	Experiment title: chiral PNA-DNA duplex	**Experiment number**:3
Beamline: ID14-1	**Date of experiment**: from: 11-02-2002 to: 12-02-2002	**Date of report**: 26/7/02
Shifts: 2	**Local contact(s)**: Sigrid KOZIELSKI	*Received at ESRF:*

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Report:

Peptide nucleic acids (PNAs) are nucleic acid analogues in which the sugar-phosphate backbone has been replaced by a pseudo-peptide skeleton, made up of N-(2-aminoethyl)glycine units.

PNAs oligomers recognize with high specificity and selectively DNA and RNA sequences, leading to RNA-PNA and DNA-PNA hybrids more stables than the corresponding regular nucleic acid complexes. The high thermal stability and the high resistance to proteases and nucleases make PNAs ideal candidates as anti-sense or anti-gene therapeutic. Several variants of the basic PNA structure were proposed in order to improve the binding specificity, solubility and uptake into cells. Among those Sforza and coworkers [1] reported the synthesis of a chiral PNA decamer containing three adjacent chiral monomers based on D-lysine, in the middle of the PNA sequence. Binding studies demonstrated that this 10-mer PNA hybridizes with the complementary DNA sequence only in the antiparallel mode, and that its ability to discriminate between a full-match and single-mismatched DNA strands was greatly enhanced compared to other achiral or chiral PNA. Thus, it possesses many of the properties required for the detection of point mutation in diagnostics, and for the development of gene therapeutics.

To the best of our knowledge, no structure of complexes between chiral PNA and DNA has been reported so far. In order to understand at the molecular level the role of chirality in DNA recognition and to investigate the structure-activity relationships of chiral PNAs, we have undertaken a crystallographic study on a PNA decamer (GTAGGAD-LysTD-LysCD-LysACT) containing a chiral box, hybridized with its complementary antiparallel DNA strand (5'-AGTGATCTAC-3'). We already collected diffraction data to 1.85 Å resolution on this duplex [2]. Recently on beam line ID14-1 at ESRF we were able to improve the resolution to 1.5 Å. The crystals belong to the

$P3_1$ space group or to its enantiomorph $P3_2$. A complete data set at 100 K has been collected using one flash cooled crystal. The scaling gave a final R_{symm}=5.2 % for a completeness of 99.5 %. The unit cell dimensions at 100 K are *a* = *b* = 35.00 Å, and *c* = 35.91 Å. A solvent content of 41.7 % was calculated by the methods of Matthews, assuming the crystals to have one duplex per asymmetric unit (Vm = 2.11 A^3/Da).
Several attempts to solve the structure by molecular replacement have failed; thus we are preparing a brominated DNA-PNA duplex in order to determine the structure by Mad techniques.

References

1 Sforza, S., Corradini, R., Ghirardi, S., Dossena, A. & Marchelli, R. (2000). Eur. J. Org. Chem. 2905-2913.

2 V. Menchise, G. De Simone, R. Corradini, S. Sforza, N. Sorrentino, A. Romanelli, M. Saviano and C. Pedone. *Acta Crystallographica section D,* in corso di stampa