Building Architectures with DNA

Soumitra Athavale

SED Group Meeting

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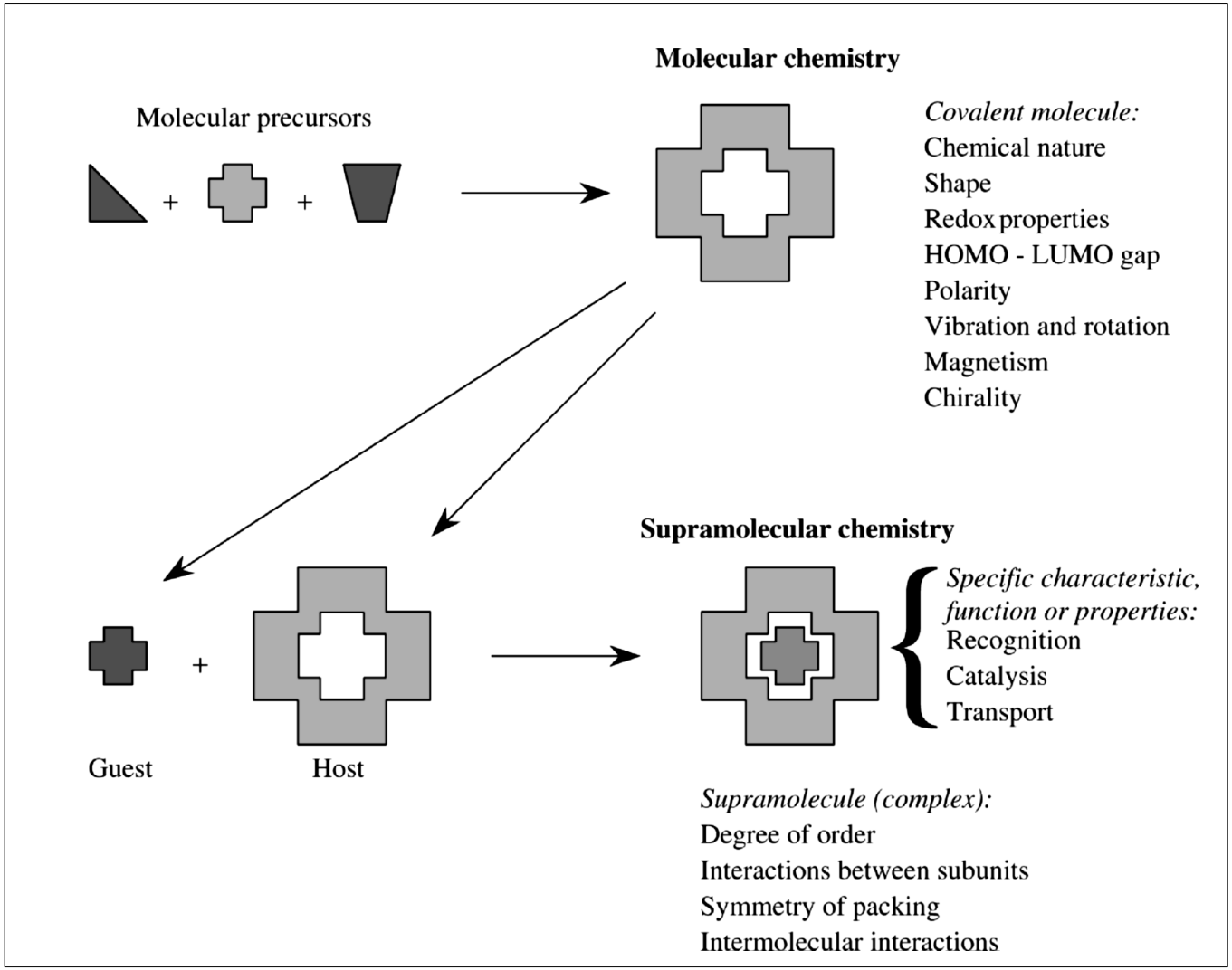
Supramolecular chemistry: Beyond Covalent bonds

'The whole is greater than its parts.'

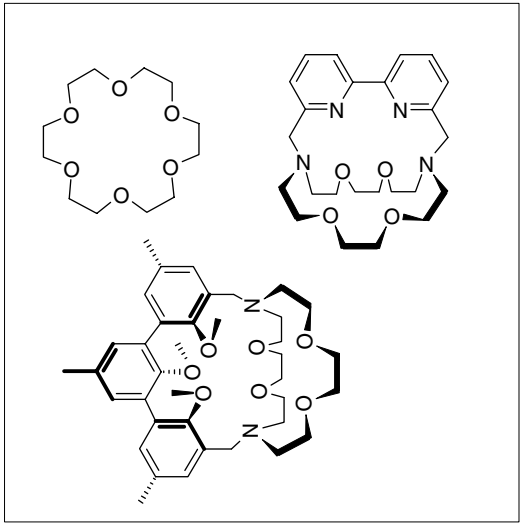
- Formal Distinction between traditional covalent chemistry and self assembly.



