ANV413-BI79-03 ARI 27 April 2010 17:56

or helper strands are added. The staple strands are used to fold the scaffolding strand into a well-defined shape. The first example of a scaffolding strand in structural DNA nanotechnology was reported by Yan et al. (38), who used a scaffolding strand to make a one-dimensional barcode array. This application of scaffolding was followed quickly by Shih et al. (30), who used a long strand of DNA with five short helper strands to build an octahedron held together by PX cohesion (39). However, neither of these two advances had the dramatic impact of Rothemund's 2006 publication (21). He demonstrated that he was able to take single-stranded M13 viral DNA and get it to fold into a variety of shapes, including a smiley face (Figure 6a). One of the great advantages of this achievement is that it creates an addressable surface area roughly 100 nm square. One can use the DX+J motif (Figure 3b) developed for patterning two-dimensional arrays (40) (see below) to place patterns on DNA origami constructs. An example is seen in Figure 6b, which shows a map of the Western Hemisphere. DNA origami has become widely used since it was introduced and has been employed for embedding nanomechanical devices (41), for making long six-helix bundles (42), for use as an aid to NMR structure determination (43), and for building three-dimensional objects (22), including a box that can be locked and unlocked, with potential uses in therapeutic delivery (44).

4. CRYSTALLINE ARRAYS

The original goal of structural DNA nanotechnology was to produce designed periodic matter (9). The first stage of this effort was the assembly of two-dimensional crystals from robust motifs. These two-dimensional crystals could be readily characterized by atomic force microscopy. Two-dimensional crystals are discussed in Section 4.1. Three-dimensional crystals have been assembled recently. They have been characterized by X-ray crystallographic methods, which require a more highly ordered sample than AFM. Three-dimensional crystals are discussed in Section 4.2.

4.1. Two-Dimensional Crystals

To generate periodic matter, it is necessary to have robust motifs that do not bend and flex readily; otherwise, a repeating pattern could fold up to form a cycle, poisoning the growth of the array. The DX molecule (see Figure 3b) was the first molecule shown to be sufficiently rigid for this purpose (13, 45). The molecule was quickly exploited to produce periodic matter in two dimensions (40). The DX+J motif was used to impose patterns on these arrays. When DX+J molecules were included specifically in the pattern, deliberately striped features could be seen in the AFM, as shown in Figure 7. DNA motifs that form two-dimensional periodic (or aperiodic) arrays are often called “tiles” because they can tile the plane. There are numerous tiles that have been developed to tile the two-dimensional plane, including the three-domain TX tile (14), the six-helix hexagonal bundle (42), and the DX triangle (46). For reasons that are not well understood, two-dimensional crystals are relatively small, typically no more than a few micrometers in either dimension. Two-dimensional crystals have proved to be an extremely good way to introduce students and other new investigators to structural DNA nanotechnology. The preparation of the AB∗ or ABCD∗ array (Figure 7), for example, is

Soumitra Athavale
SED Group Meeting
9 June 2015
Supramolecular chemistry: Beyond Covalent bonds

‘The whole is greater than its parts.’

- Formal Distinction between traditional covalent chemistry and self assembly.

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**Molecular chemistry**

- Covalent molecule:
  - Chemical nature
  - Shape
  - Redox properties
  - HOMO - LUMO gap
  - Polarity
  - Vibration and rotation
  - Magnetism
  - Chirality

**Supramolecular chemistry**

- Specific characteristic, function or properties:
  - Recognition
  - Catalysis
  - Transport

- Supramolecule (complex):
  - Degree of order
  - Interactions between subunits
  - Symmetry of packing
  - Intermolecular interactions

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*Supramolecular Chemistry (2009), 2nd ed., Steed and Atwood, John Wiley and Sons*
Since it’s formalization in the 1960-70’s, methods to build supramolecular assemblies using organic molecules and scaffolds have resulted in an astonishing array of new materials.

Molecular recognition using non covalent interactions have opened doors to host-guest complexes, topologically curious structures, dynamic molecular assemblies and responsive molecules.
DNA – A King of Self Assembly

- A polymer with a ‘nucleotide code’ to mark locations.
- Precise base pairing with an absolutely predictable structure output.
- Formation of the duplex is under thermodynamic control.
- A myriad of enzymes available to modify the resulting polymer at specific locations.
- Nucleic acid synthesis is now a mature field and oligonucleotides upto 150 bases are routinely prepared.
Solid Phase DNA synthesis

Solid Phase Phosphoramidite DNA synthesis

1. Detritylation
2. Coupling
3. Capping
4. Oxidation ($X = O$)
   OR
3. Sulfurization ($X = S$)
4. Capping

Image: http://commons.wikimedia.org/wiki/File:Oligocycle1.png
Hybridization is the basis for combining DNA strands

- DNA hybridization is highly sequence dependent. DNA molecules with complementary sequences will ‘anneal’.

