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14. ABSTRACT UNUSUAL produce unusual DNA motifs from synthetic oligonucleotides. We use these molecules individually or in combinations to produce systems to implement DNA nanotechnology. Computer optimization of DNA sequences is used to select the molecules to synthesize. Structures are assembled in solution, and 1D or 2D arrays are deposited on mica for characterization by AFM. 3D arrays are analyzed by X-ray diffraction. During the current project period, we have built a robust DNA nanomechanical device and a nanorobot that walks on a sidewalk. We have built robust 2D arrays, and have made large strides towards the control of matter in 3D.					
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FINAL REPORT

GRANT #: N00014-98-1-0093

PRINCIPAL INVESTIGATOR: Dr. Nadrian C. Seeman

INSTITUTION: New York University

GRANT TITLE: Macromolecular Design

AWARD PERIOD: Jan 01, 2002 - 30 Sep, 2004

OBJECTIVE: To develop and extend the methods of DNA nanotechnology to gain control over the structure of matter in 2D and in 3D and to produce DNA-based nanorobots.

APPROACH; Branched DNA motifs are designed by use of an algorithm that minimizes sequence symmetry. Oligonucleotides corresponding to these sequences are then assembled. These motifs are then assembled primarily by using cooling protocols. The resulting structures are examined by AFM, by gel electrophoresis or by X-ray diffraction, as appropriate to the target construct.

ACCOMPLISHMENTS:

Advances in Algorithmic Assembly: We have demonstrated successful algorithmic assembly leading to a cumulative XOR calculation (#1). This was a major step in DNA self-assembly, the first time that algorithmic assembly was demonstrated. Having shown the feasibility of aperiodic assembly, we have participated in several studies in the theory of aperiodic self-assembly (#13, #16, #26, #32, #33, #39) and other aspects of DNA-based computation (#41); this work include experimental studies (#20, #23, #29, #35) that entail making irregular graphs from DNA.

2D and 3D Arrays: We have developed (#3) and applied (#22) a quantitative ligation-closure method to demonstrate that the DX motif is about twice as stiff as ordinary DNA. We have used this information to develop robust geometrical motifs, such as the DX-triangle (#44), which forms a honey-comb array. This is a decade-old goal, on which we failed repeatedly before finally achieving this success.

We have been engaged in crystallographic studies during the current project period, partly to establish the nature of the 3D materials that we have built. Our preliminary results (#45) demonstrate the correct unit cell dimensions and space groups for a motif containing tensegrity triangles. As suggested by the results for the pseudo-hexagonal system, we are building DX versions of these triangles and we will use them to try to build crystals. We have also discovered new parallel non-Watson-Crick pairing motifs in the crystal structure of a DNA 13-mer (#42).

We were successful in demonstrating our specific aim of paranemic cohesion (#14), but this approach has not yet been shown to be useful in array construction. We are trying to get it to work in 2D.

We used AFM to demonstrate successfully the structure of 'Bowtie' junctions (#2), demonstrating the robustness of the parallelogram motif in V-shaped and 2D arrays.

Nanomechanical Devices: We have established general principles for the creation of motifs in DNA (#7); it is now straightforward to develop new motifs for different purposes, as demonstrated by our development of the PX motif (#31) and its use in both paranemic cohesion (#14), and, more importantly, in the development of the robust sequence-dependent PX-JX₂ device (#8). The development of PX-JX₂ device required the construction of edge-sharing motifs (#21) for its demonstration by AFM.

A second device that we developed is a prototypical nanorobot that walks on a sidewalk (#36). Using the Yurke method of strand removal, its attachment points to the sidewalk are altered in the course of its taking a step.

A third device measures the amount of work a DNA-distorting protein can perform as a consequence of binding to its target (#43). This device was prototyped with integration host factor (IHF), and is similar to a B-Z device developed in a previous project period. IHF binds to its recognition site, and distorts the DNA there. However, to do so, it must break base pairs, so that the amount of work it can do can be estimated by the load against which it cannot work.

We have also prototyped a translational device based on the PX-JX₂ device. It contains two such devices separating two clamps that can contain a DX motif with a continuous strand. The two different devices lead to four different states, and four different polymers, although in this case the polymers are all DNA. As a consequence of the strands added to set the states of the two devices, a variety of different polymers are made (#46).

