Par-4 (Prostate apoptosis response factor 4) is a tumor suppressor protein with pro-apoptotic function. Sequence analysis and secondary structure predictions indicated the presence of SAC domain with a nuclear localization sequence (NLS) which is of pharmaceutical interest as it induces apoptosis selectively in cancer cells but not in normal cells and a coiled coil (CC) domain with leucine heptad repeat having wide range of interactions with other proteins. Its direct involvement in the intrinsic and extrinsic pathways with a SAC domain and an interaction domain i.e., CC domain emphasized the importance of understanding the structure function of the protein. Previously it was shown to be an intrinsically disordered protein. Apparently the full length Par-4 does not fold properly and is subsequently prone to aggregation. Therefore the protein was divided into two functional domains which are investigated separately. The structural insight into this protein is desired since it is expected to aid in understanding its various functions, especially its selective induction of apoptosis in cancer cells.

The CC domain crystals (Figure 1) are extremely sensitive to exposure. Multiple wavelength anomalous diffraction (MAD) data sets with 2.7Å resolution were collected. The crystal lattice exhibits a tetragonal symmetry. Data integration, scaling and heavy atom phasing was successful in spacegroup P4_3212. Model building and refinement resulted in a model with R_free of 0.29. One of the molecules in the asymmetric unit that forms a crystallographic dimer and one of the non-crystallographic dimers have very high B-factors in comparison with other molecules in the structure. Considering this information as a possible indicator of some pathology, the data sets were also analyzed in non-isomorphic subgroups of P4_3212 i.e., P4_3, P2_1212_1 and C2221. In these subgroups one crystallographic symmetry element of P4_3212 must be replaced by a non-crystallographic symmetry element. C2221 was later excluded as no heavy partial structure solution could be found. In the remaining space groups model building and the refinement also resulted in the model with R_free ~0.29. It appears that the difference between the different spacegroups is not clearly detectable because of the data resolution.

Removal of the disordered N-terminal region did not yield crystals that diffract to higher resolution, therefore the space group ambiguity still remains.

Figure 1: CC domain crystal

References