- Conversely, if a duplex structure is heated, ‘melting’ of the two strands will occur at a sharp temperature ($T_m$).

- Generally, $T_m$ for a 30-40bp duplex will be between 60-80°C.

- This essentially means that heating the DNA sample above 90°C and cooling it to RT assures formation of a predictable, sequence-directed duplex structure.

http://www.atdbio.com/content/53/DNA-duplex-stability
Annealing gives the thermodynamically stable product

1. Heat
2. Cool

- The thermodynamically stable product is one where maximum uninterrupted base pairing is attained. The process is also kinetically enforced.
- Such a stable product is extremely easy to design/predict by looking at the sequence of participating molecules.
DNA as a building block: Foundational ideas: Nadrian Seeman (1982)

- In his cryptic 1982 paper, Seeman presented ideas to construct nucleic acid junctions.

- Naturally occurring junctions like the one shown here are unstable due to symmetry.

- He gave a list of sequence selection rules that would guarantee a stable arrangement of DNA fragments.

• A stable ‘rank 4’ junction:

(1) Every criton in the individual strands forming the junction must be unique throughout all strands, regardless of frame.
(2) The anti-criton to any criton which spans a bend in a strand must not be present in any strand, regardless of frame.
(3) Self-complementary critons are not permitted. If $N_c$ is an odd number, this junction holds for all critons of size $(N_c + 1)$.
(4) The same base pair can only abut the junction twice. If it is present twice, those two occurrences must be on adjacent arms.

Reduction of symmetry restricts junction migration.
Basically, the ‘Seeman rules’ articulated:

1. Minimizing symmetry around the junction.
2. Minimizing unwanted complementarity in participating strands
3. Prevention of long stretches of G’s
4. Avoiding homopolymer, polypurine and polypyrimidine tracks or anything which is symmetric in the broadest sense of the term.

Formalization of these rules enabled Seeman to write a FORTRAN based programme to design fragment strands

- The rigid rank 4 junction can now be stitched together with designed ‘sticky end valencies’.

Synthesis of a DNA cube

- A ‘convergent’ synthesis of a topological cube starting from 12 single strand oligos of lengths 50-80 bases.

Chen and Seeman, Nature (1991), 350, 631..
The overhanging sticky ends – C,C’ and D,D’ are designed with sequence complimentarity to guide the ligation....

...So are A,A’ and B,B’ which will be closed in the last step.

The final species was only a cube ‘topologically’. Geometrically it might have been a rhombohedral looking object.

Characterizations were essentially done by gel shift arguments.

Seeman recalls, “We went through a bunch of tricks to make the cube; it was basically a reconstitution of single-stranded species. It was a nightmare!”

Paul Weiss, ACSNano (2008), 6, 1089
Other Geometries were realized

Truncated octahedron (Seeman, 1994)
Borromean rings (Seeman, 1997)
Protein encapsulation in a tetrahedron (Turberfield, 2006)

A General Self assembly method for 3-D polyhedra, 70-90% yield (Shih, 2009)

Zhang and Seeman, JACS (1994), 116, 1661.
Mao, Sun and Seeman, Nature (1997), 386, 137.
Erben et al., ACIE (2006), 45, 7414.
Building blocks – Design motifs

• The simple rank-4 junction was not rigid enough to support a larger self assembled network.

• The ‘Crossover tile’ was designed as a stiffer, stronger, building block.
2-D arrays with DX tiles

- DAO is a synonym for the DX tile.
- Each tile has 6bp- sticky ends which enable self assembly
- Thus, the tiles are stitched together to form a 2-D network.
- Characterization can now be done with AFM

• AFM images of the self-assembled array.

• Periodicity of tiling can be seen (e).

• The process is extremely simple:

“All strands are mixed stoichiometrically, heated to 95°C and cooled slowly over 40hrs. Even undergrads and high school students usually produce beautiful AFM images on the first pass. There are some experiments that can discourage new investigators in DNA assembly but making 2-D arrays is not one of them.”

DNA strand Displacement

- Strand displacement is the process through which two strands with partial or full complementarity hybridize to each other, displacing one or more pre-hybridized strands in the process.

- The ‘reaction’ is generally rapid and quantitative since the process is highly thermodynamically favourable.

- The resulting new duplex (L) must have more continuous base pairs than the original one (S).

