Use of xenon and krypton for phasing in protein crystallography

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Xenon derivatisation is classically performed with hydrophilic additives (e.g. glycerol) to prevent nucleation of ice when the cryoprotectant buffer containing the crystal is flash cooled. The difficulties of obtaining a suitable cryoprotectant buffer, in which crystals are stable for a period of several minutes are, however, rarely reported. Crystals of porcine pancreatic elastase have been used to demonstrate that cryoprotection using dry paraffin oil [1] or Panjelly^{TM} allows with equal or better efficacy derivatisation under a xenon atmosphere prior to shock cooling [2].

Although the xenon *K* or *L*-edges are not readily accessible, the anomalous signal of xenon is appreciable even at remote energies. Phasing procedures for xenon derivatised porcine pancreatic elastase crystals, using data sets measured at three wavelengths easily accessible on a tunable beamline, have been investigated. The importance of highly redundant data in measuring precise anomalous differences is demonstrated and it is shown that an SIRAS procedure yields a better phase set than those generated by SAS or pseudo-MAD procedures [3].

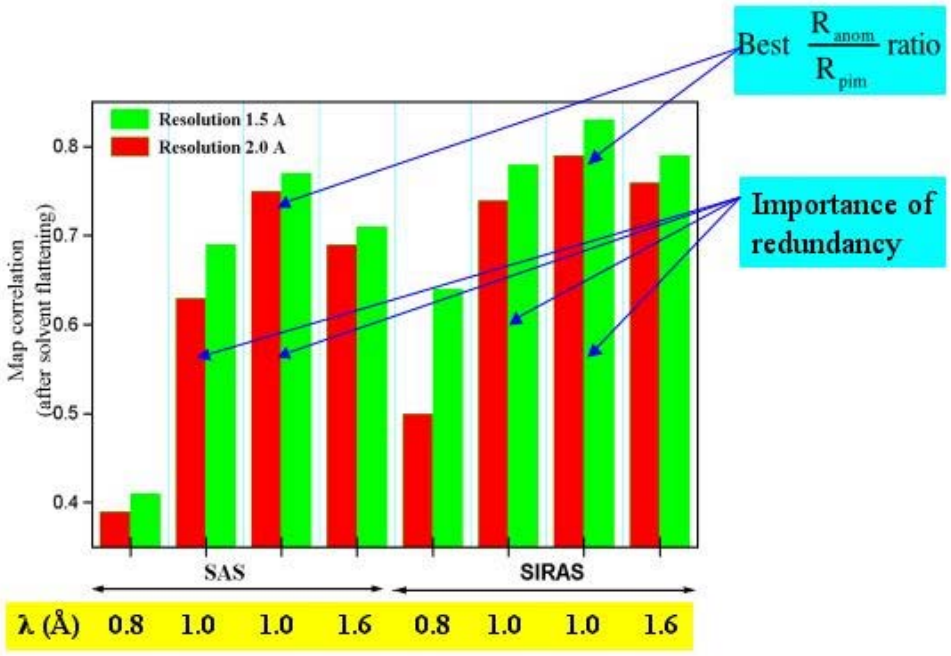

Figure 1: Indication of the efficacy of phasing [2] using data sets of xenon derivatised porcine pancreatic elastase measured at different wavelengths with different redundancies.

Crystals derivatised by soaking in bromide solutions have been subsequently derivatised under a xenon atmosphere. Intensity data collected below the bromine absorption edge is used to determine the xenon position and the resultant phase information used to determine the bromine substructure from data collected above the bromine absorption edge. This method would appear to have general applicability where large substructures need to be determined[4].

The the time course for loss of xenon and krypton from porcine pancreatic elastase has been investigated (Figure 2) and it clear that if the trends observed also apply to other proteins that it is not necessary to shock cool crystals under pressure of noble gas , because even for krypton the outgassing rate is sufficiently slow to retain useful derivatisation.

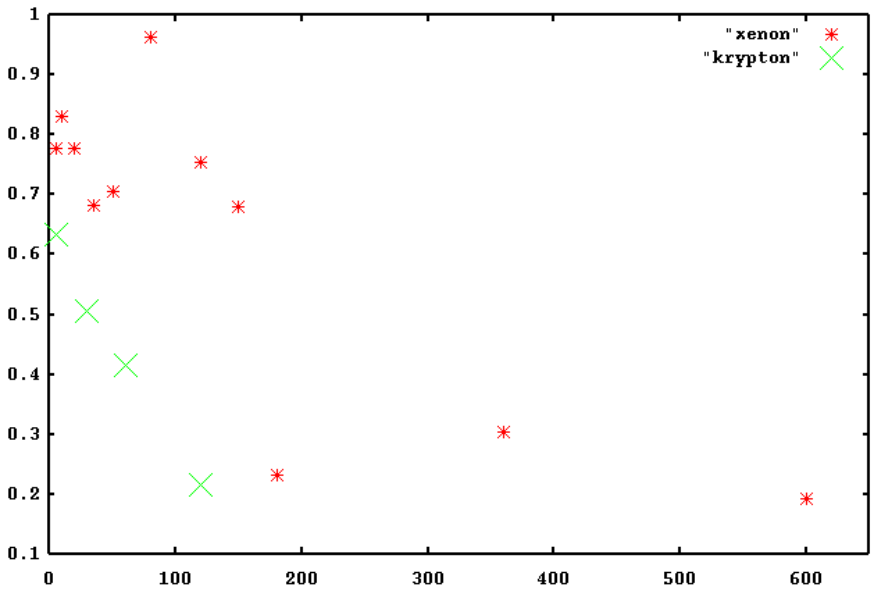

Figure 2: Desorption of xenon and kryton from porcine pancreatic elastase as measured from the refined occupancy of the noble gas in the crystal structure

The data used in these studies has been measured at various times during the year on the EMBL hamburg beamlines X11, X13, BW7A and X31.

Reference

[1] A. Riboldi-Tunnicliffe, A. & R. Hilgenfeld. J. Appl. Cryst. 32, 1003 (1999)
[2] S. Panjikar & P.A. Tucker. J. Appl. Cryst. 35, 117 (2002).
[3] S. Pajikar & P.A.Tucker, J. Appl. Cryst., 35, 261(2002)
[4] S. Panjikar & P.A. Tucker, Acta. Crsyt . D58, 1413 (2002)