1 Crystallisation and Derivatisation

Chemicals: Hen egg-white lysozyme (M ≈ 14600 g/mol, Fluka cat. no. 62970)
CH_3COONa (M = 82.03 g/mol, Sigma cat. no. S2889)
CH_3COOH (M = 60.0 g/mol, Sigma cat. no. 537020)
NaCl (M = 58.44 g/mol, Sigma cat. no. S7653)
Ethylene glycol (M = 62.07 g/mol, Merck, cat. no. 109621)
$KAuCl_4$ (M = 377.88 g/mol, Aldrich, cat. no. 33,454-5)
Milli-Q water

Tetragonal crystals of HEWL were grown as described by Weiss *et al*. (2000) by mixing 4 µl of protein solution (30 mg/ml in water) and 4 µl of reservoir solution containing 50 mM Na acetate pH 4.5 and 5% (w/v) NaCl and equilibrating the drop against the reservoir. The crystals belong to space group $P4_32_12$ (space group number 96) and exhibit the usual unit-cell parameters of a = 78.8 Å and c = 37.2 Å (Figure 3). They appeared within few days after setting up the experiment. Prior to flash cooling to 100 K, they were transferred into a solution containing 25% (v/v) ethylene glycol, 10% (w/v) NaCl and 100 mM Na acetate pH 4.5. They typically diffracted X-rays to a resolution better than 1.6 Å.

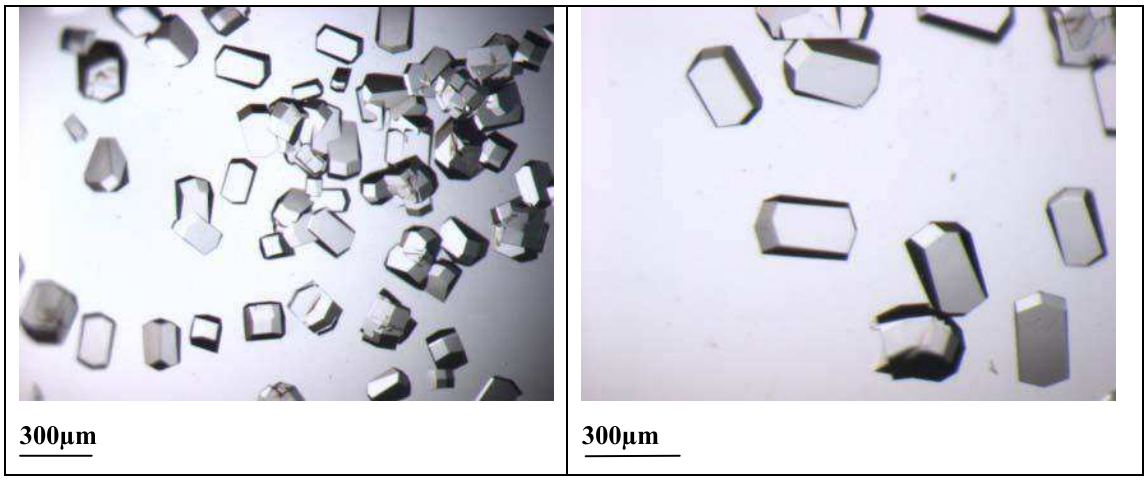

Figure 3: Tetragonal HEWL crystals.

A 10 mM solution of $KAuCl_4$ in reservoir solution was freshly prepared and one crystal was soaked in this solution for 1 minute (Sun *et al*., 2002). This crystal was then also cryo-protected in a solution containing 25% (v/v) ethylene glycol, 10% (w/v) NaCl and 100 mM Na acetate pH 4.5. The diffraction properties of such derivatized crystals are significantly worse than the ones for the native crystals but still very much acceptable (Figure 4).