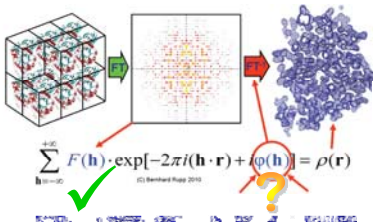


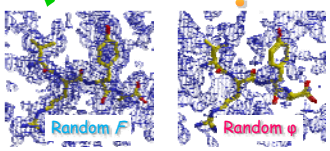
Figure 9-15 The crystallographic phase problem. The measurable component of the Fourier transform of the crystal is only the scalar structure factor amplitude $|\mathbf{h}|$ proportional to the square root of $I(\mathbf{h})$. The missing phases $\varphi(\mathbf{h})$ must be supplied by additional phasing experiments or in the form of model phases via molecular replacement. The two necessary Fourier coefficients in the back-transform formula are emphasized in blue.

$$\sum_{\mathbf{h}=-\infty}^{+\infty} F(\mathbf{h}) \cdot \exp[-2\pi i(\mathbf{h}\cdot\mathbf{r}) + i\varphi(\mathbf{h})] = \rho(\mathbf{r})$$

(C) Bernhard Rupp 2010

Which is worse?
Random amplitudes or random phases?

Phases dominate the Fourier reconstruction of the electron density map.



Effect of random intensity noise

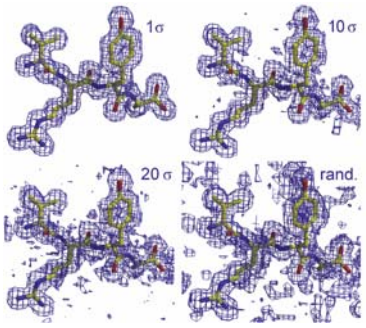
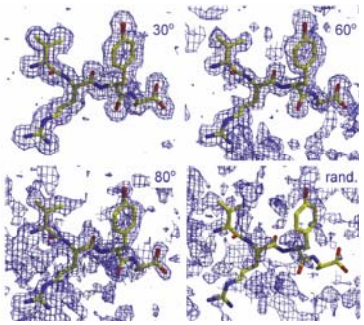


Figure 9-16 Effect of intensity measurement noise on electron density reconstruction. The electron density is calculated for data with increasing random measurement (amplitude) error while keeping correct phases. With increasing error, the maps become progressively noisy, but even at absurd error levels and even with random intensities (obeying only the Wilson distribution), the outline of the molecule remains surprisingly distinct. The mean measurement error is given in number of standard deviations $\sigma(I)$.

Effect of phase error

Figure 9-17 Influence of phase error on electron density map reconstruction. Even in the case of perfectly correct intensities or structure factor amplitudes, past about ~70° of mean phase error, the maps become uninterpretable. Random phases finally produce a random map, in contrast to random amplitudes (Figure 9-16). Poor data combined with poor phases obviously creates a particularly ghastly situation for electron density construction.



Poor phases are worse than poor data, but with poor data no phases (Chapter 10)!

$$\frac{r.m.s.(F,phs)}{r.m.s.(F,amp)} = \left(\frac{2}{(4-\pi)/2}\right)^{1/2} = 1.41/0.66 = 2.16$$

Phase dominate: phase (model) bias

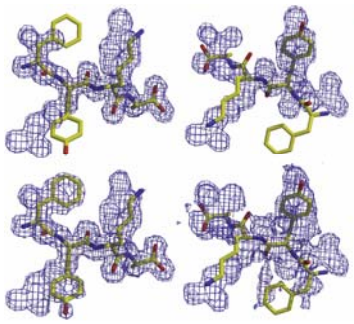


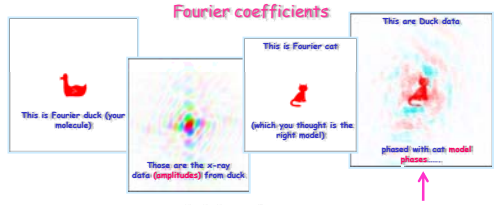
Figure 9-18 Phase bias in electron density maps. The upper panels show a mutant peptide Phe-Tyr-Lys-Ala (left) and the same peptide rotated (leading to reverse chain direction) simply superimposed on the electron density of the original Val-Arg-Tyr-Ala peptide. The lower panels show the electron density reconstructed using the diffraction data from the new models above, but using the old starting phases from the original peptide. The result is quite sobering: the shape of the electron density is still dominated by the starting model, and only weak outlines of the correct molecule density are visible. In the lower left panel, not even the direction of the peptide could be assigned for the reversed peptide with any certainty.

Better suitable map types and better map coefficients improve but not remove the problem.

Dominance of phases on Fourier synthesis: model (phase) bias.

$$\rho(\mathbf{x}) = \frac{1}{V}\sum_{-hkl}^{+hkl} F(obs)\cdot\exp(i\varphi(calc))\cdot\exp[-2\pi i(\mathbf{hx})]$$

Fourier coefficients



Full credit for the Fourier duck idea is due to Kevin Cowtan http://www.ysbl.york.ac.uk/~cowtan/fourier/fourier.html

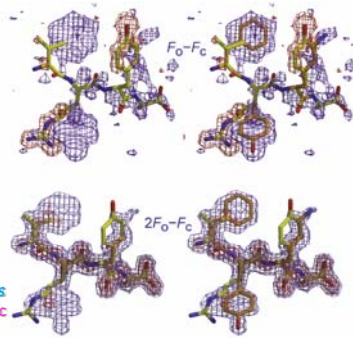
Bad phases (wrong model) overpower the experimental data:

$F(duck)\cdot\exp(i\varphi(cat))$

Fourier coefficients

Difference maps and 2Fo-Fc maps

Figure 9-19 $F_o - F_c$ difference maps and $2F_o - F_c$ maps. The $F_o - F_c$ difference maps in the top panel show negative difference density (where there should not be any density) in red and positive difference density (where there should be density) in purple. Both regions correctly reveal the difference between the starting model (yellow sticks) and the correct model (orange sticks, shown in the right panels). The sensitive difference maps are thus particularly valuable for detailed model correction. The $2F_o - F_c$ maps in the bottom panel can be interpreted as a combination of the difference map $(F_o - F_c)\exp(i\varphi_c)$ and a $(F_o)\exp(i\varphi_c)$ map. The $2F_o - F_c$ map is contoured to amplify positive density and is well suited to early model building stages. Another common color scheme is red for negative difference density and green for positive.



Best coefficients: Maximum likelihood (sigma-A) coefficients mFo-DFc or 2mFo-DFc (Chapter 10)