Scaffolding Non-DNA Materials: For several project periods, we have suggested that DNA nanotechnology would be a feasible method for scaffolding other materials. During this project period, we have begun to prototype this chemistry through various collaborators. These efforts have led to the organization of gold nanoparticles (with Rick Kiehl of Minnesota) (#15) and the scaffolding of industrial polymers on a nucleic acid backbone (with Jim Canary of NYU) (#17, #25). In addition, we have explored neutralizing components of DNA arrays by incorporating peptide-nucleic acid units (PNA) with the arrays (#37). The essence of these studies is the addition of functionality to nucleic acid arrays.

Dissemination of Progress:

The development of structural DNA nanotechnology has struck the fancy of the scientific community, resulting in many requests for review articles (#4, #5, #6, #9, #10, #11, #12, #18, #19, #24, #27, #28, #30, #34, #38, #40), where # is the serial number in the publications list.

CONCLUSIONS: Structurally based DNA nanotechnology has been shown to be an extremely potent method of controlling the structure of matter on the nanometer scale. Particularly noteworthy advances during this project period include the establishment of the robustness of DX cohesion (#44), the construction of robust sequence-dependent devices (#8 and #46), the use of DNA in algorithmic assembly (#1), the development of paranemic cohesion (#14), the one-pot synthesis of an irregular graph (#35), and the incorporation of non-DNA materials in DNA-based constructs.

SIGNIFICANCE: The results presented here demonstrate that DNA-based nanotechnology is a powerful way of creating designed structures and devices.

PATENT INFORMATION:

1. N.C. Seeman, E. Winfree, F. Liu and L. Wenzler Savin, Periodic Two- and Three-Dimensional Nucleic Acid Structures, U.S. Patent #6,255,469, Issued July 03, 2001.
2. N.C. Seeman, H. Yan, X. Zhang, & Z. Shen, A Polynucleic Acid Nanomechanical Device Controlled by Hybridization Topology, #10/370/,101, Filed 02/22/02.
3. L. Zhu, J. Canary, P. Lukeman & N.C. Seeman, Nucleic Acid-Nylon Ladder Polymers, #10/855,893, Filed 05/29/03.
4. W.B. Sherman & N.C. Seeman, A Nucleic Acid-Based Nanorobotic System, #60/511,120, Filed 10/15/03.
5. B. Ding, R. Sha & N.C. Seeman, Polygonal Nanostructures of Polynucleic Acid Multi-Crossover Molecules and Assembly of Lattices Based on Double Crossover Cohesion, #60/578,306, Filed 06/10/04.
6. S. Liao & N.C. Seeman, A Polynucleotide Nanomechanical Device that Acts as an Artificial Ribosome and Translates DNA Signals into Polymer Assembly Instructions, #60/592,402, Filed, 08/02/04.

AWARD INFORMATION: Appointed to the Margaret and Herman Sokol Chair in Chemistry at NYU; recipient of the Tulip Award as the outstanding scientist of the year by the DNA-Based Computation community.

PUBLICATIONS:

1. C. Mao, T. LaBean, J.H. Reif and N.C. Seeman, Logical Computation Using Algorithmic Self-Assembly of DNA Triple Crossover Molecules, *Nature* **407**, 493-496 (2000).
2. R. Sha, F. Liu, D.P. Millar and N.C. Seeman, Atomic Force Microscopy of Parallel DNA Branched Junction Arrays, *Chem. & Biol.* **7**, 743-751 (2000)
3. A. Podtelezchnikov, C. Mao, N.C. Seeman & A. Vologodskii, Multimerization-Cyclization of DNA Fragments as a Method of Conformational Analysis, *Biophys. J.* **79**, 2692-2704 (2000).
4. N.C. Seeman, C. Mao, F. Liu, R. Sha, X. Yang, L. Wenzler, X. Li, Z. Shen, H. Yan, P. Sa-Ardyen, X. Zhang, W. Shen, J. Birac, P. Lukeman, Y. Pinto, J. Qi, B. Liu, H. Qiu, S.M. Du, H. Wang, W. Sun, Y. Wang, T.-J. Fu, Y. Zhang, J.E. Mueller and J. Chen, Nicks, Nodes, and New Motifs for DNA Nanotechnology, *Frontiers of Nanoelectronic Systems*, ed. by L. Pavesi & E. Buzanova, Kluwer, Dordrecht, 177-198 (2000).