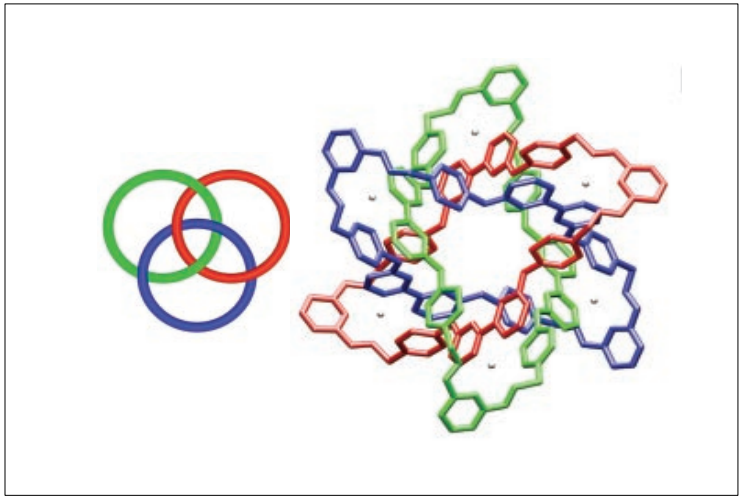
Supramolecular Chemistry (2009), 2nd ed., Steed and Atwood., John Wiley and Sons

- 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.

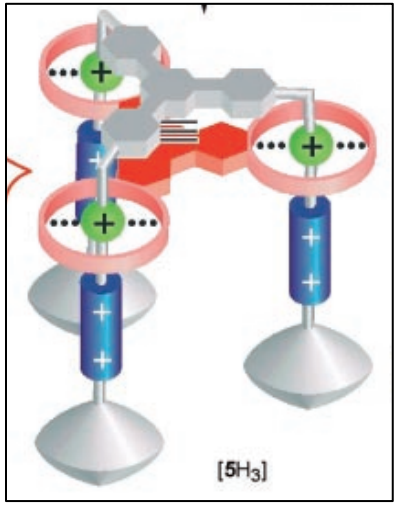
- Molecular recognition using non covalent interactions have opened doors to host-guest complexes, topologically curious structures, dynamic molecular assemblies and responsive molecules.



Supramolecular hosts (Cram, Lehn, Pederson, 1960-1975)



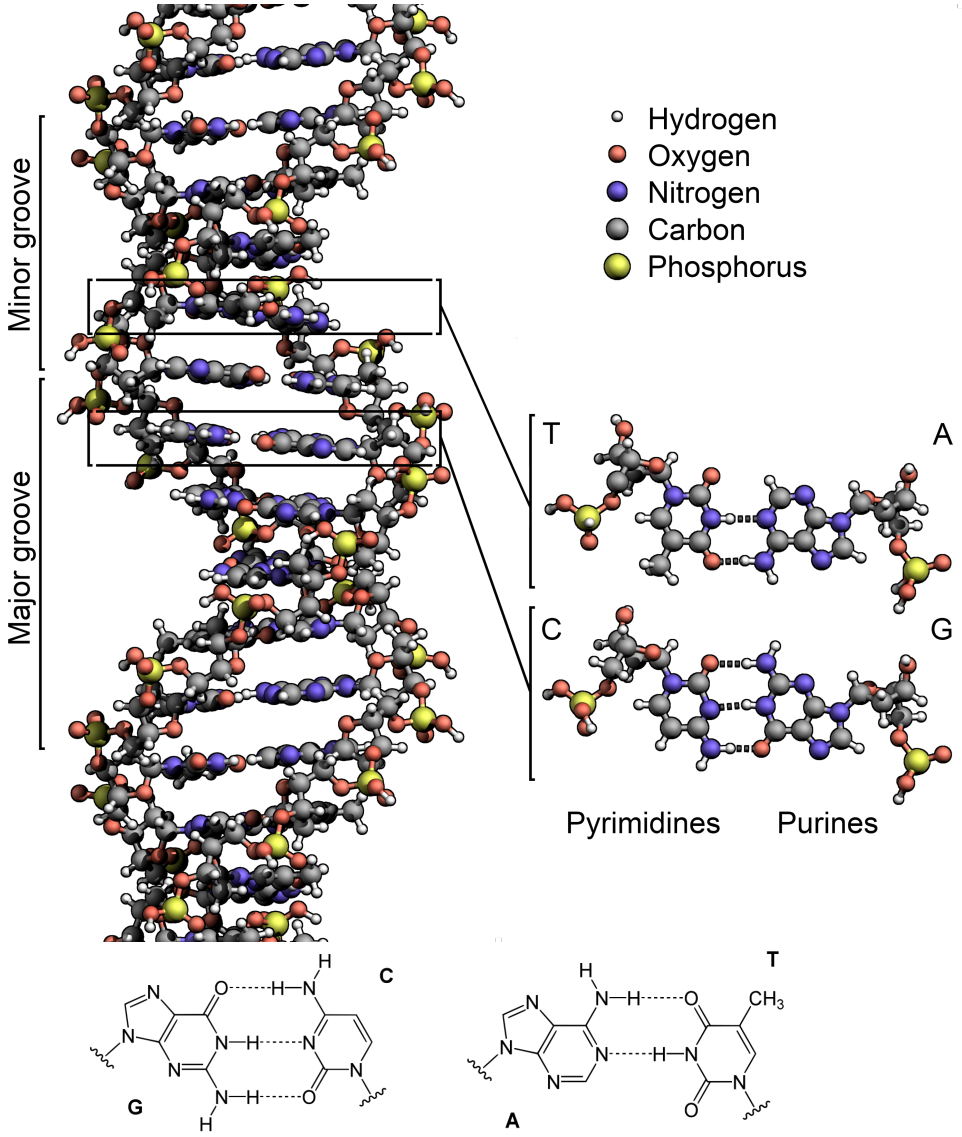
Borromean rings (Stoddard, 2004)



