The Wending Rhombus: Self-Assembling 3D DNA Crystals

Condensed Running Title: Self-Assembling 3D DNA Crystals

Brandon Lu,[1] Simon Vecchioni,[1] Yoel P. Ohayon[1], James W. Canary,[1] and Ruojie Sha[1]
[1]Department of Chemistry, New York University, New York, NY 10003, USA.

Abstract

In this perspective, we provide a summary of recent developments in self-assembling 3D DNA crystals. Starting from the inception of this subfield, we describe the various advancements in structure that have led to an increase in the diversity of macromolecular crystal motifs formed through self-assembly, and we further comment on the future directions of the field which exploit non-canonical base pairing interactions beyond Watson-Crick. We then survey the current applications of self-assembling 3D DNA crystals in reversibly active nanodevices and materials engineering, and provide an outlook on the direction researchers are taking these structures. Finally, we compare 3D DNA crystals with DNA origami and suggest how these distinct subfields might work together to enhance biomolecule structure solution, nanotechnological motifs, and their applications.

Introduction

Since Ned Seeman's proposal to use branched DNA as a structural tool in 1982 (1), DNA nanotechnology has allowed materials scientists and chemical engineers to program the assembly of structures using DNA base pairing (2-12). DNA nanotechnology exploits the reliable binding and known structural parameters of the double helix, taking DNA out of its biological context into a material for bottom-up self-assembly. These structures enable applications ranging from biomarker detection (13), therapeutics delivery (14-16), molecular switches (17), and more (18,19). In these applications, DNA acts as a template (20) or scaffold (21) for co-assembly of other materials (9,14,22) as well as programmable (23) and dynamic (24) functional material. The fundamental design principle in DNA nanotechnology is simple: One need only understand the logic of programming with oligonucleotides to create any designed structure. This adage drove Ned Seeman's dream to assemble 3D crystals from a lattice of branched DNA junctions. He

imagined that six-arm junctions with helices pointing in three dimensions could come together with sticky end cohesion to form a 3D lattice. This design suffered from a lack of structural rigidity.

Rigid structures based on the double-crossover (DX) tile paved the way for predictable 2D crystals, and 3D DNA nanostructures were subsequently made from DNA star motifs (25). The 3D tensegrity triangle motif designed by Mao (26) was the first rationally-designed, self-assembling 3D DNA crystal motif **(Fig. 1a)** (PDB ID: 3GBI) (27). The tensegrity triangle contains three double helices that are 21 bp in length connected by three four-arm Holliday junction analogs. The crossover regions proscribe rigidity into the triangle frame. In effect, the tensegrity triangle is a rigid counterpart to the six-armed junction: Double helices point in three dimensions with sticky ends that connect neighboring triangles into a rhombohedral lattice. From this first pivotal 3D tensegrity triangle structure, the ensuing "bottom-up" approaches to macromolecular self-assembly utilized exquisite control over the triangle unit in order to fine-tune crystal assembly (28), precise placement of guest molecules (29), and enhanced functionality (30). This "bottom-up" strategy prescribes designed parameters (symmetry and cavity size) onto the macroscale unit (the crystal) based on the sequence and geometry of the single unit (the triangle). Crystals then self-assemble with control over the space group, unit cell parameters, and packing, thus bypassing much of the traditional guesswork of structure determination. In essence, the structures form from a single unit in which the sequence and geometry of the single unit control how that unit binds to its neighbors and the macroscale material that results from that single unit. With these structures, Ned Seeman hoped to easily crystallize and solve the structures of guest macromolecules.

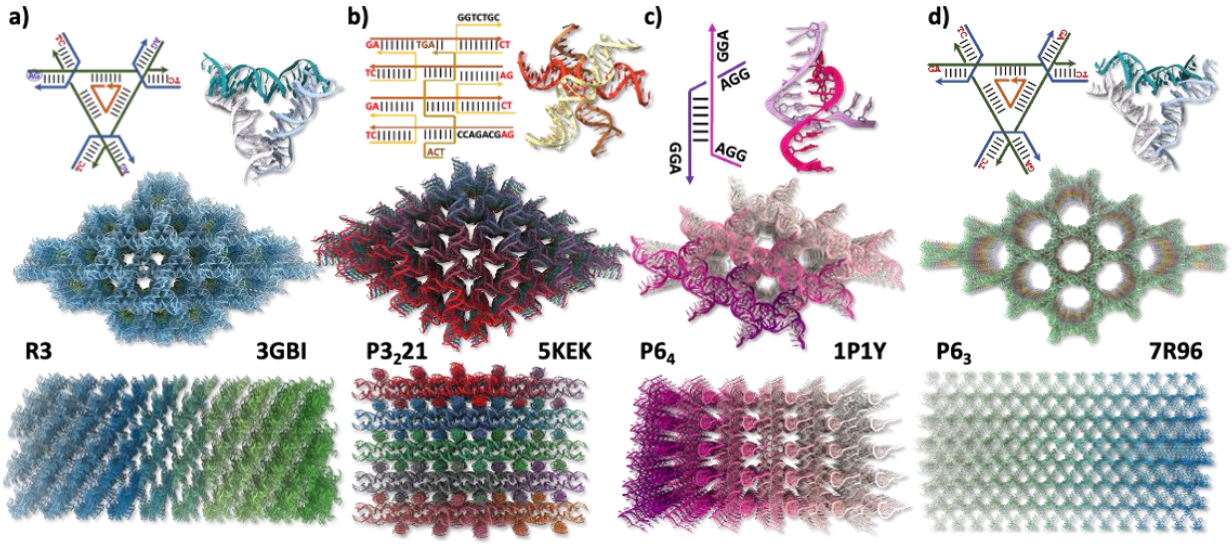

Figure 1. DNA motifs that self-assemble into 3D DNA crystals. The top row depicts the schematic design with the single unit structure; the middle row shows the view from atop the lattices; and the bottom row shows the side view of the long-range crystal lattices, with PDB IDs inset. **a)** A tensegrity triangle motif self-assembles into a rhombohedron (R3 space group) *via* sticky-end cohesion (27), PDB ID: 3GBI. **b)** An unwrapped tensegrity triangle motif with a central five-nucleotide repeating sequence results into a layered structure with $P3_221$ symmetry (31-34), PDB ID: 5KEK. **c)** A DNA 13-mer self-assembles into the stacked layers of parallel helices connected with parallel-stranded base pairing in $P6_4$ symmetry (35), PDB ID: 1P1Y. **d)** Non-Watson-Crick sticky end interactions result into an alternate, 3D hexagonal arrangement ($P6_3$ space group) of tensegrity triangles (36), PDB ID: 7R96.

