Packing of all A-T oligonucleotides in crossed layers

Francisco Acosta-Reyes[1], Raquel Sánchez-Giraldo[1], Lucy Malinina[2], Joan Pous[3], Núria Saperas[1], Juan A. Subirana[1], J. Lourdes Campos[1]*

[1] Departament d'Enginyeria Química, Universitat Politècnica de Catalunya, Barcelona, Spain.
[2] Unidad de Biología Estructural - Derio, CICbioGUNE, Vizcaya, Spain.
[3] Plataforma Automatitzada de Cristal.lografia, Institut de Recerca Biomèdica de Barcelona, PCB-CSIC, Barcelona, Spain
*email: lourdes.campos@upc.edu http://macrom.upc.edu

Summary

The major importance of DNA relies on the storage of information in genes to produce proteins but it is not its only role. Non-coding DNA first was considered as junk DNA, without any apparent function. Non-coding DNA represents a larger portion of genomes than exon regions. It has high adenine and thymine content. Now we know that some non-coding regions play an important role in regulation, for example by transcription of iRNA. However many features of non-coding DNA remain unclear. Therefore it appears of interest to study A-T rich oligonucleotides at the atomic structural level.

In this work we present the structure of two related 100% AT oligonucleotides d(AATAAATTTATT) and d(AATAATTATT). Crystals of all-AT oligonucleotides usually have a low resolution. In the presence of guanine intermolecular contacts are stabilized by hydrated divalent cations which help to stabilize the crystal lattice.

In the case of the decamer, we had previously reported the structural features of several crystals, which show the common arrangement of A-T rich oligonucleotides by forming parallel columns of stacked oligonucleotides. The crystal structure we present here was obtained in the presence of H-PRGRP-NH_2. It has a large unit cell with approximately 24 duplexes in the asymmetric unit, which are also organized as columns of oligonucleotides organized in two directions in space.

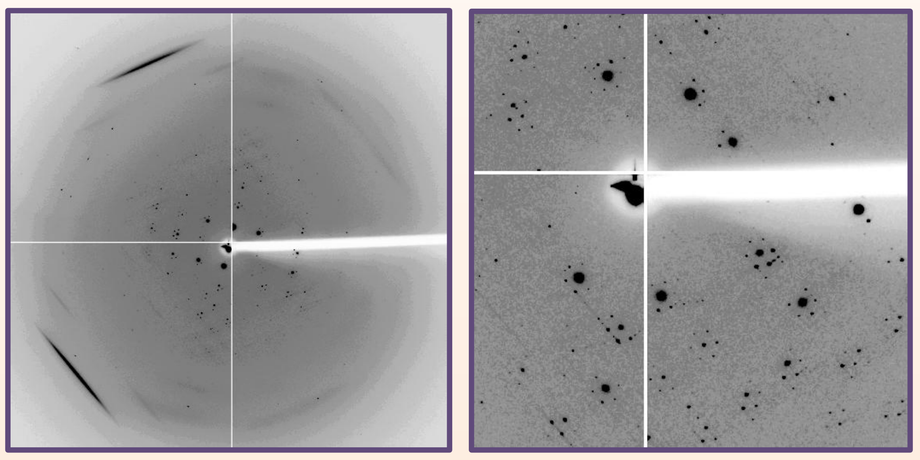
The dodecamer forms intercalated layers of columns with a shift to its neighbors. Two crystal forms are found, with a different angle between neighbor columns of DNA oligonucleotides.

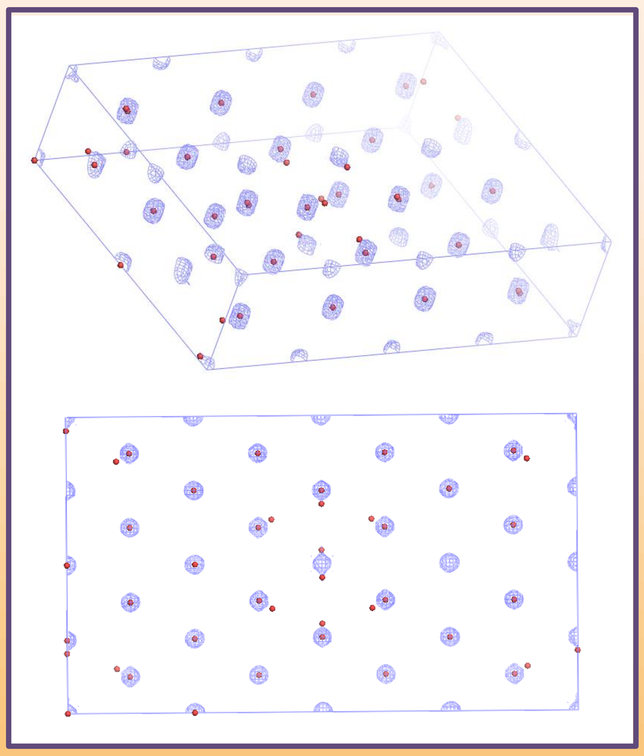
Knowing the particularities in the DNA packing and its relation to DNA sequence is important for future applications of DNA storage and also to understand the DNA packing in chromosomes.