A molecular elevator (Stoddard, 2004)

Supramolecular Chemistry (2009), 2nd ed., Steed and Atwood., John Wiley and Sons
Chichak et.al, Science (2004), 304,1308.
Badjic et. al., Science (2004), 303, 1845

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.

Image: http://upload.wikimedia.org/wikipedia/commons/4/4c/DNA_Structure%2BKey%2BLabelled.pn_NoBB.png

Solid Phase DNA synthesis

Solid Phase Phosphoramidite DNA synthesis

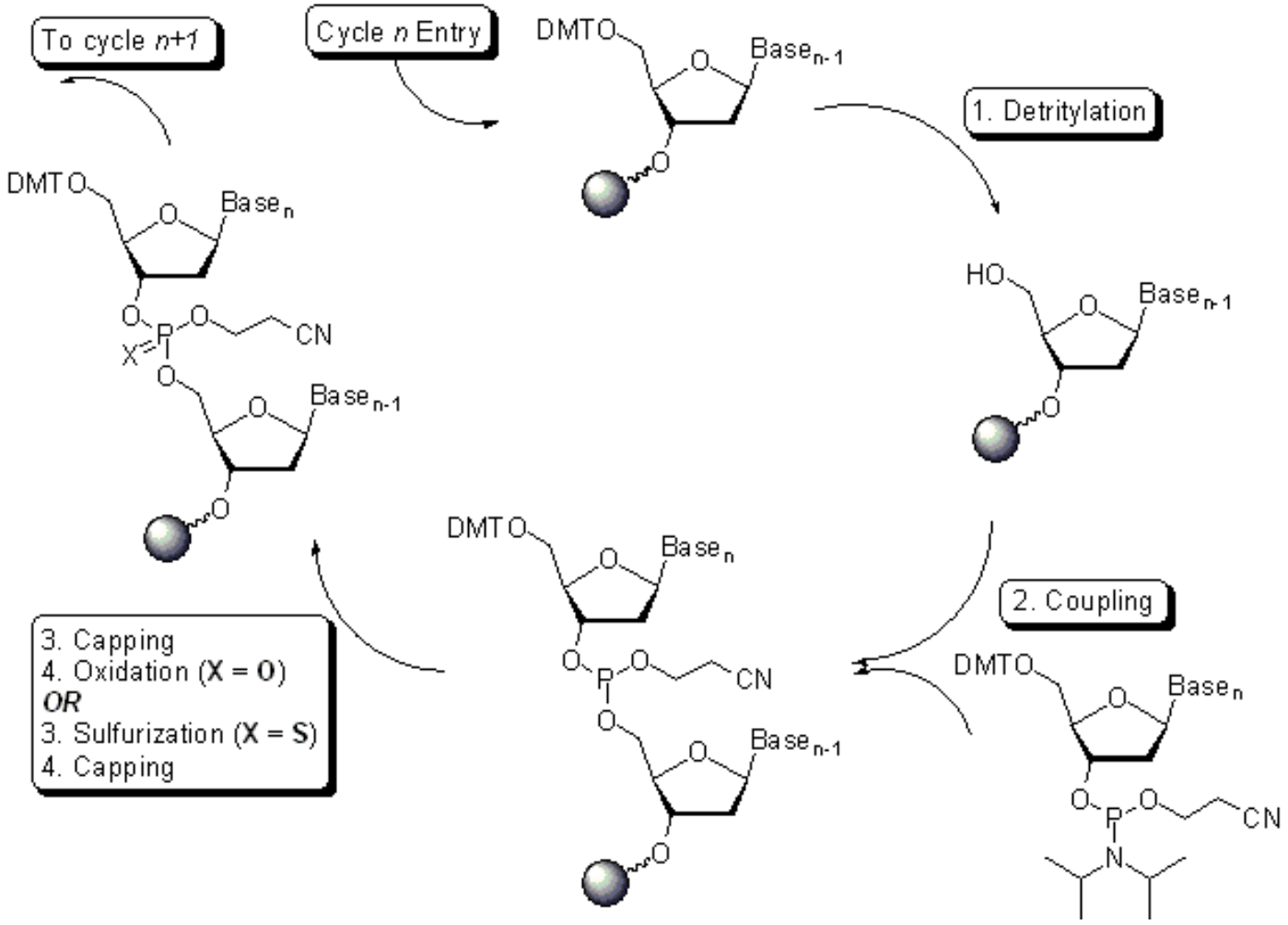
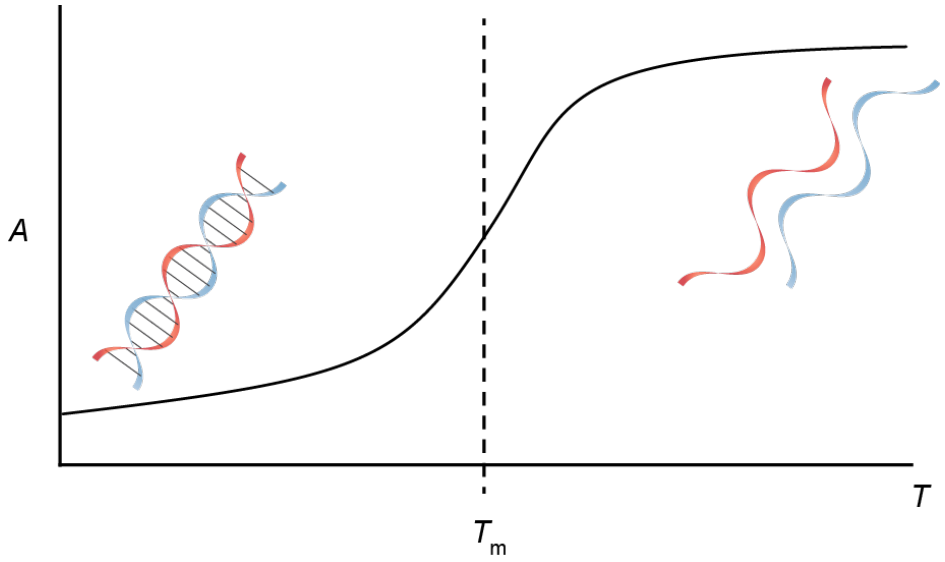


Image: http://commons.wikimedia.org/wiki/File:Oligocycle1.png

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).