A Watershed for Crystal Structures

The publication of the tensegrity triangle crystal in 2009 opened the way to a multitude of self-assembling 3D DNA crystal motifs and structures. Whereas many initial forays into self-assembled 3D DNA crystals had been a matter of trial and error, the large number of subsequent papers describe advances in programmatic control. A quick search of "3D DNA" on the Protein Data Bank reveals pages full of tensegrity triangles and tensegrity-inspired DNA structures. These coordinates all point to a common theme: An increase in control over the self-assembly pathways of DNA. The original triangle contained only three unique strands, taking advantage of the threefold symmetry to minimize aberrant interactions. However, spatial control over the crystalline lattice results from asymmetry: To program heterogeneous nanomaterials, researchers increased the size and non-periodicity of the subunits in order to prescribe greater spatial precision over crystalline guests. Instead of a single repeating triangle, two different triangles with unique sticky ends were programmed to assemble in an "A-B" pattern (37). From there, the number of unique oligonucleotides in a unit cell can be increased further, limited only by the number of strands with which one is willing to work. There exists the unexplored potential to build the entire rhombohedral unit cell out of 56 oligonucleotide strands to create the tensegrity triangle analog to Seeman's original DNA cube (38), giving access to all eight triangles for independent functionalization.

The Seeman group explored another route to control the geometry of the tensegrity triangle by altering the number of base pairs between junctions. This interaction affects the cavity size, unit

cell angle, and stress within the DNA structure (27). A seminal 2009 paper described the crystal structure of the **2**-turn (21 base pairs per edge) **T**riangle with **7** base pairs between junctions (2T7) (27). In addition, the paper also included description of a larger, **3**-turn **T**riangle with either 31 or 32 base pair per edge and with either **17** or **18** base pairs between junctions (3T17 or 3T18), a **4**-turn **T**riangle with 42 base pairs and **17** base pairs (4T17) and a **4**-turn **T**riangle with 42 base pairs and **28** base pairs between junctions (4T28), albeit without solved crystal structures (27). The data indicated that as the size of the triangle motif increases, unit cell cross-sectional area and cavity size both greatly increase while diffraction resolution decreases. The 3T17 motif structure was later solved in a separate paper that confirmed these findings (PDB ID: 3UBI) (39). Additional exploration of varying interjunction distances yielded a motif with eight base pairs between junctions (2T8), more than 2/3 of a helical turn, which resulted in torsional stress at the center of the triangle with underwound DNA (40). Motifs with 13, 14, 15, and 16 base pairs between junctions have been analyzed for topology, but no crystal structure has been solved as of yet—such a structure could provide crucial information on the helicity of DNA near the junction (22). Surprisingly, tensegrity triangle-based structures are robust to heterogeneity and can be relatively tolerant of stress (41). As such, modifying the interjunction base pairs alters the angles and cavity size without inhibiting self-assembly, allowing iterative customization of the tensegrity triangle motif.

More substantial alterations to the tensegrity triangle motif resulted in unpredictable results. Hao Yan's group worked to produce a tensegrity "square" in a similar vein as the tensegrity triangle by replacing the triangle center with a designed square center, but this strategy did not produce a similar packing arrangement of square-like units. Instead, the result was a series of tubes caused by the unwinding of the center strand that remained bundled together by sticky-end cohesion **(Fig. 1b),** (PDB ID: 5KEK) (33). The repeating unit of four tubes is capable of forming structures in the $P3_2$, $P3_221$ and R3 space groups depending on junction sequence (34). Yan and colleagues hypothesized that the structure results from the strain induced on the motif when there are fewer interjunction bases than the design can tolerate, causing an overall overwinding. With similar tunability of the interjunction distance (32) and arm length (31) to the tensegrity motif, this structure diversifies the assembly toolbox of structural DNA nanotechnology.

We have come a long way in designing structures that assemble by programmed and predictable interactions. The addition of designed hairpins of various lengths on the tensegrity triangle allows control over the shape and quality of crystals (42), while post-crystallization modifications such as triplex-forming oligonucleotides increase the stability of crystals (43). Moreover, Hao Yan's group designed an entirely new structure with six-fold symmetry that diffracted to very high resolution based on the layering from an unwound center strand (44). However, the Seeman group's recent discovery of a new hexagonal packing arrangement of the tensegrity triangle reveals that the field still has a long way to go to reach a comprehensive understanding of DNA self-assembly in 3D **(Fig. 1d)** (PDB ID: 7R96) (36). The small alteration to the sticky end on the helical strand from 5'-GA to 5'-AG reconfigures the self-assembling mechanism of the tensegrity triangle (36). It is with these serendipitous findings that we approach a complete picture of the various assembly processes of 3D DNA crystals. We expect to find more non-canonical interactions that yield additional self-assembling geometries that further expand the structural diversity of 3D DNA structures. The more modifications introduced into the assembly process, the more emergent and previously unknown factors we discover. We came from one self-assembling motif capable of forming 3D DNA crystals in 2009 (27) to more than four distinct classes of motifs spanning many more individual structures in the past three years (27,33,36,44). We expect systematic studies such as a recent paper from the Yan group that studies the effect all possible junction sequences to further reveal the mystery of self-assembly pathways (34).

With such an increase in structural diversity over the past few years, we expect a continued exploration of tensegrity-based structures, but we also expect a more thorough investigation of non-canonical base pair interactions. While Paukstelis's first self-assembling DNA crystal resulted from homopurine non-Watson-Crick interactions (35), there has mainly been a focus on the paradigmatic [A:T, G:C] base pairing. With the introduction of non-Watson-Crick motifs such as the poly-T-melamine duplex (45), DNA triplexes (46,47), wobble base pairs (36), paranemic crossovers (48), silver and mercury-mediated base pairs (49,50), and many more. Countless opportunities add diversity and complexity to the toolbox of DNA nanotechnology. There exists a tradeoff between the structure diversity and ease of design for these structures. Owing to this challenge, no current rationally-designed structures rely exclusively upon *non*-Watson-Crick interactions, but we foresee that this will change in the very near future as more groups take note of the enhanced possibilities beyond the Watson-Crick canon.