d(AATAATTATT)$_2$

Crystallographic data	
Crystallization conditions (Hanging drop)	Oligonucleotide: 0.3 mM AATAATTATT Peptide: 1.3 mM PRGRP 5 mM $MgCl_2$, 0.25 mM $NiCl_2$, 25 mM Tris-HCl pH= 7.5 (MPD) 4ºC
Resolution	5.5 Å
Space group	C2
Unit-cell parameters	a=148.8 Å, b=251.8 Å, c=51.0 Å β=103.7º
Contents of ASU (approx)	24 duplexes (96 in cell)

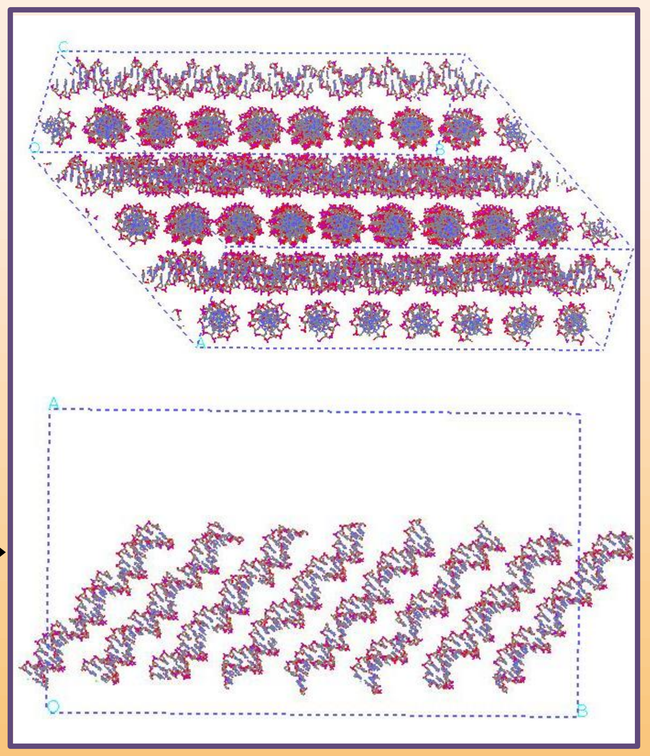
Two diffraction patterns of the crystal (12º oscillation). An enlarged view is shown in one of them. Strong spots are surrounded by weak spots. The strong spots can be indexed in a smaller subcell with a'=a/2 and b'=b/4.



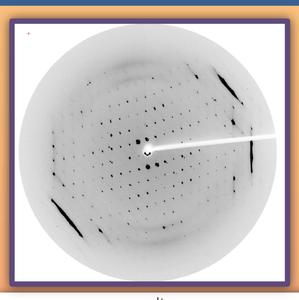


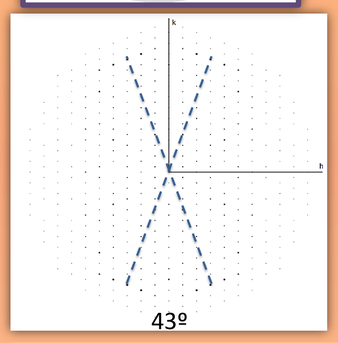
Patterson density maps. A perspective view and a projection onto the *ab* plane are shown. The map clearly shows that the structure has an internal subcell.

Tentative model of the AATAATTATT crystal. Views of the asymmetric unit and a whole cell. Parallel columns of duplexes form the structure. They are organized in layers which cross at about 80º. Each individual duplex has a different rotational setting.



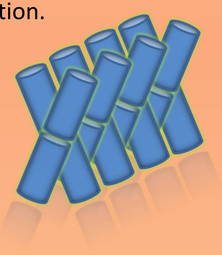
43º Crossed d(AATAAATTTATT)$_2$ 80º Crossed

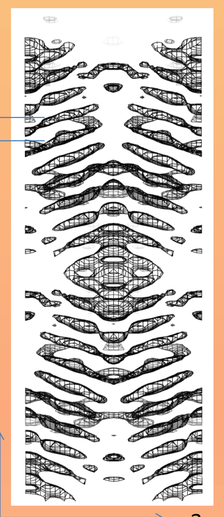




Clusters of high intensity diffractions about 3.26 Å from stacked oligonucleotides in two directions. The approximated cross angle between the two stacking is 43 degrees.