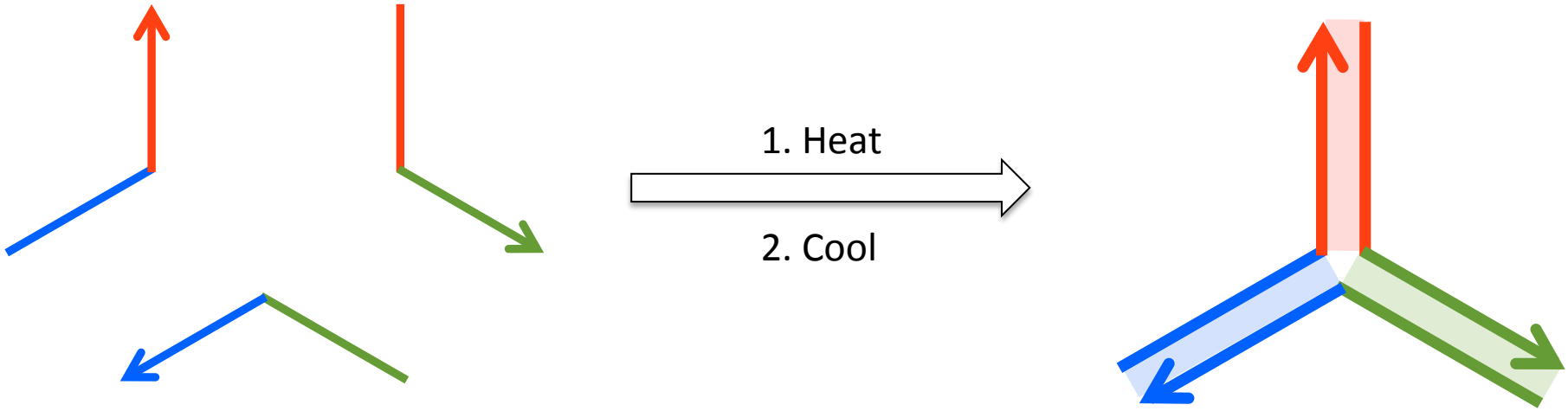


A typical DNA melting curve

- 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

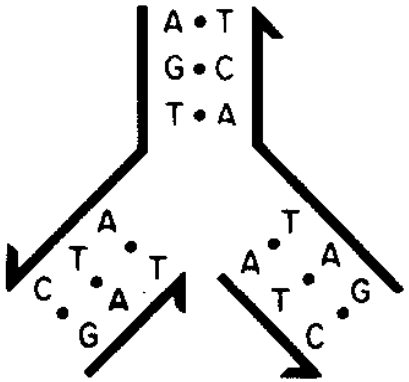


- The thermodynamically stable product is one where maximum uninterrupted base pairing is attained. The process is also kinetically enforced.

- 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.

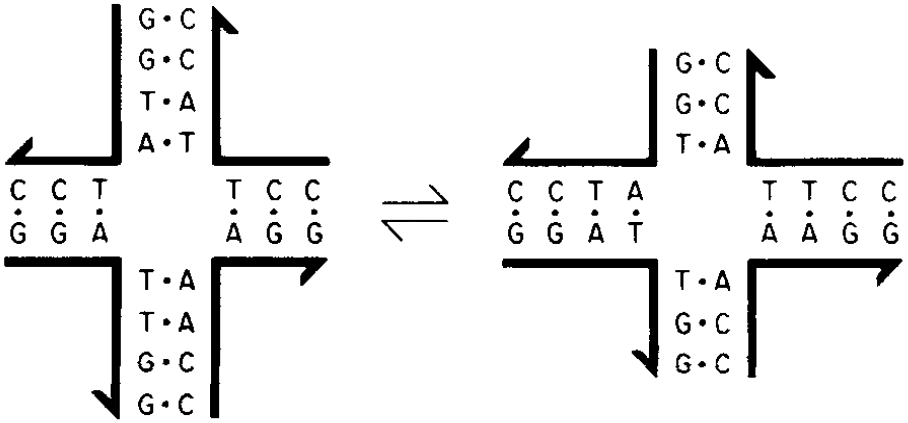


Ned Seeman, 2008

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

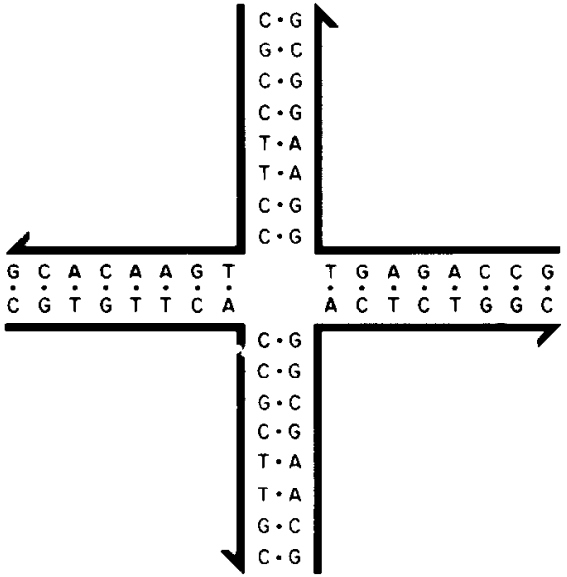
- He gave a list of sequence selection rules that would guarantee a stable arrangement of DNA fragments.

Paul Weiss, ACSNano (2008), 6, 1089.
Seeman, J. Theor. Bio. (1982), 99, 237.



