ESRF	**Experiment title:** HNA-RNA, a new antisense construct	**Experiment number**: LS 1484
Beamline: BM 30	**Date of experiment**: from: 25.11.99 to: 27.11.99	**Date of report**:
Shifts: 3 Shifts	**Local contact(s)**: Philippe Carpentier (Pluo A)	*Received at ESRF:*

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Report:

Antisense oligonucleotides have become a major research tool for life sciences during the last years. Great effort is made to establish antisense technology in human medicine. Currently, a large number of different modified oligonucleotides is being synthesized and tested for their antisense properties in *in vitro* systems. To rationalize the future design of a group of promising antisense oligonucleotides (Vandermeeren et al., 2000, Flores et al., 1999) the structure of a hexitol-nucleic acid was to be determined in this experiment. The evolutionary preference for five-membered sugar rings in nucleic acids has been discussed for a long time. The structure of a hexitol-nucleic acid containing a six-membered sugar alcohol ring is expected to provide new insights to possible conformational organization of nucleic acids.
Data were collected at BM30 for a native hybrid duplex of a decameric HNA strand and the complementary RNA, the active form of HNA as an antisense agent. For phasing a 4-wavelength MAD dataset was recorded for the same HNA-RNA duplex modified by incorporating a single brominated uridine in the RNA strand. Optimal wavelength for MAD measurements were determined using a XAFS scan of the same crystal that was used for data collection. The crystals were transferred from mother liquor to Paratone N and flash-frozen. Data were recorded under cryogenic conditions on a MAR Image plate to 2.6 Å resolution for both the native and the MAD dataset. The native dataset is 99.4 % (95.5 %) complete, with

I/ = 2.1 for the highest resolution shell and R_{sym}=0.06. The spacegroup was $P4_12_12$ with cell dimensions of a = b = 114 Å and c = 55 Å.
Phasing was carried out with *mlphare*, MAD phases were improved with *dm*. For refinement of the structure *cns* was used. The structure was refined to a R / R_{free} = 0.22 / 0.25 at 2.6 Å resolution. The asymmetric unit contains 1688 oligonucleotide atoms in four hybrid duplexes of identical composition and 166 water atoms. The average rmsd between the four duplexes is 1.2±0.2 $Å^2$. The differences between the individual duplexes are due to crystal packing effects that lead to local structural differences at interaction sites of two duplexes. The average atomic B-factor for nucleic acid atoms is 47 $Å^2$. Large variations in the atomic B-factor are observed between the individual duplexes (30 $Å^2$ to 60 $Å^2$) and between different regions within one duplex, but there are only minor deviations of the B-factors of base-paired HNA- and RNA-strands. In a few regions, mainly at the end of duplexes, alternate conformations or stronger disorder is observed.
The overall helical structure of the HNA-RNA hybrid duplex is an A-form helix in its A`-subform concluded from a helical rise of around 2.6 Å, a helical twist of less or equal than 31° for three out of the four duplexes and a displacement of basepairs in x-direction of around 5 Å. All duplexes are composed of completely and regularly basepaired strands. Despite the resolution of only 2.6 Å and the high B-factor in some regions of the molecules a considerable number of water molecules could be detected. A general pattern of hydration (first shell water molecules) revealed differences in the hydration of the HNA- and the RNA-strands, that could be confirmed from diffraction data for crystals with lower average B factor recorded in-house. In summary, evidence was obtained for the stability and ordered structure of a nucleic acid analogue with a six-membered ring instead of a ribosyl-group. Correlation of the structure determined here with biochemical properties of HNA is expected to provide guidelines and ideas for the design of improved antisense agents.

References:

Vandermeeren M., Preveral S., Janssens S., Geysen J., Saison-Behmoaras E., Van Aerschot A. and Herdewijn P. (2000) Biological activity of hexitol nucleic acids targeted at Ha-ras and intracellular adhesion molecule-1 mRNA. *Biochem. Pharmacol.* **59**, 655-663.

Flores M.V., Atkins D., Stewart T.S., van Aerschot A. and Herdewijn P. (1999) Antimalarial antisense activity of hexitol nucleic acids. *Parasitol. Res.* **85**, 864-866.