	Experiment title: The crystal structure of a D-lysine based Pna-Dna complex	**Experiment number**: LS-2199
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Report:

Peptide nucleic acids (PNAs) are oligonucleotide mimics in which the sugar-phosphate backbone has been replaced by a pseudo-peptide skeleton, composed of N-(2-aminoethyl)glycine units. Nucleobases are linked to this skeleton through a two atom carboxymethyl spacer.

PNAs bind DNA and RNA with high specificity and selectivity, forming Watson-Crick base pairs and leading to RNA-PNA and DNA-PNA hybrids more stable than the corresponding nucleic acid complexes. Because of their high thermal stability and resistance to proteases and nucleases, PNAs are ideal candidates as anti-sense or anti-gene therapeutic agents and are currently used as powerful tools in molecular biology and in diagnostics.

PNAs can bind to complementary DNA strands in both parallel and antiparallel orientations and are poorly soluble in physiological conditions, thus preventing the development of PNA based therapeutics. In order to improve the binding specificity, solubility and uptake into cells, several modifications of the basic PNA structure were proposed. A particular approach involves the introduction of stereogenic centers, most commonly based on chiral aminoethyl amino acids. By circular dichroism experiments it has been demonstrated that the configuration of the stereogenic centers induces preferential PNA helicity: in particular, PNAs containing monomers derived from D-amino acids (D-PNAs) induce a preferred right-handedness in the PNA:PNA duplexes, while L-PNAs induce left–handedness. As a consequence of this preferential handedness, right-handed DNA was found to bind to D-PNAs with higher affinity than L-PNAs.

In a general research project, aimed at understanding the role of chirality in DNA recognition, and at achieving insights into the structure-activity relationships of chiral PNAs, we solved the crystal structure at 1.66 Å resolution of a chiral PNA-DNA duplex (LPD), by a single wavelength anomalous diffraction experiment.

A native data set at 1.66 Å resolution was collected at the ESFR in Grenoble using one crystal flash cooled at 100 K from a precipitant solution containing 10% glycerol. Crystals belong to the space group $P3_1$ with one molecule per asymmetric unit (see Table 1).

In order to determine the structure by a single wavelength anomalous diffraction (SAD) experiment, a DNA strand containing two 5-bromouracil has been synthesized and a Br-derivative PNA-DNA duplex has been prepared. The SAD data at 1.75 Å

resolution were collected around the absorption Br K edge on a Mar CCD detector at Synchrotron source Elettra in Trieste, using one crystal flash cooled at 100 K from a precipitant solution containing 15% glycerol. Crystals belong to the space group P31 with one molecule per asymmetric unit and were isomorphous with native crystals. The data sets from the native and Br-derivative PNA-DNA were processed using the HKL crystallographic data reduction package. Determination and refinement of the two bromide sites and phase calculations were carried out with the program SOLVE, using data between 20.0 and 2.0 Å resolution. The structure was then refined against the native data set using CNS.
Several cycles of simulated annealing, minimization and B factor refinement, followed by manual model rebuilding using the program O, reduced the R_{factor} and R_{free} values for all the data in the resolution range from 20.0-1.66 Å to 0.196 and 0.230, respectively. The statistics of the refinement are given in Table 1.

Table 1

Crystal data	**Native**
Space group	$P3_1$
a (Å)	34.94
c (Å)	35.80
Independent molecule	1
Diffraction data	
Resolution limits (Å)	20.0-1.66
Total reflections	46117
Unique reflections	5617
Completeness (%)	
Overalll	97.8
Outermost data shell	95.1
I/σ(I) All	
Overall	15.4
Outermost data shell	5.1
Rmerge (%)[1]	
All	6.0
Outermost data shell	18.5
Refinement	
Reflections used	5429
Resolution (Å)	20-1.66
Rfactor (%)[2]	19.6
Rfree (%)[2]	23.0
rmsd from ideal geometry:	
Bond length (Å)	0.011
Bond angles (°)	1.1
Average B factor ($Å^2$)	
Pna strand	22.35
Dna strand	25.65
water molecules	37.06

[1] $R_{merge} = \Sigma|<I> - I| / \Sigma<I>$; over all reflections
[2] $R_{factor} = \Sigma|F_O - F_C| / \Sigma F_O$; R_{free} calculated with 5% of data withheld from refinement