Programs:

The CRANK pipeline
(ShelxC, ShelxD, SFTools, Ecalc, REFMAC5, Peakmax, Parrot, Buccaneer)

Scenario

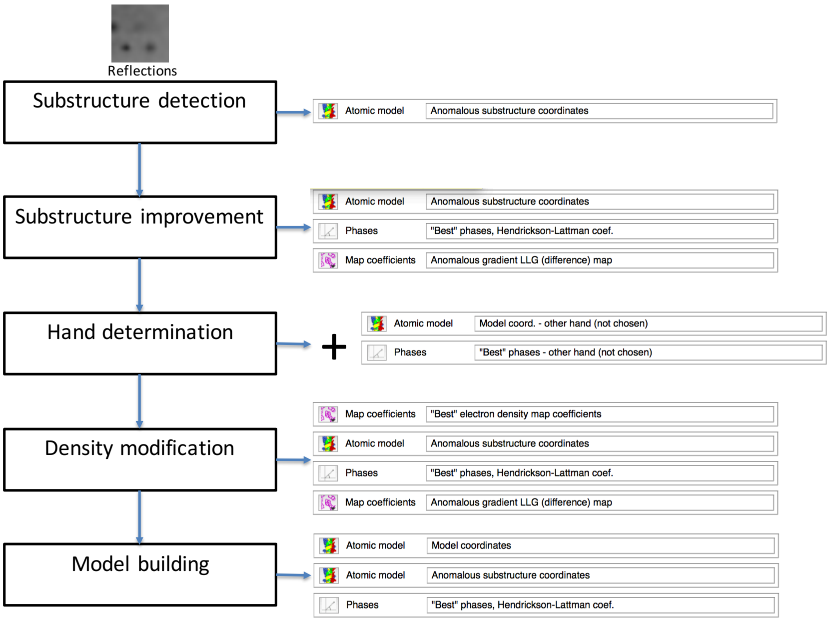
Adapter proteins of the AP2 complex select membrane protein cargo for capture into vesicles. The gamma adapter binds to peptide motifs on, amongst others, the EGF receptor, resulting in receptor internalization and recycling. Recognition of the cytoplasmic tail of EGFR is mediated by the ear domain, which was crystallized and into which xenon atoms have been introduced by pressurization of the crystals prior to crash cooling. A SAD dataset was collected on a home source from these crystals.

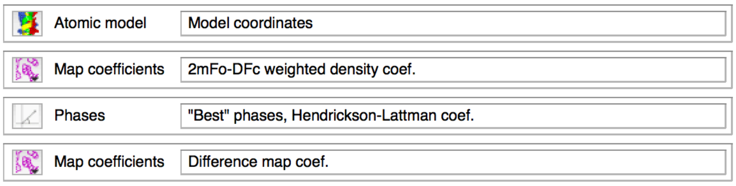
Data location

Data for this tutorial can be downloaded into a new CCP4i2 project using the Utilities menu:

Utilities-."Download test data"->gamma. Files are then available in a subdirectory of your project's top directory

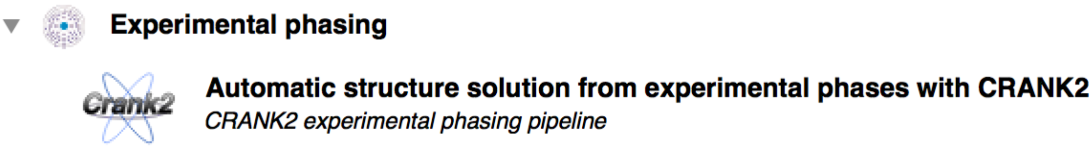
Task 1: Run CRANK to solve the structure





Atomic model	Model coordinates
Map coefficients	2mFo-DFc weighted density coef.
Phases	"Best" phases, Hendrickson-Lattman coef.
Map coefficients	Difference map coef.

Task location



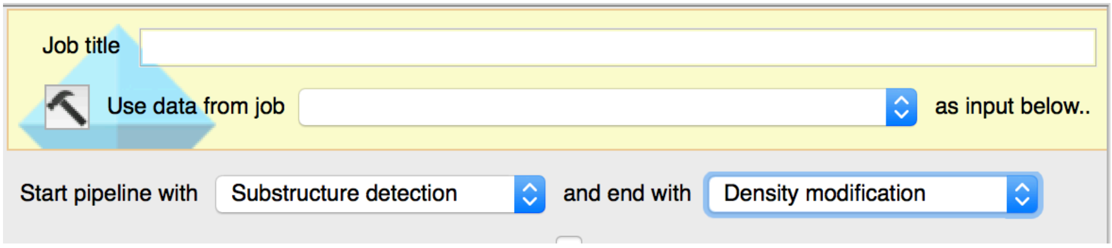
Experimental phasing

Automatic structure solution from experimental phases with CRANK2

CRANK2 experimental phasing pipeline

All you need to provide is the SAD data, a sequence file, and the identity of the anomalous scatterer.

Task 2: Run the CRANK pipeline part way …

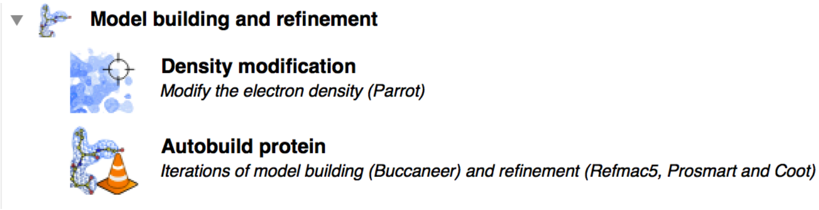


Job title

Use data from job as input below..

Start pipeline with Substructure detection and end with Density modification

Task 3: … and pick up with other tools



Model building and refinement

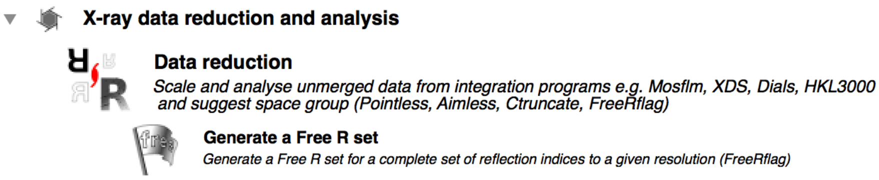
Density modification

Modify the electron density (Parrot)

Autobuild protein

Iterations of model building (Buccaneer) and refinement (Refmac5, Prosmart and Coot)

The autobuild task crashes because the FreeR set that comes out of the CRANK pipeline is not quite complete. To generate a truly complete Free R set, you will need to use the CCP4i2 FreeR set (or load one in that wou have created another way !)



X-ray data reduction and analysis

Data reduction

Scale and analyse unmerged data from integration programs e.g. Mosflm, XDS, Dials, HKL3000 and suggest space group (Pointless, Aimless, Ctruncate, FreeRflag)

Generate a Free R set

Generate a Free R set for a complete set of reflection indices to a given resolution (FreeRflag)