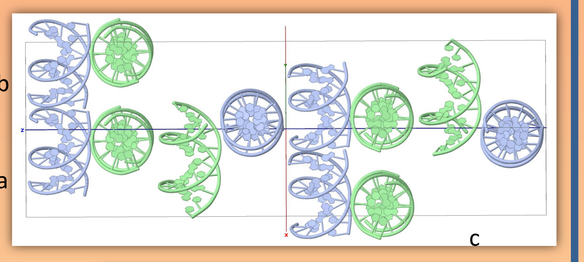
Patterson map shows the two orientations of oligos in the cell and the basepairs from 2 stacked oligonucleotides in each direction.



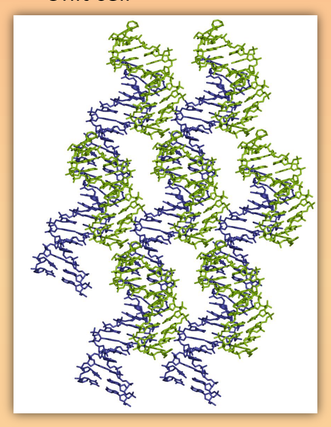


Schematic model of the unit cell packing.

Crystallographic data	
Crystallization conditions	Oligonucleotide: d(AATAAATTTATT)$_2$ Ion: $NiCl_2$ Precipitant: MPD
Resolution	3 Å
Space group	C 2 2 21
Unit-cell parameters	a=28.06 Å, b=72.66 Å, c=156.47 Å α = β = γ = 90 º
Contents of ASU	2 duplexes (16 in cell)

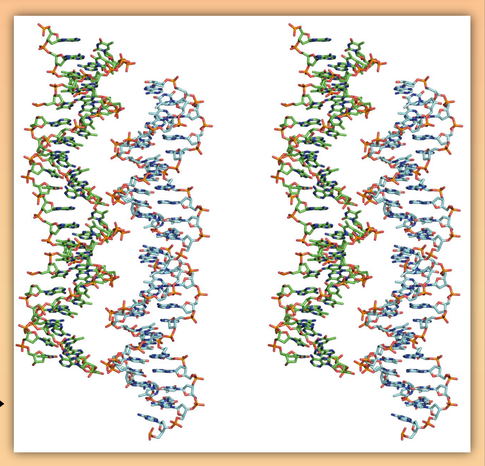


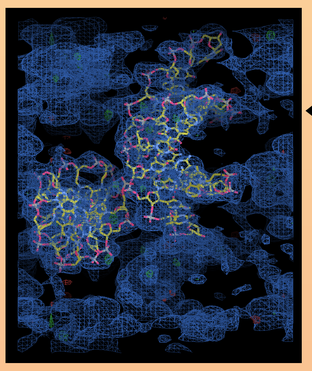
Unit cell



Interlocking of crossed helices, where the nearer helix is rotated counterclockwise leading grooves and phosphates in place to mesh major and minor grooves between neighbors.

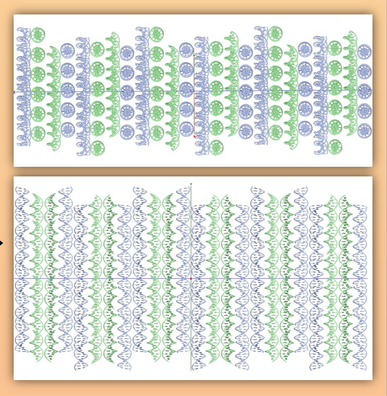
Stereoview of neighbor oligonucleotides coupling minor and major grooves.





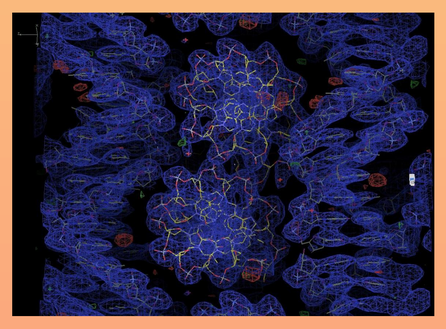
Electron map density with asymmetric unit.