Crystalline Applications

The expected increase in applications from the availability and tunability of motifs has not yet appeared. Nanotechnological applications have not grown in tandem with structural design. Harking back to the founding mission of the field in which designer DNA crystals were to be employed as hosts for biomolecular characterization by X-ray diffraction, a primary limitation of self-assembling DNA crystals lies in the diffraction resolution of these lattices. Resolution trades off with size, which hampers the precision of structure determination of larger lattices and decreases the attractiveness of performing crystallography on functionalized structures (27). As such, it remains to be seen whether the structure of a large number of guest molecules can be solved with ease at high resolutions *via* co-crystallization with a 3D DNA crystal cage per the original vision (19,51). Paukstelis encapsulated proteins in 3D DNA cages in 2006 (52), making the issue not one of getting the protein into the lattice but rather an issue of visibility of the resulting complex. Fortunately, recent advances helped to mitigate this issue, although not enough for atomic resolution. The original tensegrity triangle diffracted to 4.02 Å (27), while shortening the length of the sticky end from two to one nucleotide results in 3.40 Å resolution (53), and the addition of a 5′ -phosphate (54,55) to the crossover strand further increased the resolution to 3.06 Å (53). The highest resolution self-assembling DNA crystal is 2.60 Å, by optimizing the junctions sequence for a presumably more rigid structure (31). At such resolution, individual nucleobases are clearly visible, which significantly increases the information from a crystal structure. Currently, we are unaware of other modifications that could impact resolution, and we suspect that ultimately, resolution may be limited by the high solvent content (>90%) of these crystals (56).

As a result, many applications of DNA crystals have not utilized crystallography. These studies take advantage of the optical properties of macromolecular crystals to visualize changes resulting from modifications. Paukstelis's group used sequence design and guest molecule presence to program multi-layered self-assembly, producing discrete shells with different properties (57). Chengde Mao's group used enzymatic ligation of sticky ends and covalent attachments in a similar manner to build a matryoshka-doll-like crystal with multiple layers along with many other ligation-based experiments (46,58). These crystals act as molecular sieves for protein entrapment as well as power molecular motion through the addition of DNA single strands. They may be further used to selectively immobilize enzymes based on properties such as size and affinity for solid-state

catalysis (59). In line with this approach, we believe that DNA crystals are extremely promising as a vehicle for catalysis (60,61). Additionally, devices that take advantage of the simplicity of adding terminal dyes to synthetic DNA constructs were developed in a 3D DNA crystal system. One example of this entailed a reversible color-changing device using DNA strand displacement to exchange strands on tensegrity triangle subunits (30,62). This device was used to collect kinetic data on the dynamics of strand displacement within a crystal, opening the door to dynamic DNA nanotechnology in 3D. The detection of color changes on a crystal was also used to assemble an organic semiconductor with the properties of an electronic switch inside a tensegrity triangle scaffold (29).

Taking advantage of DNA's ability to organize complex materials based on sequence design (63), such devices enable the specific orientation of electronically-active materials within a DNA lattice, opening the door to organic semiconductors organized through a DNA scaffold (64,65). It is thus not a coincidence that the Canary and Seeman groups explored self-assembled DNA crystals as vehicles for the precise positioning of nanoelectronic components. Without the need to directly observe the high resolution electron density of highly mobile and small pieces (29,30,64), we can often confirm the construction of these nanodevices using optical means. As such, we expect to see an expansion in the diversity and efficacy of organic materials organized by DNA such as graphene nanoribbons (66), carbon nanotubes (67,68), and more complex assemblies of organic semiconductors (69). In addition to the electronic properties of guest molecules, we believe that self-assembled DNA crystals can take advantage of the emergent electronic properties of modified DNA to build more complex nanoelectronics. By incorporating novel architectures such as metal-mediated DNA base pairs into a crystal lattice, 3D DNA crystals may have the potential to organize DNA nanowires in 3D (49). In this manner, self-assembling 3D DNA crystals are forecasted to contribute significant applications in materials chemistry through the orientation of multiple complex, electrically-active components within a lattice (69).

But Why Not Use DNA Origami—A Comparison

A discussion of self-assembling 3D DNA crystals is not complete without juxtaposition with its often-conflated, fellow subfield of DNA origami. DNA origami, invented by Paul Rothemund, employs a long single-stranded DNA molecule as a scaffold that is folded by short staple strands into a desired shape (70). These structures are generally standalone, single structures of finite size

and are inherently asymmetric (71-73), rather than infinite lattices formed by self-assembling DNA crystals. In this way, DNA origami allows fine control over the size and shape of structures in a way that an infinite crystal lacks, building nanostructures from thousands of base pairs (74) to gigadalton-scale polyhedra (72). As such, origami offers a larger degree of customization with respect to 3D crystals, which has been used to design hundreds of unique structures for applications across a variety of fields (60,63,69,71-76). Though a younger subfield, this customizability is likely the reason that most applications of DNA nanotechnology employ DNA origami. Furthermore, DNA origami relies on a veritable arsenal of computational tools (77) that aid in the design of structures such as caDNAno (78) and oxDNA (79-81). The ease of designing a specific motif for a targeted application positions DNA origami structures for drug delivery (82), biomarker detection (83), catalysis (84), and much more (85). Thus, DNA origami is the current state-of-the-art approach to designing a DNA structure for a specific task.

Recently, DNA origami provided another avenue to form 3D crystals (71). These crystals enabled a scaling-up of self-assembling DNA crystals up to 10 times the unit cell length (71). In a similar manner to self-assembling DNA structural motifs, DNA origami was used to build single units that assembled to form a macromolecular crystal in the shape of a tensegrity triangle (71). This approach showcases how more complex DNA origami asymmetric units can use the principles of crystal self-assembly to form larger structures, enabling the construction of more complex architectures and more control over those architectures. Additionally, DNA origami frames can form single-crystals, enabling crystallographic approaches to the analysis of these structures (2).