5. N.C. Seeman, In the Nick of Space: Generalized Nucleic Acid Complementarity and the Development of DNA Nanotechnology, *Synlett* 2000, 1536-1548 (2000).
6. J.H. Reif, T.H. LaBean & N.C. Seeman, Challenges and Applications for Self-Assembled DNA Nanostructures, *Sixth International Workshop on DNA-Based Computers*, DNA 2000, Leiden, The Netherlands, (June, 2000) ed. A. Condon, G. Rozenberg. Springer-Verlag, Berlin, Heidelberg, Lecture Notes in Computer Science 2054, 173-198, (2001).
7. N.C. Seeman, DNA Nicks and Nodes and Nanotechnology, *NanoLett.* 1, 22-26 (2001).
8. H. Yan, X. Zhang, Z. Shen and N.C. Seeman, A Robust DNA Mechanical Device Controlled by Hybridization Topology, *Nature* 415, 62-65 (2002).
9. N.C. Seeman, DNA Nanotechnology: Life's Central Performer in a New Role, *Biol. Phys. Newslett.* 2 (1) 2-6 (2002).
10. N.C. Seeman, It Started with Watson and Crick, But it Sure Didn't End There: Pitfalls and Possibilities beyond the Classic Double Helix, *Natural Computing* 1, 53-84 (2002).
11. N.C. Seeman & A.M. Belcher, Emulating Biology: Nanotechnology from the Bottom Up, *Proc. Nat. Acad. Sci. (USA)* 99 (supp. 2), 6451-6455 (2002).
12. N.C. Seeman, Key Experimental Approaches in DNA Nanotechnology, *Current Protocols in Nucleic Acid Chemistry*, Unit 12.1, John Wiley & Sons, New York (2002).
13. A. Carbone and N.C. Seeman, Circuits and Programmable Self-Assembling DNA Structures, *Proc. Nat. Acad. Sci. (USA)* 99 12577-12582 (2002).
14. X. Zhang, H. Yan, Z. Shen and N.C. Seeman, Paranemic Cohesion of Topologically-Closed DNA Molecules, *J Am. Chem. Soc.* 124, 12940-12941 (2002).
15. S. Xiao, F. Liu, A. Rosen, J.F. Hainfeld, N.C. Seeman, K.M. Musier-Forsyth & R.A. Kiehl, Self-Assembly of Nanoparticle Arrays by DNA Scaffolding, *J. Nanopart. Res.* 4, 313-317 (2002).
16. A. Carbone and N.C. Seeman, Fractal Designs Based on DNA Parallelogram Structures, *Natural Computing* 1, 469-480 (2002).
17. L. Zhu, O. dos Santos, N.C. Seeman and J.W. Canary, Reaction of N³-Benzoyl-3', 5'-O-(di-tert-butylsilyl)uridine with Hindered Electrophiles: Intermolecular N³ to 2'-O Protecting Group Transfer, *Nucleosides, Nucleotides & Nucl. Acids* 21, 723-735 (2002).
18. N.C. Seeman, DNA in a Material World, *Nature* 421, 427-431 (2003).
19. N.C. Seeman, Structural DNA Nanotechnology: A New Organizing Principle for Advanced Nanomaterials, *Materials Today* 6 (7), 24-29 (2003).
20. P. Sa-Ardyen, N. Jonoska and N.C. Seeman, Self-Assembling DNA Graphs, *DNA-Based Computers VIII*, LNCS 2568, Springer-Verlag, Berlin, 1-9 (2003).
21. H. Yan and N.C. Seeman, Edge-Sharing Motifs in DNA Nanotechnology. *J. Supramol. Chem.* 1, 229-237 (2003).
22. P. Sa-Ardyen, A.V. Vologodskii and N.C. Seeman, The Flexibility of DNA Double Crossover Molecules. *Biophys. J.* 84, 3829-3837 (2003).
23. N. Jonoska, P. Sa-Ardyen and N.C. Seeman, Computation by Self-Assembly of DNA Graphs, *J. Genetic Programming & Evolvable Machines* 4, 123-137 (2003).