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Hang and Winfree, JACS (2009), 131, 17303.
Use of ‘toeholds’ is critical for controlling the kinetics of strand displacement

1. Toehold domains initiate binding

2. Domain 2 undergoes branch migration

3. Strand displacement completes

- Toehold mediated strand displacement results in rate constants upto 6 magnitudes higher for the forward reaction.

- In this case, the toehold domain (3) allows strand A to localize specifically on complex X. Subsequent equilibrium branch migration is followed by an irreversible displacement of B.

Dynamic DNA assemblies

- A DNA molecular tweezer fuelled by DNA (Turberfield, 2000)

- The open, linear tweezer is shown on the left. Objective is to bring the two fluorophores TET and TAMRA together.

- Strand F has complementary regions to the blue and green sticky ends and closes the the tweezer.

- F also has a toehold (in orange) and can be removed by using its complementary strand F’.

- Thus, a sequential addition of F and F’ can open and close the tweezer.

The idea is to use a modified DX tile that can alter its dimension by strand displacement (Hao Yan, 2003).

The orange and blue strands in the tile can be interchanged, thus changing the length of the tile.
The nanoactuator device is incorporated into a 2D DNA lattice constructed previously by Mao et al.,[3] which was demonstrated to display a rhombuslike cavity with size of ~14 nm in each of the two dimensions. The unit of the previously demonstrated parallelogram contains four four-arm branched junctions (Figure 4 a), which were fused into a rhombuslike molecule. The branch points, which define vertices, are each separated by four double-helical turns. The rhombuses were directed to self-assemble by hydrogen bonding into a two-dimensional periodic array, whose spacing is six turns in each direction. In our design, we modified the molecule and incorporated two nanoactuator devices into two opposite edges of the unit (Figure 4 b). The operation of the nanoactuator devices will result in a contraction/extension motion of the 2D lattice assembled from the designed unit, as illustrated in lower panel of Figure 4 b.

We performed the interconversion of the nanoactuator device in solution and demonstrated the motion of the lattice by imaging samples deposited on mica using atomic force microscopy (AFM). The AFM images in Figure 5 illustrate the dynamic DNA array.

- Strand displacement reaction on the entire array transforms individual tiles from S1 to S2.
- The dimensional changes at the tile level translates to a collective ‘breathing’ of the array.

Feng et al., ACIE (2003), 42, 4342.
DNA walkers

- **Autonomous multistep Organic Synthesis with a DNA walker.** (David Liu, 2010)
  - Synthesis of a tripeptid from activated precursors on a self assembled DNA platform. Inspired by the ribosome.
  - S1-S3 are assembled on template T. The walker W facilitates stepwise formation of the coupling products by moving along the track autonomously.

*He and Liu, Nature Nanotechnology (2010), 5, 778.*
• The Walker has complimentary regions to the fragment anchors (D1-D1’). This enables proximity based coupling to the amine terminus. An inbuilt DNAzyme domain allows cleavage of anchors to move forward.
• Once the Walker is added, no intervention is needed to obtain the final product. An overall tripeptide yield of 45% is reported.
Summary till now

- Hybridization guides predictable self assembly of DNA strands.
- Early examples of DNA structures included polyhedrons constructed by imaginative use of DNA hybridization.
- Introduction of stiff design motifs gave rise to assembly of 2-D network structures.
- Strand Displacement reactions enabled dynamic DNA architectures capable of function.

By this time, DNA had established itself as a superior building block. However, as in the case of classical supramolecular designs, new architectures depended on ingenious use of DNA hybridization and imagination. Larger self assembled structures were limited by the repetitive nature of their building blocks.

*The field was to be transformed by Paul Rothemund’s revolutionary publication in 2006.*
Folding DNA to create Nanoscale shapes and patterns (Paul Rothemund, 2006).

Instead of using building blocks for a ‘bottom-up’ construction, the idea here is to use a long single stranded DNA molecule (~7kb in this case) of known sequence and then fold it into a desired shape by using hundreds of designed, smaller ‘staple’ strands (20-40 bases):
DNA Origami

1. The desired shape is approximated by parallel helices with regular crossovers.
2. The scaffold strand runs through every helix and forms more crossovers.
3. Staple strands with crossovers realize these helices and produce the complete structure.
4. The whole process is executed by a computer written programme.
5. Resulting structures are characterized by AFM imaging.
Any desired shape can be accessed. Yields are greater than 70%

Patterning with staple strands:
Staple strands specify location on the scaffold. Thus, the shape can be regiospecifically marked by modifying the necessary staple strand.
Patterning with staple strands:
Markers on staple strands are seen as distinct topological features on the AFM.
3-D DNA Origami

- The folding of a scaffold strand was soon extended to attain 3-D architectures.
- Shih and coworkers developed ‘caDNAno’ an open source software for design of DNA architectures. The software designs all staple strands based on the desired shape.