The question then becomes, why use 3D crystals at all? *Prima facie*, DNA origami appears to be an easier tool to make use of with a multitude of sequence design tools and predictability not seen with 3D crystals. However, we believe that there are many applications that cannot be achieved by DNA origami, even though sequence design may be more challenging. One of these is precision: There is no way to precisely orient atoms in a DNA construct on an angstrom scale that can be measured and reconstructed without 3D DNA crystals (86). In a similar vein, the only way to control the formation of structures orders of magnitudes larger than the single unit is through crystalline arrangement, as modifying the asymmetric unit is enough to propagate the modification into the entire structure (36,58,64). Additionally, alterations to one unit cell results in parallel alterations to the entire crystal, enabling the discovery of emerging metamaterial properties that

are far more difficult to visualize and measure in single units of DNA origami (30,64). These all point to a common theme: Controlling the nanoscale interactions of a self-assembling single unit. As such, we believe that a combination of crystal self-assembly approaches with DNA origami-like constructs could bring additional functionality to DNA nanodevices, combining atomic control with macroscale assembly (87,88). This strategy, which could be executed with the DNA "brick" method (89), would allow for the propagation of unique DNA strands in 3D crystal motifs, overcoming the issue of periodicity for crystals and allowing fine atomic control over each section of the unit cell.

The "brick" method also constructed six-armed junction lattice with sufficient rigidity for structure solution *via* cryogenic electron microscopy (cryo-EM), achieving one of Ned Seeman's original goals (89). This underscores recent advances in cryo-EM that can solve relatively small structures of RNA to sub-3-Å using kissing loops to mitigate flexibility (90). With cryo-EM bringing resolutions comparable to the highest possible resolutions that x-ray crystallography can achieve on self-assembling 3D DNA crystals, DNA crystals are no longer the only avenue for high resolution structural solution. As such, some structures that cannot crystallize or have poor diffraction such as larger lattices may be determined through cryo-EM in the future. Thus, combining the precise design and orientation of self-assembling structures with the macroscale control of DNA origami to produce assemblies of finite size can harness cryo-EM for structure solution where crystallization fails.

Conclusion

Self-assembling 3D DNA crystals expand the toolbox for nanotechnology by using the semantomorphic programmability of DNA as a structural material distinct from its genetic roots. These principles were used to construct a diverse range of crystals from branched DNA motifs. Recent years have seen a marked increase in the number and diversity of 3D crystal types that can be self-assembled from various DNA motifs, enhancing our 3D rational design capabilities. From the original tensegrity triangle, we now have variations in size, shape, and packing arrangement, not to mention a wide array of new 3D motifs inspired by the tensegrity triangle. Now that there have been such advancements in *structure*, use of these structures in *functional* applications may soon follow. We expect the field to shift from the studies of structure to studies on functions of self-assembling 3D DNA crystals. We anticipate the capabilities Seeman predicted 40 years ago

being put to the test through DNA nanoelectronics, catalysis, organic scaffolding, DNA computing, protein organization, and beyond.

Author Contributions

All authors wrote the manuscript.

Acknowledgements

We acknowledge support of grants N000141912596 from the Office of Naval Research, DE-SC0007991 from the Department of Energy, 2106790 from the National Science Foundation, RPG0010/2017 from Human Frontiers Science Program, DMR-1420073 from the MRSEC Program of the National Science Foundation, and the 2020 NASA Center Innovation Fund (CIF) Program.

References

1. Seeman, N. C. 1982. Nucleic acid junctions and lattices. *J Theor Biol*. 99(2):237-247, doi: 10.1016/0022-5193(82)90002-9.
2. Wang, Y., L. Dai, Z. Ding, M. Ji, J. Liu, H. Xing, X. Liu, Y. Ke, C. Fan, P. Wang, and Y. Tian. 2021. DNA origami single crystals with Wulff shapes. *Nat Commun*. 12(1):3011, doi: 10.1038/s41467-021-23332-4.
3. Wang, S., X. Xie, Z. Chen, N. Ma, X. Zhang, K. Li, C. Teng, Y. Ke, and Y. Tian. 2021. DNA-Grafted 3D Superlattice Self-Assembly. *Int J Mol Sci*. 22(14), doi: 10.3390/ijms22147558.
4. Wang, D., L. Yu, B. Ji, S. Chang, J. Song, and Y. Ke. 2020. Programming the Curvatures in Reconfigurable DNA Domino Origami by Using Asymmetric Units. *Nano Lett*. 20(11):8236-8241, doi: 10.1021/acs.nanolett.0c03348.
5. Martin, T. G., T. A. Bharat, A. C. Joerger, X. C. Bai, F. Praetorius, A. R. Fersht, H. Dietz, and S. H. Scheres. 2016. Design of a molecular support for cryo-EM structure determination. *Proc Natl Acad Sci U S A*. 113(47):E7456-E7463, doi: 10.1073/pnas.1612720113.
6. Zhang, Q., Q. Jiang, N. Li, L. Dai, Q. Liu, L. Song, J. Wang, Y. Li, J. Tian, B. Ding, and Y. Du. 2014. DNA origami as an in vivo drug delivery vehicle for cancer therapy. *ACS Nano*. 8(7):6633-6643, doi: 10.1021/nn502058j.
7. Shrestha, P., D. Yang, T. E. Tomov, J. I. MacDonald, A. Ward, H. T. Bergal, E. Krieg, S. Cabi, Y. Luo, B. Nathwani, A. Johnson-Buck, W. M. Shih, and W. P. Wong. 2021. Single-molecule mechanical fingerprinting with DNA nanoswitch calipers. *Nat Nanotechnol*. 16(12):1362-1370, doi: 10.1038/s41565-021-00979-0.
8. Bian, X., Z. Zhang, Q. Xiong, P. De Camilli, and C. Lin. 2019. A programmable DNA-origami platform for studying lipid transfer between bilayers. *Nat Chem Biol*. 15(8):830-837, doi: 10.1038/s41589-019-0325-3.
9. Shetty, R. M., S. R. Brady, P. W. K. Rothemund, R. F. Hariadi, and A. Gopinath. 2021. Bench-Top Fabrication of Single-Molecule Nanoarrays by DNA Origami Placement. *ACS Nano*. doi: 10.1021/acsnano.1c01150.
10. Wang, W., C. Chen, S. Vecchioni, T. Zhang, C. Wu, Y. P. Ohayon, R. Sha, N. C. Seeman, and B. Wei. 2021. Reconfigurable Two-Dimensional DNA Lattices: Static and Dynamic Angle Control. *Angew. Chem. Int. Ed.* 60(49):25781-25786, doi: 10.1002/anie.202112487.
11. Mei, L., B. Chen, R. Fan, M. Wu, C. Weng, A. Tong, B. Zou, H. Yang, C. Nie, and G. Guo. 2022. Magic of Architecting Oligo-DNAs: 3D Structure-Dependent Stability and Programmable Specificity to Tumor Cells. *Adv. Funct. Mater.*, doi: 10.1002/adfm.202112544.
12. Zhang, C., M. Zheng, Y. P. Ohayon, S. Vecchioni, R. Sha, N. C. Seeman, N. Jonoska, and C. Mao. 2022. Programming DNA Self-Assembly by Geometry. *J. Am. Chem. Soc.*, doi: 10.1021/jacs.2c02456.
13. Yang, Q., F. Yang, W. Dai, X. Meng, W. Wei, Y. Cheng, J. Dong, H. Lu, and H. Dong. 2021. DNA Logic Circuits for Multiple Tumor Cells Identification Using Intracellular