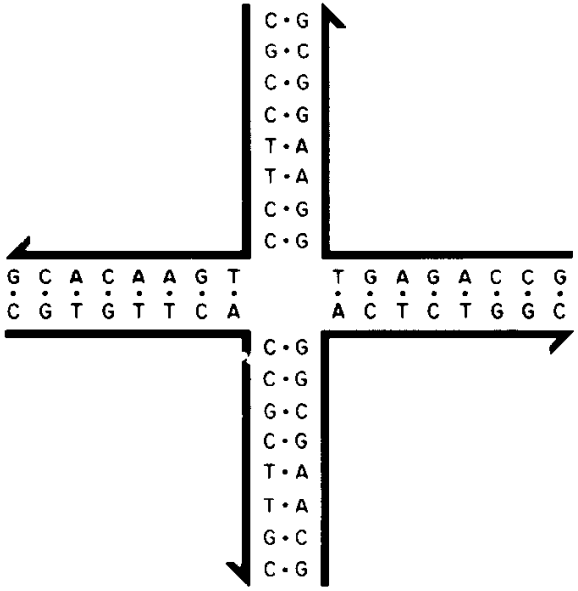
Reduction of symmmetry restricts junction migration.

- 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 injunction 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.

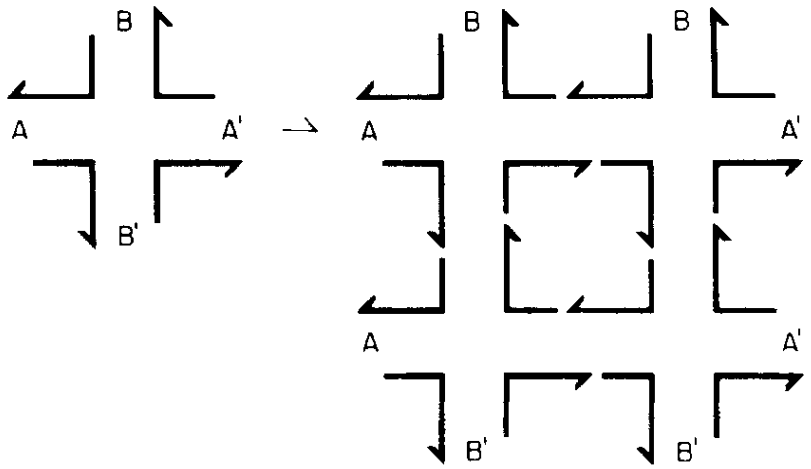
Seeman, J. Theor. Bio. (1982), 99, 237.



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.

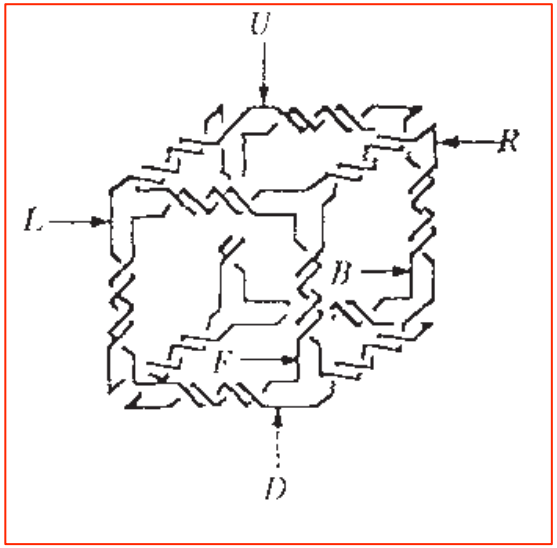
Formalization of these rules enabled Seeman to write a FORTRAN based programme to design fragment strands