24. N.C. Seeman, Biochemistry and Structural DNA Nanotechnology: An Evolving Symbiotic Relationship, *Biochem.* **42**, 7259-7269 (2003).
25. L. Zhu, P.S. Lukeman, J. Canary & N.C. Seeman, Nylon/DNA: Single-Stranded DNA with Covalently Stitched Nylon Lining, *J. Am. Chem. Soc.* **125**, 10178-10179 (2003).
26. A. Carbone and N.C. Seeman, Coding and Geometrical Shapes in Nanostructures: a Fractal DNA-Assembly, *Natural Computing* **2**, 133-151 (2003).
27. N.C. Seeman, DNA: Beyond the Double Helix, *Macromolecular Symposia* **201**, 237-244 (2003).
28. N.C. Seeman, At the Crossroads of Chemistry, Biology and Materials: Structural DNA Nanotechnology, *Chem. & Biol.* **10**, 1151-1159 (2003).
29. P. Sa-Ardyen, N. Jonoska and N.C. Seeman, Self-Assembling DNA Graphs, *Natural Computing*, **2**, 427-438 (2003).
30. N.C. Seeman, DNA Nanostructures for Mechanics and Computing: Nonlinear Thinking With Life's Central Molecule. *NanoBiotechnology*. Editors, Chad Mirkin and Christof Niemeyer, Wiley-VCH Verlag GmbH & Co., Weinheim, Chapter 20, pp 308-318 (2004).
31. Z. Shen, H. Yan, T. Wang and N.C. Seeman, Paranemic Crossover DNA: A Generalized Holliday Structure with Applications in Nanotechnology *J. Am. Chem. Soc.* **126**, 1666-1674 (2004).
32. N. Jonoska, S. Liao, & N.C. Seeman, Transducers with programmable input by DNA Self-assembly, *Aspects of Molecular Computing, Lecture Notes in Computer Science* **2340**, Springer-Verlag, Berlin, 219-240 (2004).
33. A. Carbone & N.C. Seeman, Molecular Tiling and DNA Self-Assembly, *Aspects of Molecular Computing, Lecture Notes in Computer Science* **2340**, Springer-Verlag, Berlin, 61-83 (2004).
34. N.C. Seeman. Nanotechnology and the Double Helix, *Sci. Am.*, **290**, (6) 64-75 (2004).
35. P. Sa-Ardyen, N. Jonoska and N.C. Seeman, The Construction of Graphs Whose Edges are DNA Helix Axes, *J. Am. Chem. Soc.*, **126**, 6648-6657 (2004).
36. W.B. Sherman and N.C. Seeman, A Precisely Controlled DNA Bipedal Walking Device, *NanoLett.* **4**, 1203-1207 (2004).
37. P.S. Lukeman, A. Mittal, & N.C. Seeman, Two Dimensional PNA/DNA Arrays: Estimating the Helicity of Unusual Nucleic Acid Polymers, *Chem. Comm.* **2004**, 1694-1695 (2004).
38. N.C. Seeman, DNA Nanotechnology, *Encyclopedia of Supramolecular Chemistry*, Marcel Dekker, New York, 475-483 (2004).
39. A. Carbone, C. Mao, P. Constantinou, B. Ding, J. Kopatsch, W. B. Sherman and N.C. Seeman, 3D Fractal DNA Assembly from Coding, Geometry and Protection, *Natural Computing* **3**, 235-252 (2004).
40. N.C. Seeman. Structural DNA Nanotechnology: An Overview. *Bionanotechnology Protocols*, Editors, Sandra Rosenthal and David W. Wright, in press (2004).
41. M. Cavaliere, N. Jonoska and N.C. Seeman, Implementing Computing Devices with Unbounded Memories Using Biomolecules *DNA-10*, in press (2004).
42. P. Paukstelis, J. Nowakowski, J.J. Birktoft and N.C. Seeman, An Oligonucleotide that Self-Assembles into a 3DCrystalline Lattice, *Chem. & Biol., Biology* **1**, 1119-1126 (2004).
43. W. Shen, M. Bruist, S. Goodman & N.C. Seeman, A Nanomechanical Device for Measuring the Excess Binding Energy of Proteins that Distort DNA, *Angew. Chem Int. Ed.* **43**, 4750-4752 (2004).

44. B. Ding, R. Sha & N.C. Seeman, Trigonal Motifs from DX DNA Triangles, *J. Am. Chem. Soc.* **126**, 10230-10231 (2004).
45. C. Mao, P.E. Constantinou, F. Liu, Y. Pinto, J. Kopatsch, P.S. Lukeman, T. Wang, B. Ding, H. Yan, J.J. Birktoft, R. Sha, H.Zhong, L. Foley, L.A. Wenzler, R. Sweet, M. Becker and N.C. Seeman, The Design of Self-Assembled 3D DNA Networks, *Proc. Electrochem. Soc.* in press (2004).
46. S. Liao & N.C. Seeman, Translation of DNA Signals into Polymer Assembly Instructions, *Science*, in press (2004).