# Crystal Structure of a Rationally Designed Six-Fold Symmetric DNA Scaffold for the Precise Organization of Biomolecules

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X-ray crystallography has been the most prominent method of determining biomolecular structure for decades. The key bottleneck in the determination of these structures, however, has been the ability to form adequate crystals of the target species. Our goal is to use robust nucleic acid motifs to build designed host lattices that can organize guest molecules such as proteins, peptides, or nanoparticles without having to crystallize them first. The Watson-Crick programmability of DNA makes it an ideal material for the rational design of three-dimensional crystal scaffolds that can be assembled into prescribed architectures. However, only a handful of DNA crystals have been reported to date, and the creation of arbitrary lattices has not yet been fully achieved with DNA nanotechnology. In this work, we present a novel DNA scaffold that was designed to self-assemble into a layered hexagonal lattice containing only two 21 base-pair (bp) oligonucleotides organized in a repeating array of Holliday junctions. The two oligonucleotides hybridize to form an 11-bp duplex, leaving four unpaired single stranded DNA (ssDNA) regions that further organize into specified crossover sites within the motif. Two of these four regions are designed to pair with the neighboring layers, by creating one four arm junction between every two layers. The adjacent ssDNA regions are used to interact with one another along the duplex in each layer. The resulting three-dimensional lattice contains a 21-bp duplex with two Holliday junctions that comprise the asymmetric unit (ASU). Self-assembly is facilitated using designed (and programmable) sticky-end cohesion of complementary DNA sequences, which form an interconnected network of continuous double helical arrays that result in the crystal scaffold. The six-fold symmetry, along with the chirality of the crystal, arise from the configuration and the arrangement of these layers organized by the repeating junctions. In our design, we use these features to not only dictate the symmetry of the crystal, but also to control the handedness of the resulting lattice. We determined the crystal structure of the design to 3.05 Å using bromine derivatized crystals with unit cell dimensions a = b = 68 Å c = 137 Å $\alpha = \beta = 90^\circ$ $\gamma = 120^\circ$, in the hexagonal space group *P*6 with a right-handed six-fold helical screw axis resulting in $P6_1$ symmetry, corresponding perfectly to the intended design. The unit cell was comprised of six asymmetric units with one 21-bp duplex per ASU, connected at crossover points, forming a series of four arm junctions at intervals of 120˚ along the six-fold c-axis. The net result was a “spiraling” of helical layers formed in both the a- and b-direction. The crystal lattice contained a highly ordered array of discrete cavities that could allow us to determine the *a priori,* site-specific attachment of macromolecular guest species for structural determination. These designer crystals will also be excellent candidate materials for transformative applications ranging from catalysis, molecular absorption and separation, and as vehicles for drug delivery to cellular systems.