



# Absolute Structure of L-Alanine

## NOVEL TECHNIQUES IN DETERMINING THE ABSOLUTE STRUCTURE OF LIGHT ATOM-CONTAINING MOLECULES

Determining the absolute structure of light-atom organic molecules is a developing field with several research groups working on novel statistical methods in data refinement. All of these methods require precise experimental determination of very small differences in the intensities of related reflections. With high intensity sources and ultra-sensitive, low noise detectors coupled with efficient data collection strategies Rigaku Oxford Diffraction's micro-focus SuperNova X-ray diffractometer is well suited to this field.

### THEORY

Using X-rays to assign chirality relies on anomalous scattering effects. These cause the intensities of reflections related by inversion symmetry (Bijvoet pairs) to differ due to the chirality of the sample. If a molecule contains a sulfur (or heavier) atom, the observable difference in anomalous scattering is relatively high (and greatly amplified by using Cu radiation) and so absolute configuration studies can be straightforward. For molecules which only contain elements lighter than sulfur, conclusive data becomes difficult to obtain, even for substances of known chirality. L-alanine,  $C_3H_7NO_2$ , is a good example of this and therefore makes a good test case.

The Flack parameter<sup>1</sup> is the universally accepted method of determining the quality of an absolute configuration result. For publication quality assignment of chirality, the Flack parameter must be close to 0 with an error (esd) of <0.1 (10%). In recent years, alternative methods such as the Hooft<sup>2</sup> parameter (employing Bayesian statistics) have been developed to provide statistically more conclusive results from more challenging samples.

**Simon Parsons**, professor of crystallography at the University of Edinburgh and a Rigaku Oxford Diffraction SuperNova user, developed a novel technique (now used in current versions of ShelXL) using quotient restraints. It has been shown that the highest resolution reflections contain the most anomalous scattering information. By selecting and weighting those 20 reflections which have the most influence on the quality of the absolute configuration refinement, it is possible to improve the quality of the result.



### EXPERIMENTAL

For challenging absolute configuration samples, high quality data is always required, with good quality crystals, high signal to noise ( $I/\sigma$ ) ratios and high redundancy factors. Redundancies of 10-15 give the best results for most light atom absolute configuration data, but using traditional fine-focus, sealed tube instruments, experiments of this type can be extremely long. However, with the micro-focus Cu Nova X-ray source on Rigaku Oxford Diffraction's SuperNova instrument, such experiments can be carried out in a matter of hours.

The data were collected in collaboration with Professor Parsons using a Rigaku Oxford Diffraction SuperNova dual wavelength (Mo, Cu) X-ray diffractometer with CrysAlis<sup>Pro</sup> data collection and refinement software. Measurements were conducted both on a SuperNova at our demo facility and on the SuperNova instrument installed at the University of Edinburgh.

Data were collected on L-alanine (a substance of known chirality). High quality crystals were used, and data were collected at 100K. A strategy for a high redundancy data set can be calculated in seconds using the strategy module in CrysAlis<sup>Pro</sup>, and for L-alanine, two data sets were collected; with redundancies of 5.3 and 14.9. The same exposure times were used for both data sets.

## RESULTS

The effect of high redundancy is extremely important, with significantly improved results for all refinement techniques. The error value associated with the Flack parameter is indicative but not conclusive, whereas both Hooft and Quotient techniques yield excellent quality results.

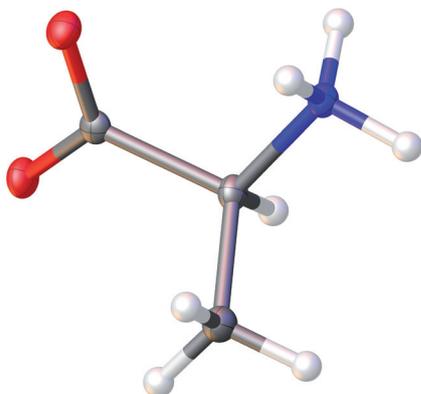


Figure 1 - The structure of L-alanine

Exp. Time (hrs)	4	16
Merged I/sig	70.39	176.77
Redundancy	5.3	14.9
Flack	-0.28(25)	0.06(21)
Hooft	-0.16(9)	0.06(5)
Quotient	-0.14(12)	0.08(4)



Figure 2 - The SuperNova diffractometer system

## CONCLUSIONS

The Nova micro-focus X-ray source combined with a high redundancy data collection affords absolute configuration results of excellent quality, even for light atom-containing samples such as alanine. Where the Flack parameter does not confirm a conclusive result, alternative techniques including the use of Quotient restraints can yield statistically more conclusive data.

Quotient restraints may allow the user to obtain conclusive absolute structure data from combining routine data with repeated measurement of a subset of high angle reflections. This would dramatically reduce the time involved in collecting such high redundancy data collections, making experiments of this type a more routine procedure.

## REFERENCES

1. Flack, H. D. (1983), *Acta Cryst.*, A39, 876–881
2. Hooft, R.W.W., Straver, L.H., Spek, A.L. (2008). *J. Appl. Cryst.*, 41, 96-103

## ACKNOWLEDGMENTS

We would like to thank Prof. Simon Parsons for contributing data, analysis and text to this application note.

## Rigaku Oxford Diffraction

9009 New Trails Drive, The Woodlands, TX 77381-5209  
Tel.: (281) 362-2300 | FAX: (281) 364-3628 | [www.Rigaku.com](http://www.Rigaku.com) | [info@Rigaku.com](mailto:info@Rigaku.com)