“With caDNAno, an individual with no prior knowledge of programming or DNA structure can complete a short tutorial and then be capable of generating sequences within a day for building a new shape comparable in complexity to the examples demonstrated here.”

**DNA based self Assembly of Chiral plasmonic nanostructures**

a) Functionalized staple strands on the cylindrical backbone allow precise attachment of gold nanoparticles.

b) Left and right handed helices seen in TEM.

c) CD for the chiral nanostructures

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*Kuzyk et. al., Nature (2012), 483, 311.*
Dynamic Origami Architectures

Self Assembly of a nanoscale DNA box with a controllable lid

- A single scaffold folded into a cube and lid.
- Lid could be opened by strand displacement of the ‘lock’ strands with ‘key’ strands.
- AFM images of an individual box; closed and open.
DNA Nanorobot

A logic gated nanorobot for targeted transport of molecular payloads.

- A ‘mousetrap’ container design that can carry cargo inside (in this case, antibodies).
- The container is locked by aptamer keys to a target protein.
- The protein will bind to the aptamer and open the container, releasing stored cargo.

Douglas, Bachelet and Church, Science (2012), 335, 831.
One obstacle to overcome in constructing a DNA nanorobot was to ensure assembly guide staples were inserted into the intended cargo (Fig. 1D). After folding and purification, the cargo was loaded in molar excess to attachment sites and purified by adding a 10:1 excess of fully complementary oligos to dissociate any remaining locked complexes. The lock mechanism is thus equivalent to a logical AND gate, with possible inputs of cell surface antibody fragments or HLA-A/B/C and various Fab’ antibody fragments expressed in human leukocyte antigen (HLA) classes I and II. To examine nanorobot function, we selected a type of cargo to load: 5-nm gold nanoparticles. Both locks needed to be opened simultaneously to activate the robot. The robot remained inactive when only one of the two locks was opened. The lock can be stabilized in an inactive state by the antigen key (red). Unless otherwise noted, the lock and a partially complementary strand (or stapled) were in the rear by the scaffold hinges. (Black). Nanorobots can be subsequently activated by interaction with antigen ligand-bearing 8-base toeholds (magenta) can be loaded inside the nanorobot. (Blue). The lock mechanism thus ensures assembly of the robot to 97.5% yield in its closed state as assessed by manual counting of nanorobots images by TEM (fig. S17). We used key-neutralizing antibodies in competitive inhibition controls to confirm that the lock mechanism is working as intended.

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DNA nanorobot

- The robot can recognize and conditionally deliver cargo to cell types depending on their cell surface proteins (keys).

**B**

![Diagram](image)

**C**

![Diagram](image)
• DNA origami can be used to create any 3-D shape of one’s choice.

• The staple strands are also regiospecific markers for functionality.

• The extreme simplicity of this procedure has ushered a new era in nano-architecture.

• The researcher can now concentrate his creative faculties in deciding ‘what’ to build rather than ‘how’ to build it.

• Highly complex and responsive structures can be built based upon Origami guided construction and dynamic nucleic acid chemistry.
• The explosive growth of DNA nanotechnology in the past few decades
Current limitations and future prospects

• Proof of concept studies with DNA nanotechnology have been successful but large scale applications hinge on a simple limitation: the scale of DNA oligonucleotide synthesis.

• The current cost of DNA synthesis is about $0.1 per base for oligonucleotide synthesis on a 25nm scale.

• The overall material cost for preparing ~10nmole of a DNA origami structure then comes out to about $700.

• The price for DNA synthesis has to come down by several orders of magnitude to enable industrial scale production.

• Ultimately, innovations in basic synthetic methods and nucleic acid chemistry will play a crucial role in driving prices down.

• The DNA nanorobot mentioned earlier has been tested in a live animal and is slated for human clinical trials.

Conclusion

• Structural DNA nanotechnology is currently the most robust method to produce precisely controlled nanoscale assemblies.

• In a sense, DNA is now a *topological synthon* in the nanoscale just the way atoms are the building blocks for small molecules.

• The technical aspect of methodologies is relatively simple and today, guided by well designed softwares, many new researchers are entering this highly interdisciplinary field. Many exciting advances are expected in the coming decades.

• Large scale applications will depend on fundamental advances in nucleic acid synthesis methodologies to make the technology commercially viable.

“It is all chemistry of one sort or another, just moving atoms around, whether they are in large groups, small groups, whatever—it is just chemistry.”

– Ned Seeman

*Paul Weiss, ACSNano (2008), 6, 1089.*