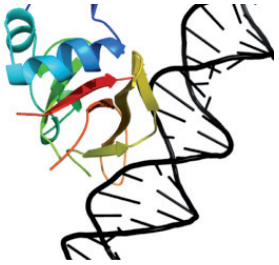
In this study, we present the X-ray structure of the NAC:DNA complex to 4.4 Å. The structure was solved with molecular replacement (MR) using as search models the apo-structure of NAC (1UT4) and a B-DNA model of the oligonucleotide (X.-J. Lu and W. K. Olsen (2003) Nucleic Acids Res 31(17):5108-5121). Refinement proved complicated due to the low resolution. The best strategy turned out to include NCS restraints on all atoms, reference-model restraints on protein atoms, and TLS. Phase restraints were available but did not improve refinement. Neither did simulated annealing.

The exact position of the individual DNA bases along the main DNA axis could not be determined by MR alone. Instead, the DNA sequence was assigned based on a uranyl photoprobing assay. The resulting model could be refined to R_{work}=24.1% and R_{free}=34.4%.

The structure provides for the first time experimental evidence for the speculated evolutionary relationship between the plant-specific NAC proteins and other transcription factors with a surprising phylogenetic breath. The structure shows how the NAC domain inserts the edge of its core beta-sheet in the major groove (figure), while leaving the DNA largely un-distorted. Recognition of DNA with the edge of a beta-sheet is also believed to be a feature of the WRKY family of transcription factors (Rushton et al. (2010) Trends Plant Sci 15(5):247-258), which are found in early eukaryotes and plants. Further, the animal GCM transcription factors use the same binding motif (Cohen et al. (2003) EMBO J 22(8):1835.1845). In addition, these three transcription factors share a core beta-sheet with a very similar topology.

The presented NAC:DNA complex structure provides a framework for studying the effects of single amino acids as well as structural features on DNA binding affinity and specificity. Further, we have evidence of limited flexibility of the NAC dimer arrangement, which could explain the limited tolerance in NAC binding site spacing that has been reported (Olsen et al. (2005) Plant Sci 169:785-797, Xue (2005) Plant J 41:638-649)



Keywords: transcription factor, NAC, low resolution

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Optimising low resolution structural biology techniques

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The investment in modern equipment and the development of highly automated beamline control software on the public MX-beamlines at the ESRF now allows standard X-ray diffraction experiments, even multiple anomalous diffraction (MAD), to be easily and robustly performed. However the success of X-ray diffraction experiments is still dependent on the quality of the crystals obtained. Most modern structural biology projects have become ever more complex and their success is now often dependent on a combination of low resolution techniques such as EM, X-ray small angle scattering and macromolecular crystallography. Many large and complex macromolecular assemblies often fail to crystallise or at best form few poorly diffracting crystals that are radiation sensitive. Such projects require newly developed equipment and a much more careful approach for data collection [1, 2]. In Grenoble we are developing improved instruments and methods for optimised low resolution data collection possibilities. Here I will present our current abilities and some future developments for new and challenging structural biology experiments.

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Preferential enrichment of cocrystals of amino acids and achiral dicarboxylic acid classified as racemic compound

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Preferential enrichment is a symmetry-breaking chiral separation phenomenon that is initiated by the solvent-assisted solid-to-solid transformation of the first-formed metastable polymorphic form into a thermodynamically stable one during crystallization from the supersaturated solution of certain kinds of racemic mixed crystals (i.e., solid solutions or pseudoracemates) composed of two enantiomers in organic solvents without the aid of any external chiral element [1].

We anticipated that the preferential enrichment phenomenon might be observed even for a so-called 'racemic compound', if the following three requirements are satisfied: i) The solubility of the enantiopure sample is higher than that of the corresponding racemic sample. ii) Upon recrystallization of racemic or nearly racemic sample from organic solvents under kinetic conditions, a solid-to-solid polymorphic transition of the initially-formed metastable mixed crystals composed of the heterochiral 1D *R* and *S* chains into the stable mixed crystals mainly comprising heterochiral (*R,S*) chains occurs. iii) In the resulting deposited crystals, the fairly random arrangement of two enantiomers can be retained, without undergoing further solvent-mediated polymorphic transition to give exact racemic compound crystals.

Recently, we reported that other racemic crystals having a regular packing of a pair of *R* and *S* enantiomers in their crystal could also display the 'preferential enrichment' phenomenon. We have found that neutral amino acids, alanine and leucine, exhibited a very similar phenomenon to 'preferential enrichment' [2]. Here we report the successful enantiomeric resolution of other amino acids, such as phenylalanine, histidine, and cysteine with a racemic compound structure, which could be spontaneously resolved into its enantiomeric components by co-crystallization with achiral dicarboxylic acid. For example, repeated recrystallization of the cocrystals of DL-phenylalanine and fumaric acid from the 6-fold supersaturated aqueous solution led to a remarkable alternating enrichment of the two enantiomers up to 85% *ee* in the mother liquor, together with slight enrichment (< 6% *ee*) of the opposite enantiomer in the deposited crystals [3]. The mechanism of preferential enrichment is proposed on the basis of i) the observation of polymorphic transition during crystallization by in situ ATR-FTIR and Raman spectroscopy, ii) the characterization of deposited crystals by X-ray crystallographic analysis and powder XRD measurement, and iii) the optical microscopic and AFM observations of the crystal shape and surface, respectively.

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