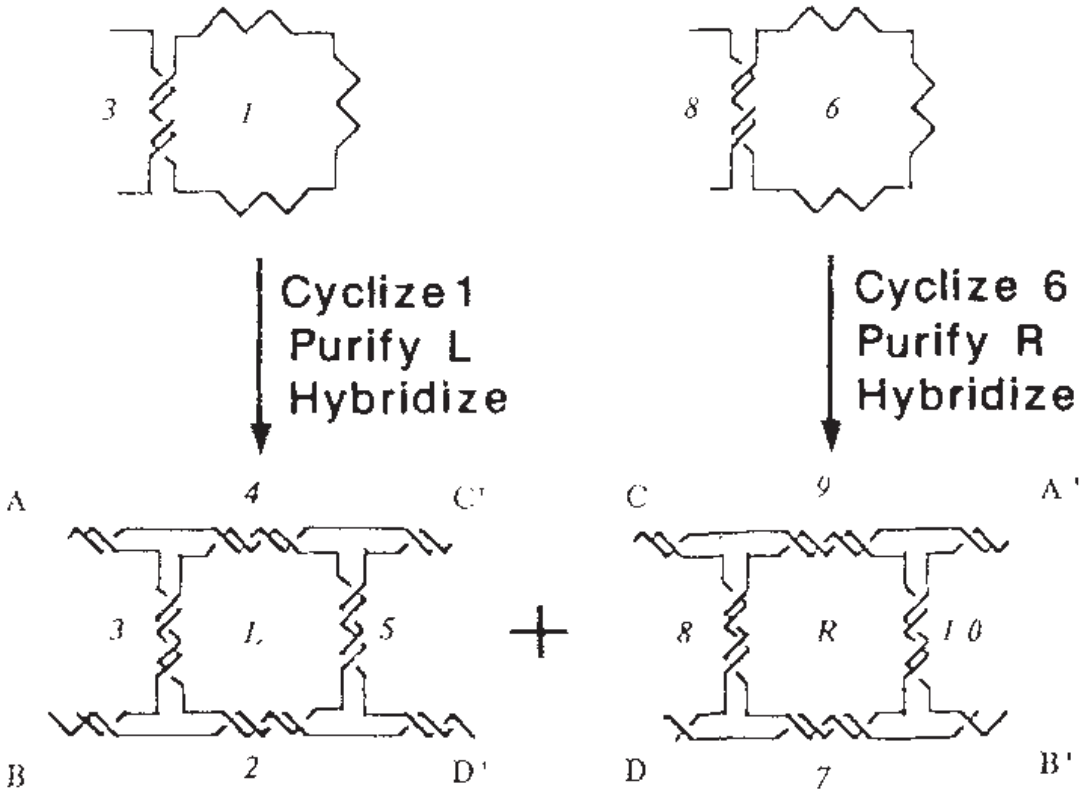


- The rigid rank 4 junction can now be stitched together with designed 'sticky end valencies'.

Seeman, J. Theor. Bio. (1982), 99, 237.

