APS workshop
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Using weak anomalous signals for phasing

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Anomalous scattering

If the energy of X-rays is close to the excitation energy of inner electrons
Structure factor and anomalous effect

\[
F(h) = \sum_j f_j \exp(2\pi i h \cdot r_j)
\]

\[
f_j = f_0^j(\theta) + f'_j(\lambda) + i f''_j(\lambda)
\]

Anomalous correction \(f''\) is proportional to absorption and fluorescence and \(f'\) is its derivative.
Anomalous corrections $f'$ and $f''$ for Se

- Black - theory for single atom in vacuum
- Blue - measured curve from real sample

Selenium K edge
Friedel pair: $F(h)$ and $\ast F(-h)$

$|F(h)| \neq |F(-h)|$

Friedel’s Law is broken
Bijvoet difference

\[ \Delta F^\pm = |F^+| - |F^-| \]
Bijvoet ratio

\[
\frac{\langle \Delta F^\pm \rangle}{\langle F \rangle} = 2^{1/2} \cdot \frac{(N_A)^{1/2} \cdot f''}{(N_T)^{1/2} \cdot Z_{\text{eff}}}
\]
Friedel pair: \( F(h) \) and \( \ast F(-h) \)
Friedel pair more realistically

\[ f^\circ(S) = 16 \]
\[ f''(S) = 0.56 \quad \text{for} \quad \lambda = 1.54 \text{ Å} \]

\[ f^\circ(\text{Hg}) = 82 \]
\[ f''(\text{Hg}) \approx 4.5 \quad \text{for} \quad \lambda < 1.0 \text{ Å} \]
Bijvoet ratio more realistically

\[
\frac{\Delta F}{F} \approx 3 - 6\% \text{ for Se} \\
\approx 2\% \text{ for P in DNA} \\
\approx 1\% \text{ for S}
\]
In 1985 B.C. Wang concluded (on the basis of the simulated, error-free data) that signal one S-S bridge in 120 a.a. protein is enough to solve its structure

\[ 2 \text{ S per 120 a.a. } \frac{<\Delta F>}{<F>} \approx 0.6 \% \]

\(0.6 \% = \text{so called Wang limit}\)

MAD is not possible for S or P since their absorption edges are at \(\lambda > 4 \text{ Å}\)
Partial structure of anomalous atoms

Anomalous atoms can be located by Patterson or direct methods, since:

\[ \Delta F^\pm = 2 \cdot F''_A \cdot \sin (\varphi_T - \varphi_A) = 2 \cdot F_A \cdot (f''/f^o) \cdot \sin (\varphi_T - \varphi_A) \]

\[ \Delta F^\pm \approx F_A \quad \text{for large Bijvoet differences} \]

anomalous atoms are mutually distant (even low resolution is “atomic”)
Two solutions for SAD (Single-wavelength Anomalous Diffraction)

If anomalous sites are known \((\Delta F^\pm, F_A, F'_A, F''_A, \phi_A)\)

there are two possible phase solutions
Selection of mean phase
Electron density map is then sum of correct structure and noise

\[ F_1 + F_2 \]

Iterative solvent flattening indicates correct phase
With errors in measured $|F^+|$ and $|F^-|$ and inaccurate anomalous sites, the phase indications are not sharp.
Bijvoet ratio vs. resolution

\[ \langle \Delta F^\pm \rangle / \langle F \rangle = (2 \frac{N_A}{N_p})^{1/2} \cdot (f_{A''}/Z_{\text{eff}}) \]

Four data sets from glucose isomerase

1 Mn + 10 S in 375 a.a.
SAD result - d(CGCGCG)₂

Hexamer duplex of Z-DNA

10 P among 12 bases

\[ \lambda = 1.54 \text{ Å}, \quad f''(P) = 0.48 \]

\[ \langle \Delta F^\pm \rangle / \langle |F| \rangle = 2.0 \% \]

resolution 1.5 Å

phased with SHELXD/SHARP
SAD result - xylanase

5 S among ~ 2570 atoms
\( \lambda = 1.49 \, \text{Å}, \quad f''(S) = 0.52 \)

\( \langle \Delta F^\pm \rangle / |F| = 0.56 \% \)

resolution 1.75 Å
phased with OASIS2004
SAD result - proteinase K

10 S + 1 Ca among in 279 a.a.

\( \lambda = 0.98 \text{ Å} \), \( f''(S) = 0.23 \)

\( \langle \Delta F^\pm \rangle / \langle |F| \rangle = 0.43 \% \)

resolution 1.3 Å

330 degrees of data

phased with SHELX/D/E

with 330...120°

failed with 90°

Mean phase error 27.5°
Weak anomalous signal

Very weak anomalous signal can be used successfully, but data have to be complete and measured very accurately, with $R_{\text{merge}}$ comparable with expected $\langle \Delta I \rangle / \langle I \rangle$

Resolution, anomalous scatterer type etc. are not so crucial
The only good indicator

only one satisfactory indicator of anomalous signal exists
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of anomalous signal exists:

successful structure solution
The only good indicator

only one satisfactory indicator of anomalous signal exists:

successful structure solution

3.0-\(\lambda\) approach (MAD, 1990)
1.5-\(\lambda\) approach (Dauter, 2002)
1.0-\(\lambda\) approach (SAD, nowadays)
0.5-\(\lambda\) approach (Minor et al., 2007)

structure can be solved when crystal is still at the beam line