MicroRNA Molecular Bispecific Recognition. *Adv Healthc Mater*. 10(21):e2101130, doi: 10.1002/adhm.202101130.
14. Li, J., Y. Zhang, J. Sun, J. Ouyang, and N. Na. 2021. SiRNA-templated 3D framework nucleic acids for chemotactic recognition, and programmable and visualized precise delivery for synergistic cancer therapy. *Chem Sci*. 12(46):15353-15361, doi: 10.1039/d1sc04249a.
15. Hu, C. H., and R. Veneziano. 2022. Controlled Release in Hydrogels Using DNA Nanotechnology. *Biomedicines*. 10(2), doi: 10.3390/biomedicines10020213.
16. Hu, Y., S. Gao, H. Lu, and J. Y. Ying. 2022. Acid-Resistant and Physiological pH-Responsive DNA Hydrogel Composed of A-Motif and i-Motif toward Oral Insulin Delivery. *J. Am. Chem. Soc.* 144(12):5461-5470, doi: 10.1021/jacs.1c13426.
17. Harroun, S. G., C. Prevost-Tremblay, D. Lauzon, A. Desrosiers, X. Wang, L. Pedro, and A. Vallee-Belisle. 2018. Programmable DNA switches and their applications. *Nanoscale*. 10(10):4607-4641, doi: 10.1039/c7nr07348h.
18. Ma, W., Y. Zhan, Y. Zhang, C. Mao, X. Xie, and Y. Lin. 2021. The biological applications of DNA nanomaterials: current challenges and future directions. *Signal Transduct Target Ther*. 6(1):351, doi: 10.1038/s41392-021-00727-9.
19. Seeman, N. C., and H. F. Sleiman. 2017. DNA nanotechnology. *Nat. Rev. Mater.* 3(1), doi: 10.1038/natrevmats.2017.68.
20. O'Reilly, R. K., A. J. Turberfield, and T. R. Wilks. 2017. The Evolution of DNA-Templated Synthesis as a Tool for Materials Discovery. *Acc Chem Res*. 50(10):2496-2509, doi: 10.1021/acs.accounts.7b00280.
21. Julin, S., S. Nummelin, M. A. Kostiainen, and V. Linko. 2018. DNA nanostructure-directed assembly of metal nanoparticle superlattices. *J Nanopart Res*. 20(5):119, doi: 10.1007/s11051-018-4225-3.
22. Liu, D., G. Chen, U. Akhter, T. M. Cronin, and Y. Weizmann. 2016. Creating complex molecular topologies by configuring DNA four-way junctions. *Nat Chem*. 8(10):907-914, doi: 10.1038/nchem.2564.
23. Li, J., A. A. Green, H. Yan, and C. Fan. 2017. Engineering nucleic acid structures for programmable molecular circuitry and intracellular biocomputation. *Nat Chem*. 9(11):1056-1067, doi: 10.1038/nchem.2852.
24. Kopperger, E., J. List, S. Madhira, F. Rothfischer, D. C. Lamb, and F. C. Simmel. 2018. A self-assembled nanoscale robotic arm controlled by electric fields. *Science*. 359(6373):296-301, doi: 10.1126/science.aao4284.
25. Zhang, C., Y. He, M. Su, S. H. Ko, T. Ye, Y. Leng, X. Sun, A. E. Ribbe, W. Jiangh, and C. Mao. 2009. DNA self-assembly: from 2D to 3D. *Faraday Discuss*. 143:221-233; discussion 265-275, doi: 10.1039/b905313c.
26. Liu, D., M. Wang, Z. Deng, R. Walulu, and C. Mao. 2004. Tensegrity: construction of rigid DNA triangles with flexible four-arm DNA junctions. *J. Am. Chem. Soc.* 126(8):2324-2325, doi: 10.1021/ja031754r.
27. Zheng, J., J. J. Birktoft, Y. Chen, T. Wang, R. Sha, P. E. Constantinou, S. L. Ginell, C. Mao, and N. C. Seeman. 2009. From molecular to macroscopic via the rational design of a self-assembled 3D DNA crystal. *Nature*. 461(7260):74-77, doi: 10.1038/nature08274.
28. Wang, T., R. Sha, J. Birktoft, J. Zheng, C. Mao, and N. C. Seeman. 2010. A DNA crystal designed to contain two molecules per asymmetric unit. *J. Am. Chem. Soc.* 132(44):15471-15473, doi: 10.1021/ja104833t.

