The Rational Design and Structural Analysis of a Self-Assembled Three-Dimensional DNA Crystal

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Abstract

The precise control of the 3D structure of matter is a central concern of the natural sciences. To this end, numerous investigators have developed self-assembling systems to produce targets of interest. Taking its cue from biological systems, structural DNA nanotechnology has used branched DNA motifs combined with the molecular recognition properties of cohesive ends to produce objects, nanomechanical devices and designed 2D lattices. The details of these 2D lattices have been characterized primarily by atomic force microscopy, whose resolution is typically >4 nm. The criteria for 3D lattices (crystals) are stricter, because they are analyzed by x-ray crystallography, which can provide atomic resolution. Previous efforts to generate designed self-assembled 3D lattices have produced crystals that conformed to the design, but whose resolution was no better than 10 Å. Here, we report the crystal structure at 4 Å resolution of a designed, self-assembled, 3D crystal based on the tensegrity triangle.[1] This motif contains three helices that propagate in three linearly independent directions. The data demonstrate clearly that it is possible to design a 3D lattice using the techniques of self-assembly based on molecular recognition.

Motif Design, Data Collection and Structural Solution

Data collection
Space group:R3
Cell dimension :
a=b=c=69.4 Å
$\alpha = \beta = \gamma$ =101.1°
Redundancy:7.3(6.0)
Completeness(%):96.1(100)
Rmerge:0.067(0.594)
I/ σ (I):10.9(1.0)

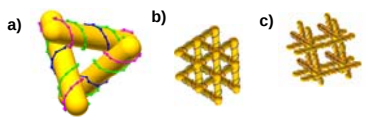
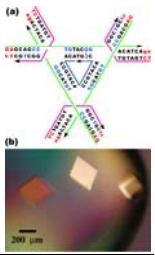
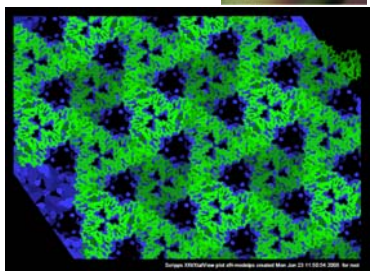


Fig.1 Symmetric 3D triangle DNA motif and lattice.



Refinement statistics
Resolution (Å): 17.29 – 4.01 Å
R_{work}(number of reflections): 0.215 (2888)
R_{free} (number of reflections) :0.280 (323)
Number of nucleic acid atoms :854
Number of non-nucleic acid atoms :0
R.M.S deviations: bonds:0.018 Å
angles:1.434°



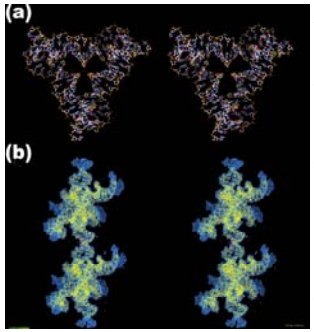
The 3-fold symmetric triangle DNA motif as shown in Fig.1a, is inherently 3D. It spans 3-space and consists of three 4-arm junctions. If you follow the horizontal helix from the left to right, it starts on the top of its adjacent helix on the left and ends up on the bottom of the helix on the right. When all three helices have sticky-ends, the arrays can grow into 3D. The resulting lattice is rhombohedra as shown in Fig. 1b.

Fig.2 Schematic design, sequence, and crystal pictures. (a) schematic of the tensegrity triangle. (b) Crystals of the tensegrity triangle

Crystals (Fig.2b) of iodinated derivatives were screened and X-ray data were collected with 1.7 Å X-rays to 4 Å resolution. Diffraction images were processed using HKL2000. the resulting data were fed into hkl2map, a graphic interface for the Shelxc/d/e programs. The resulting map is shown in Fig.3.

Fig.3 Electron Density map together with model

Overall Structure and Structural Analysis



Although the asymmetric unit of the unit cell is one third of the triangle, it is most useful for comprehending the structure to think about the triangular structures. One of these triangles is shown in a stereoscopic view Fig. 4a, where its over-and-under motif is readily visible. Fig. 4b shows a view of two of the triangles in electron density, with emphasis on their connection by sticky ended cohesion.

Fig.4 View of the tensegrity triangle. (a) A stereoscopic view of the triangle down its3-fold axis. (b) a stereoscopic view of two triangles in electron density.

This crystal structure demonstrates the viability of designing periodic nucleic acid structures in three dimensions. The three directions that define the lattice are readily seen by the red, green and yellow color coding in Fig.5, which shows the surroundings of a given tensegrity triangle.

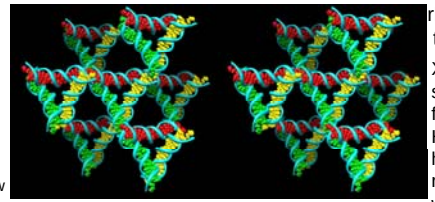


Fig.5 The surroundings of an individual triangle.

The open nature of this stick-like lattice is shown in Fig.6, which illustrates the rhombohedron that is flanked by eight of the triangles.The unit cell is outlined in Fig.6.

X3DNA[2] was used to analyze the DNA triangle DNA structure. A B to A DNA transition map[3] was used for criteria. The DNA is largely in the B-form. However, seven of the independent nucleotides have characteristics closer to A-form nucleotides rather than B-form, as estimated by comparisons with Ho et al.'s A-B series of crystal structures; these nucleotides are indicated by blue lettering in Fig.2a.

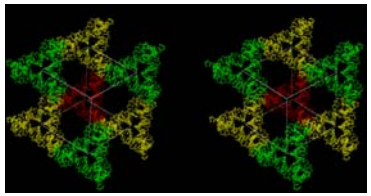


Fig.6 The rhombohedral cavity formed by the tensegrity triangles.

Conclusion and Outlooks

As a further demonstration of the ability to program crystalline DNA arrangements in 3D, we have built eight other rhombohedral lattices from related tensegrity triangles. In addition to edge-lengths of two double helices, some of these crystals contain three or four double helical turns per rhombohedral edge. The crystals are summarized in Fig.7. The applications that have been suggested previously for designed 3D nucleic acid crystalline systems include the scaffolding of biological systems for crystallographic structure determination, as well as the organization of nanoelectronics. Both of these applications will be most usefully realized with scaffolding that is not 3-fold disordered.The crystallographic application will likely require somewhat higher resolution and perhaps the larger unit cells listed in Fig. 7.

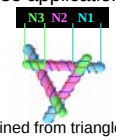


Fig.7 Crystals were obtained from triangle motif with different unit length and inter-junction length.

	Symmetry	Unit Length (base)	Length between Crossovers(base)	Cell Dimmension (in R_H)	Resolution (Å)	Cross Sectional Size(nm^2)	Cavity Size (nm^3)
1	+	21	7	a=68.3, alpha=102.4	4.0	23	103
2	-	21	7	a=68.0, alpha=102.6	5.0	23	101
3	+	31	17	a=102.0, alpha=112.7	6.1	62	366
4	-	31	17	a=100.9, alpha=111.6	6.3	61	373
5	+	32	18	a=103.6, alpha=113.6	6.5	64	367
6	-	32	18	a=103.3, alpha=112.2	6.5	64	395
7	+	42	17	a=134.9, alpha=110.9	11.0	123	1104
8	-	42	17	a=133.7, alpha=111.3	14.0	120	1048
9	+	42	28	a=134.9,alpha=117.3	10.0	117	643

Reference

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This work is supported by the National Institute of General Medical Sciences, the National Science Foundation, the Army Research Office, the Office of Naval Research and a grant from the W.M. Keck Foundation