Theoretical background

The program SHELX estimates initial native phases $\varphi$, by the simple yet effective approximation:

$$\varphi_f = \varphi_n + \alpha$$

where $\varphi_n$ is the phase calculated for the heavy (or anomalous) atom substructure and $\alpha$ is a phase shift (90 or 270° for SAD, general for MAD and SIRAS phasing). These initial phases are then improved by density modification and chain tracing [Sheldrick, 2010]. $\alpha$ and the heavy atom structure factor $|F_h|$ are estimated from the experimental data by SHELXC. SHELXD uses the $|F_h|$ values to solve the substructure.

ANODE

In the program ANODE [Thorn & Sheldrick, 2011] this procedure is reversed by applying:

$$\varphi_n = \varphi_f - \alpha$$

to obtain an estimate of the anomalous or heavy atom phase from the native phase, which is calculated from the known structure provided as a PDB file. A map calculated using phases $\varphi_f$ and amplitudes $|F_h|$ is often colloquially referred to as an ‘anomalous Fourier’. Such maps were probably first used by Strahs [1968]. However, this approach is equally valid for SIR, MAD and SIRAS data, for which a heavy atom density map might be a more appropriate description.

ANODE may be started from a command line containing a file-name stem and optionally one or more switches:

- **anode name**
- reads a PDB format file name.ent (or if that is not found name.pdb), and extracts the unit-cell, space group name and atom coordinates from this file. The file name_fa.hkl from SHELXC or XPAR is read to extract the reflection indices, $|F_h|$ and $\alpha$ values. In addition to writing the $\varphi_f$ and $|F_h|$ values to file in a format suitable for Coot [Emsley et al., 2010], ANODE prints a summary of the highest peaks in the map and the average heavy atom density for different types of atom. The origin of this map is always consistent with the input PDB file. If alternative reflection indexing is possible, ANODE prints a warning and can re-index the data automatically if required so that the reflection indexing is consistent with the PDB file.

**REFERENCES**


**Example 1: Anomalous signal from sulfur**

Weak anomalous scatterers, such as sulfur, can be clearly identified with ANODE, even when the anomalous differences are too noisy for a structure solution directly by sulfur-SAD (see Fig. 2, 3). In such cases the heavy atom sites found by ANODE can provide extra phase information that can be added (using SHELX) to the phases from a molecular replacement solution [MR-SAD, Schuermann et al. 2003].

**Example 2: Element types**

Despite the assumption that all anomalous scatterers are the same element, in practice other anomalous scatterers are also visible and by applying ANODE separately to the different wavelengths in a MAD experiment, from the relative peak-heights it is possible to make deductions about the elements present. In the example shown in Fig. 4, the peak-height ratio of the new site to that of the known zinc site was constant for the different wavelengths, in contrast to the behavior of the calcium sites, indicating that the new site is a partially occupied zinc atom.

**Conclusion**

Despite the rather drastic approximations made in this approach, e.g. that for SAD data $\alpha$ is restricted to 90 or 270° - or that only one type of anomalous scatterer is present, ANODE proves to be useful as a diagnostic tool, for example by confirming molecular replacement solutions by locating the sulfur atoms or distinguishing between water molecules and chloride ions in a refined structure.