29. Wang, X., R. Sha, M. Kristiansen, C. Hernandez, Y. Hao, C. Mao, J. W. Canary, and N. C. Seeman. 2017. An Organic Semiconductor Organized into 3D DNA Arrays by "Bottom-up" Rational Design. *Angew. Chem. Int. Ed.* 56(23):6445-6448, doi: 10.1002/anie.201700462.
30. Hao, Y., M. Kristiansen, R. Sha, J. J. Birktoft, C. Hernandez, C. Mao, and N. C. Seeman. 2017. A device that operates within a self-assembled 3D DNA crystal. *Nat Chem*. 9(8):824-827, doi: 10.1038/nchem.2745.
31. Simmons, C. R., T. MacCulloch, F. Zhang, Y. Liu, N. Stephanopoulos, and H. Yan. 2020. A Self-Assembled Rhombohedral DNA Crystal Scaffold with Tunable Cavity Sizes and High-Resolution Structural Detail. *Angew. Chem. Int. Ed.* 59(42):18619-18626, doi: 10.1002/anie.202005505.
32. Simmons, C. R., F. Zhang, T. MacCulloch, N. Fahmi, N. Stephanopoulos, Y. Liu, N. C. Seeman, and H. Yan. 2017. Tuning the Cavity Size and Chirality of Self-Assembling 3D DNA Crystals. *J. Am. Chem. Soc.* 139(32):11254-11260, doi: 10.1021/jacs.7b06485.
33. Simmons, C. R., F. Zhang, J. J. Birktoft, X. Qi, D. Han, Y. Liu, R. Sha, H. O. Abdallah, C. Hernandez, Y. P. Ohayon, N. C. Seeman, and H. Yan. 2016. Construction and Structure Determination of a Three-Dimensional DNA Crystal. *J. Am. Chem. Soc.* 138(31):10047-10054, doi: 10.1021/jacs.6b06508.
34. Simmons, C. R., T. MacCulloch, M. Krepl, M. Matthies, A. Buchberger, I. Crawford, J. Sponer, P. Sulc, N. Stephanopoulos, and H. Yan. 2022. The influence of Holliday junction sequence and dynamics on DNA crystal self-assembly. *Nat Commun*. 13(1):3112, doi: 10.1038/s41467-022-30779-6.
35. Paukstelis, P. J., J. Nowakowski, J. J. Birktoft, and N. C. Seeman. 2004. Crystal structure of a continuous three-dimensional DNA lattice. *Chem Biol*. 11(8):1119-1126, doi: 10.1016/j.chembiol.2004.05.021.
36. Lu, B., S. Vecchioni, Y. P. Ohayon, R. Sha, K. Woloszyn, B. Yang, C. Mao, and N. C. Seeman. 2021. 3D Hexagonal Arrangement of DNA Tensegrity Triangles. *ACS Nano*. 15(10):16788-16793, doi: 10.1021/acsnano.1c06963.
37. Rothemund, P. W., N. Papadakis, and E. Winfree. 2004. Algorithmic self-assembly of DNA Sierpinski triangles. *PLoS Biol*. 2(12):e424, doi: 10.1371/journal.pbio.0020424.
38. Chen, J. H., and N. C. Seeman. 1991. Synthesis from DNA of a molecule with the connectivity of a cube. *Nature*. 350(6319):631-633, doi: 10.1038/350631a0.
39. Nguyen, N., J. J. Birktoft, R. Sha, T. Wang, J. Zheng, P. E. Constantinou, S. L. Ginell, Y. Chen, C. Mao, and N. C. Seeman. 2012. The absence of tertiary interactions in a self-assembled DNA crystal structure. *J Mol Recognit*. 25(4):234-237, doi: 10.1002/jmr.2183.
40. Hernandez, C., J. J. Birktoft, Y. P. Ohayon, A. R. Chandrasekaran, H. Abdallah, R. Sha, V. Stojanoff, C. Mao, and N. C. Seeman. 2017. Self-Assembly of 3D DNA Crystals Containing a Torsionally Stressed Component. *Cell Chem Biol*. 24(11):1401-1406 e1402, doi: 10.1016/j.chembiol.2017.08.018.
41. Stahl, E., F. Praetorius, C. C. de Oliveira Mann, K. P. Hopfner, and H. Dietz. 2016. Impact of Heterogeneity and Lattice Bond Strength on DNA Triangle Crystal Growth. *ACS Nano*. 10(10):9156-9164, doi: 10.1021/acsnano.6b04787.
42. Zhao, J., Y. Zhao, Z. Li, Y. Wang, R. Sha, N. C. Seeman, and C. Mao. 2018. Modulating Self-Assembly of DNA Crystals with Rationally Designed Agents. *Angew. Chem. Int. Ed.* 57(50):16529-16532, doi: 10.1002/anie.201809757.

43. Zhao, J., A. R. Chandrasekaran, Q. Li, X. Li, R. Sha, N. C. Seeman, and C. Mao. 2015. Post-Assembly Stabilization of Rationally Designed DNA Crystals. *Angew. Chem.* 127(34):10074-10077, doi: 10.1002/ange.201503610.
44. Zhang, F., C. R. Simmons, J. Gates, Y. Liu, and H. Yan. 2018. Self-Assembly of a 3D DNA Crystal Structure with Rationally Designed Six-Fold Symmetry. *Angew. Chem. Int. Ed.* 57(38):12504-12507, doi: 10.1002/anie.201807223.
45. Li, Q., J. Zhao, L. Liu, S. Jonchhe, F. J. Rizzuto, S. Mandal, H. He, S. Wei, H. F. Sleiman, H. Mao, and C. Mao. 2020. A poly(thymine)-melamine duplex for the assembly of DNA nanomaterials. *Nat Mater*. 19(9):1012-1018, doi: 10.1038/s41563-020-0728-2.
46. Li, Z., L. Liu, M. Zheng, J. Zhao, N. C. Seeman, and C. Mao. 2019. Making Engineered 3D DNA Crystals Robust. *J. Am. Chem. Soc.* 141(40):15850-15855, doi: 10.1021/jacs.9b06613.
47. Rusling, D. A., A. R. Chandrasekaran, Y. P. Ohayon, T. Brown, K. R. Fox, R. Sha, C. Mao, and N. C. Seeman. 2014. Functionalizing designer DNA crystals with a triple-helical veneer. *Angew. Chem. Int. Ed.* 53(15):3979-3982, doi: 10.1002/anie.201309914.
48. Wang, X., A. R. Chandrasekaran, Z. Shen, Y. P. Ohayon, T. Wang, M. E. Kizer, R. Sha, C. Mao, H. Yan, X. Zhang, S. Liao, B. Ding, B. Chakraborty, N. Jonoska, D. Niu, H. Gu, J. Chao, X. Gao, Y. Li, T. Ciengshin, and N. C. Seeman. 2019. Paranemic Crossover DNA: There and Back Again. *Chem Rev*. 119(10):6273-6289, doi: 10.1021/acs.chemrev.8b00207.
49. Vecchioni, S., M. C. Capece, E. Toomey, L. Nguyen, A. Ray, A. Greenberg, K. Fujishima, J. Urbina, I. G. Paulino-Lima, V. Pinheiro, J. Shih, G. Wessel, S. J. Wind, and L. Rothschild. 2019. Construction and characterization of metal ion-containing DNA nanowires for synthetic biology and nanotechnology. *Sci Rep*. 9(1):6942, doi: 10.1038/s41598-019-43316-1.
50. Sebera, J., J. Burda, M. Straka, A. Ono, C. Kojima, Y. Tanaka, and V. Sychrovsky. 2013. Formation of a thymine-Hg(II)-thymine metal-mediated DNA base pair: proposal and theoretical calculation of the reaction pathway. *Chemistry*. 19(30):9884-9894, doi: 10.1002/chem.201300460.
51. Seeman, N. C. 2020. DNA Nanotechnology at 40. *Nano Lett*. 20(3):1477-1478, doi: 10.1021/acs.nanolett.0c00325.
52. Paukstelis, P. J. 2006. Three-dimensional DNA crystals as molecular sieves. *J. Am. Chem. Soc.* 128(21):6794-6795, doi: 10.1021/ja061322r.
53. Ohayon, Y. P., C. Hernandez, A. R. Chandrasekaran, X. Wang, H. O. Abdallah, M. A. Jong, M. G. Mohsen, R. Sha, J. J. Birktoft, P. S. Lukeman, P. M. Chaikin, S. L. Ginell, C. Mao, and N. C. Seeman. 2019. Designing Higher Resolution Self-Assembled 3D DNA Crystals via Strand Terminus Modifications. *ACS Nano*. 13(7):7957-7965, doi: 10.1021/acsnano.9b02430.
54. Sha, R., J. J. Birktoft, N. Nguyen, A. R. Chandrasekaran, J. Zheng, X. Zhao, C. Mao, and N. C. Seeman. 2013. Self-assembled DNA crystals: the impact on resolution of 5'-phosphates and the DNA source. *Nano Lett*. 13(2):793-797, doi: 10.1021/nl304550c.
55. Li, Z., M. Zheng, L. Liu, N. C. Seeman, and C. Mao. 2021. 5'-Phosphorylation Strengthens Sticky-End Cohesions. *J. Am. Chem. Soc.* 143(37):14987-14991, doi: 10.1021/jacs.1c07279.

