D-xylose isomerase (XI; D-xylose ketol-isomerase, EC 5.3.1.5) is a metal-ion dependent bacterial enzyme that catalyses the conversion of D-xylose to D-xylulose and D-glucose to D-fructose[1]. The catalytic mechanism of XI is believed to proceed in four steps [2]: 1) preferential binding of the α-anomer 2) ring opening, 3) extension of the substrate and, finally 4) metal-mediated hydride-shift [3]. Despite the extensive crystallographic studies on the catalytic mechanism of XI, only the linear forms of glucose or xylose have been observed so far in substrate-soaked crystals. Insights into the orientation of the substrate have been obtained by using thio-α-D-glucopyranose (THG), a closed-ring sugar analogue[4]. No crystal structure has been reported for the closed-forms of the natural substrates (glucose and xylose) of xylose isomerase.

In order to gain further insight into the catalytic mechanism of the enzyme, particularly of what is happening during the first moments of the reaction we have collected high resolution (<1.5 Å) data in the presence of glucose after soaking. Data were collected on station X11 at EMBL Hamburg using the MARCCD detector. Difference electron density maps showed binding of glucose in its cyclic form. Longer soaking times and possibly different concentrations may be required to get a complete picture of the reaction. Data from xylose-soaked crystals have been collected on stations X11 and X13 under different conditions but no binding of the cyclic form has been seen yet.

During the course of our studies, we examined crystals of XI grown in the presence of PEG 400. These crystals are quite sensitive and disintegrate after 3-4 days. A data set to 1.5 Å was collected and the structure has been refined. The structure has shown the presence of a PEG 400 molecule on the surface of the protein close to the active site of the enzyme and to the crystal contacts. Detailed inspection of the structure and comparison with the atomic resolution structure of XI is currently underway to understand better the behavior of the crystals and the role of different precipitants in crystallisation.
References