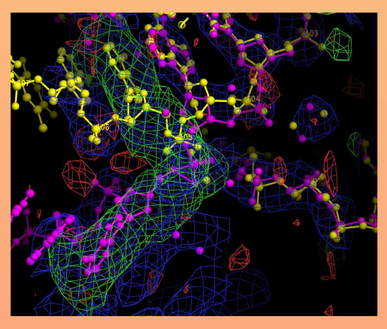
Packing of the oligonucleotide with intercalated layers of columns with a shift of 43 degrees to its neighbors.

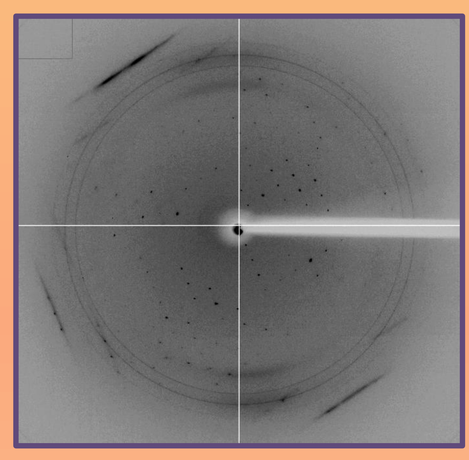


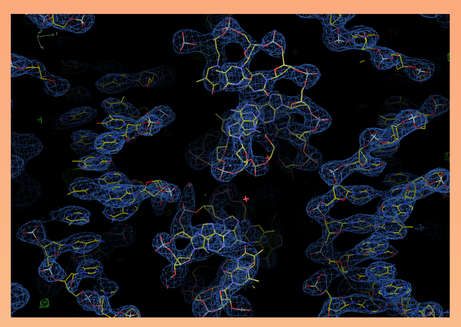
CROSS PACKING

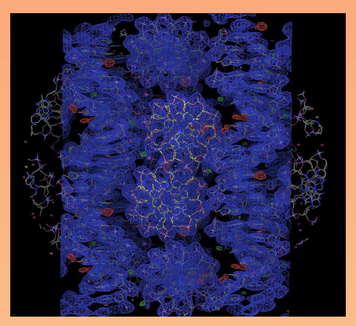
All images show different views of the compact packing in the crystal. The molecules form parallel columns, which cross at about 90º.

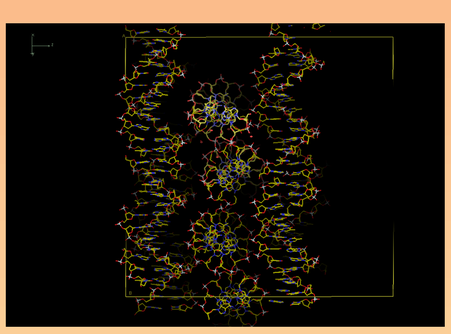


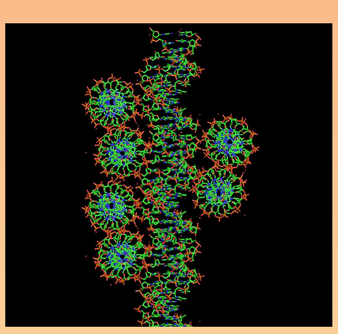












Crystallographic data	
Crystallization conditions (Hanging drop)	Oligonucleotide: 0.5 mM d(AATAAATTTATT)$_2$ 10 mM $MgCl_2$, 25 mM NaCac pH= 6.5 (37% MPD) 11ºC
Resolution	2.9 Å
Space group	C 2 2 2 1
Unit-cell parameters	a=49.86 Å, b=60.60 Å, c=79.87 Å α=β=γ=90º
Contents of ASU	2 duplexes
Completeness	100 % (3.0)
R_{work}	0.235
R_{free}	0.297

Conclusions

- The two oligonucleotides studied here present crystal structures with crossed layers of parallel columns of molecules.
- Their detailed organization depends on the divalent cations or peptides used for crystallization.
- Previous studies had shown that these and most all-AT oligonucleotides usually crystallize as parallel columns of duplexes (Acta Crys D61, 1587, 2005; Bioph. J. 91, 892, 2006).
- The versatility of these structures may help to understand DNA packing in viruses, as well as its applications for nanotechnology.

Acknowledgements

Comissionat per a Universitats i Recerca (CUR) del Departament d'Innovació, Universitats i Empresa (DIUE) de la Generalitat de Catalunya i del Fons Social Europeu, fellowships FI to EF-S and RS-G.

Generalitat de Catalunya, project SRG2009-1208.

Ministerio de Ciencia e Innovación, project BFU-2009-10380.

Consejo de Ciencia y Tecnología (CONACYT) fellowship, reg: 212993, to FAR.