56. Weichenberger, C. X., P. V. Afonine, K. Kantardjieff, and B. Rupp. 2015. The solvent component of macromolecular crystals. *Acta Crystallogr. D*. 71(Pt 5):1023-1038, doi: 10.1107/S1399004715006045.
57. McNeil, R., Jr., and P. J. Paukstelis. 2017. Core-Shell and Layer-by-Layer Assembly of 3D DNA Crystals. *Adv Mater*. 29(28), doi: 10.1002/adma.201701019.
58. Zheng, M., Z. Li, C. Zhang, N. C. Seeman, and C. Mao. 2022. Powering a approximately 50-mum Motion by a Molecular Event in DNA Crystals. *Adv Mater*.e2200441, doi: 10.1002/adma.202200441.
59. Geng, C., and P. J. Paukstelis. 2014. DNA crystals as vehicles for biocatalysis. *J. Am. Chem. Soc.* 136(22):7817-7820, doi: 10.1021/ja502356m.
60. Zhao, Z., J. Fu, S. Dhakal, A. Johnson-Buck, M. Liu, T. Zhang, N. W. Woodbury, Y. Liu, N. G. Walter, and H. Yan. 2016. Nanocaged enzymes with enhanced catalytic activity and increased stability against protease digestion. *Nat Commun*. 7:10619, doi: 10.1038/ncomms10619.
61. Fu, J., Y. R. Yang, S. Dhakal, Z. Zhao, M. Liu, T. Zhang, N. G. Walter, and H. Yan. 2016. Assembly of multienzyme complexes on DNA nanostructures. *Nat Protoc*. 11(11):2243-2273, doi: 10.1038/nprot.2016.139.
62. Zhang, D. Y., and G. Seelig. 2011. Dynamic DNA nanotechnology using strand-displacement reactions. *Nat Chem*. 3(2):103-113, doi: 10.1038/nchem.957.
63. Jiang, S., F. Zhang, and H. Yan. 2020. Complex assemblies and crystals guided by DNA. *Nat Mater*. 19(7):694-700, doi: 10.1038/s41563-020-0719-3.
64. Wang, X., R. Deshmukh, R. Sha, J. J. Birktoft, V. Menon, N. C. Seeman, and J. W. Canary. 2022. Orienting an Organic Semiconductor into DNA 3D Arrays by Covalent Bonds. *Angew. Chem. Int. Ed.* 61(5):e202115155, doi: 10.1002/anie.202115155.
65. Wang, X., R. Sha, W. B. Knowlton, N. C. Seeman, J. W. Canary, and B. Yurke. 2022. Exciton Delocalization in a DNA-Templated Organic Semiconductor Dimer Assembly. *ACS Nano*. doi: 10.1021/acsnano.1c09143.
66. Reuven, D. G., H. B. Shashikala, S. Mandal, M. N. Williams, J. Chaudhary, and X. Q. Wang. 2013. Supramolecular Assembly of DNA on Graphene Nanoribbons. *J Mater Chem B*. 1(32):3926-3931, doi: 10.1039/C3TB20397B.
67. Demirer, G. S., H. Zhang, N. S. Goh, E. Gonzalez-Grandio, and M. P. Landry. 2019. Carbon nanotube-mediated DNA delivery without transgene integration in intact plants. *Nat Protoc*. 14(10):2954-2971, doi: 10.1038/s41596-019-0208-9.
68. Pei, H., R. Sha, X. Wang, M. Zheng, C. Fan, J. W. Canary, and N. C. Seeman. 2019. Organizing End-Site-Specific SWCNTs in Specific Loci Using DNA. *J. Am. Chem. Soc.* 141(30):11923-11928, doi: 10.1021/jacs.9b03432.
69. Cui, C., D. H. Park, and D. J. Ahn. 2020. Organic Semiconductor-DNA Hybrid Assemblies. *Adv Mater*. 32(51):e2002213, doi: 10.1002/adma.202002213.
70. Rothemund, P. W. 2006. Folding DNA to create nanoscale shapes and patterns. *Nature*. 440(7082):297-302, doi: 10.1038/nature04586.
71. Zhang, T., C. Hartl, K. Frank, A. Heuer-Jungemann, S. Fischer, P. C. Nickels, B. Nickel, and T. Liedl. 2018. 3D DNA Origami Crystals. *Adv Mater*. 30(28):e1800273, doi: 10.1002/adma.201800273.
72. Wagenbauer, K. F., C. Sigl, and H. Dietz. 2017. Gigadalton-scale shape-programmable DNA assemblies. *Nature*. 552(7683):78-83, doi: 10.1038/nature24651.

73. Veneziano, R., S. Ratanalert, K. Zhang, F. Zhang, H. Yan, W. Chiu, and M. Bathe. 2016. Designer nanoscale DNA assemblies programmed from the top down. *Science*. 352(6293):1534, doi: 10.1126/science.aaf4388.
74. Engelhardt, F. A. S., F. Praetorius, C. H. Wachauf, G. Bruggenthies, F. Kohler, B. Kick, K. L. Kadletz, P. N. Pham, K. L. Behler, T. Gerling, and H. Dietz. 2019. Custom-Size, Functional, and Durable DNA Origami with Design-Specific Scaffolds. *ACS Nano*. 13(5):5015-5027, doi: 10.1021/acsnano.9b01025.
75. Praetorius, F., B. Kick, K. L. Behler, M. N. Honemann, D. Weuster-Botz, and H. Dietz. 2017. Biotechnological mass production of DNA origami. *Nature*. 552(7683):84-87, doi: 10.1038/nature24650.
76. Jiang, S., Z. Ge, S. Mou, H. Yan, and C. Fan. 2021. Designer DNA nanostructures for therapeutics. *Chem*. 7(5):1156-1179, doi: 10.1016/j.chempr.2020.10.025.
77. Jun, H., X. Wang, M. F. Parsons, W. P. Bricker, T. John, S. Li, S. Jackson, W. Chiu, and M. Bathe. 2021. Rapid prototyping of arbitrary 2D and 3D wireframe DNA origami. *Nucleic Acids Res*. 49(18):10265-10274, doi: 10.1093/nar/gkab762.
78. Douglas, S. M., A. H. Marblestone, S. Teerapittayanon, A. Vazquez, G. M. Church, and W. M. Shih. 2009. Rapid prototyping of 3D DNA-origami shapes with caDNAno. *Nucleic Acids Res*. 37(15):5001-5006, doi: 10.1093/nar/gkp436.
79. Sulc, P., F. Romano, T. E. Ouldridge, L. Rovigatti, J. P. Doye, and A. A. Louis. 2012. Sequence-dependent thermodynamics of a coarse-grained DNA model. *J Chem Phys*. 137(13):135101, doi: 10.1063/1.4754132.
80. Snodin, B. E., F. Randisi, M. Mosayebi, P. Sulc, J. S. Schreck, F. Romano, T. E. Ouldridge, R. Tsukanov, E. Nir, A. A. Louis, and J. P. Doye. 2015. Introducing improved structural properties and salt dependence into a coarse-grained model of DNA. *J Chem Phys*. 142(23):234901, doi: 10.1063/1.4921957.
81. Poppleton, E., R. Romero, A. Mallya, L. Rovigatti, and P. Sulc. 2021. OxDNA.org: a public webserver for coarse-grained simulations of DNA and RNA nanostructures. *Nucleic Acids Res*. 49(W1):W491-W498, doi: 10.1093/nar/gkab324.
82. Wiraja, C., Y. Zhu, D. C. S. Lio, D. C. Yeo, M. Xie, W. Fang, Q. Li, M. Zheng, M. Van Steensel, L. Wang, C. Fan, and C. Xu. 2019. Framework nucleic acids as programmable carrier for transdermal drug delivery. *Nat Commun*. 10(1):1147, doi: 10.1038/s41467-019-09029-9.
83. Cervantes-Salguero, K., M. Freeley, J. L. Chavez, and M. Palma. 2020. Single-molecule DNA origami aptasensors for real-time biomarker detection. *J Mater Chem B*. 8(30):6352-6356, doi: 10.1039/d0tb01291b.
84. Klein, W. P., R. P. Thomsen, K. B. Turner, S. A. Walper, J. Vranish, J. Kjems, M. G. Ancona, and I. L. Medintz. 2019. Enhanced Catalysis from Multienzyme Cascades Assembled on a DNA Origami Triangle. *ACS Nano*. 13(12):13677-13689, doi: 10.1021/acsnano.9b05746.
85. Li, Y., Z. Liu, G. Yu, W. Jiang, and C. Mao. 2015. Self-assembly of molecule-like nanoparticle clusters directed by DNA nanocages. *J. Am. Chem. Soc.* 137(13):4320-4323, doi: 10.1021/jacs.5b01196.
86. Silvester, E., B. Vollmer, V. Prazak, D. Vasishtan, E. A. Machala, C. Whittle, S. Black, J. Bath, A. J. Turberfield, K. Grunewald, and L. A. Baker. 2021. DNA origami signposts for identifying proteins on cell membranes by electron cryotomography. *Cell*. 184(4):1110-1121 e1116, doi: 10.1016/j.cell.2021.01.033.

87. Liu, W., H. Duan, D. Zhang, X. Zhang, Q. Luo, T. Xie, H. Yan, L. Peng, Y. Hu, L. Liang, G. Zhao, Z. Xie, and J. Hu. 2021. Concepts and Application of DNA Origami and DNA Self-Assembly: A Systematic Review. *Appl Bionics Biomech*. 2021:9112407, doi: 10.1155/2021/9112407.
88. Yan, X., S. Huang, Y. Wang, Y. Tang, and Y. Tian. 2020. Bottom-Up Self-Assembly Based on DNA Nanotechnology. *Nanomaterials (Basel)*. 10(10), doi: 10.3390/nano10102047.
89. Wang, W., S. Chen, B. An, K. Huang, T. Bai, M. Xu, G. Bellot, Y. Ke, Y. Xiang, and B. Wei. 2019. Complex wireframe DNA nanostructures from simple building blocks. *Nat Commun*. 10(1):1067, doi: 10.1038/s41467-019-08647-7.
90. Liu, D., F. A. Thelot, J. A. Piccirilli, M. Liao, and P. Yin. 2022. Sub-3-A cryo-EM structure of RNA enabled by engineered homomeric self-assembly. *Nat Methods*. 19(5):576-585, doi: 10.1038/s41592-022-01455-w.