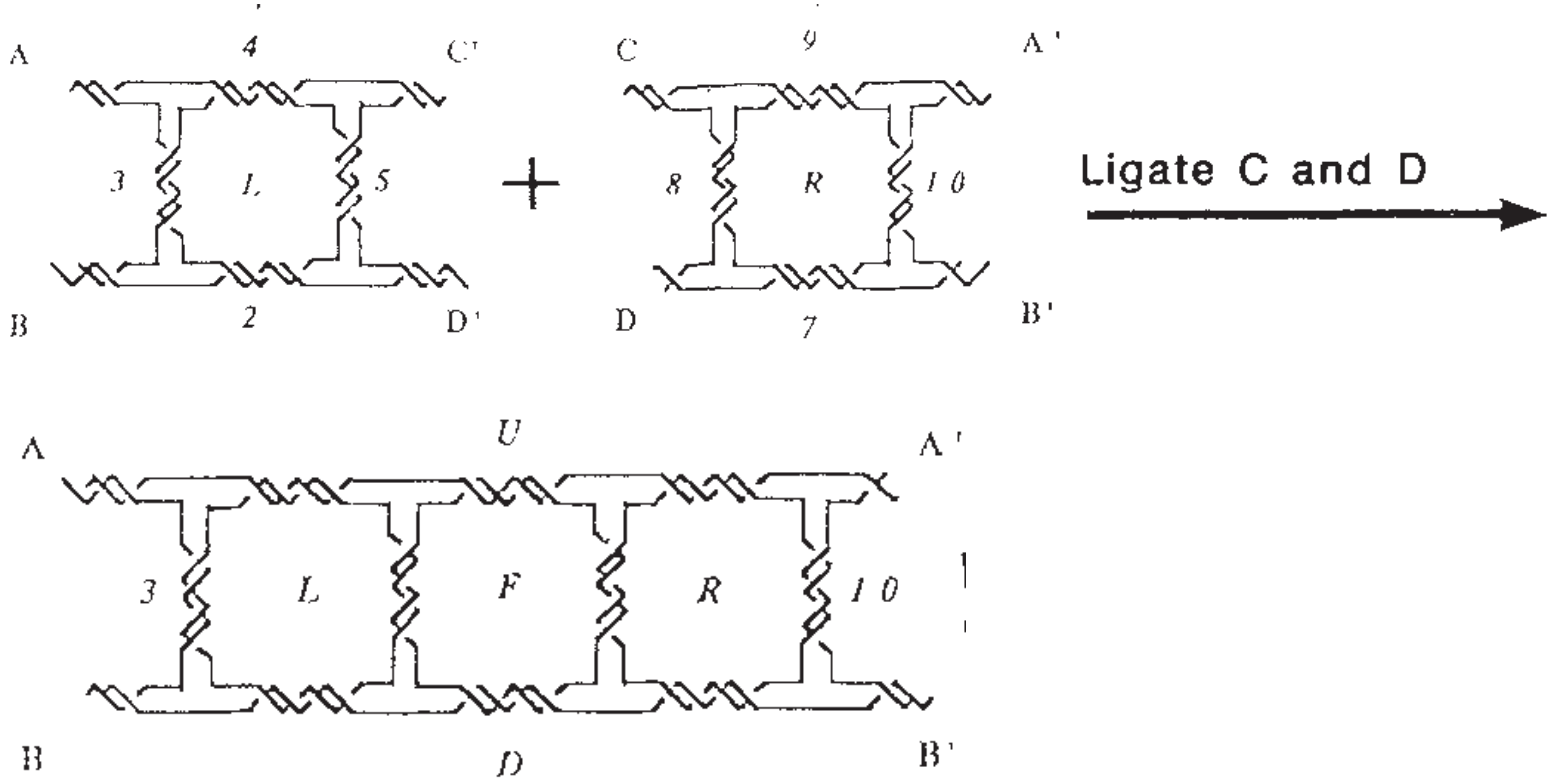
Synthesis of a DNA cube





- 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..

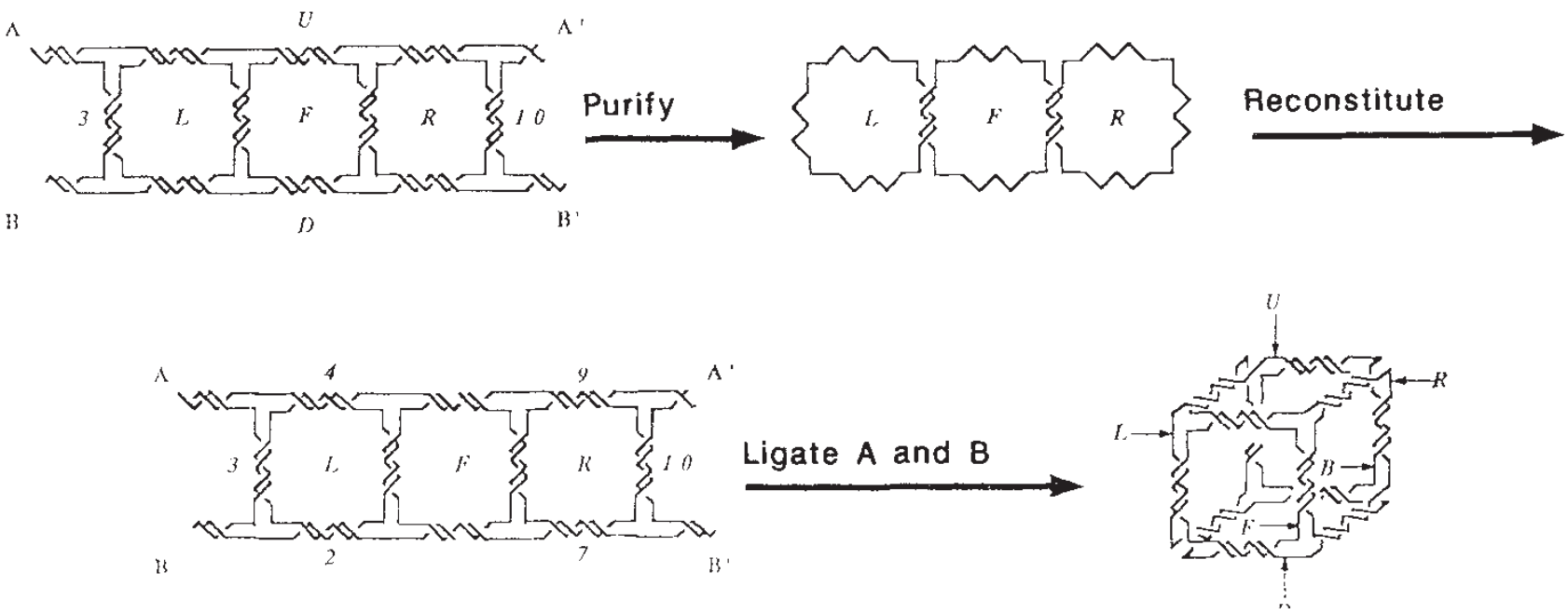


The overhanging sticky ends – C,C' and D,D' are designed with sequence complimentarity to guide the ligation….

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

Chen and Seeman, Nature (1991), 350, 631..

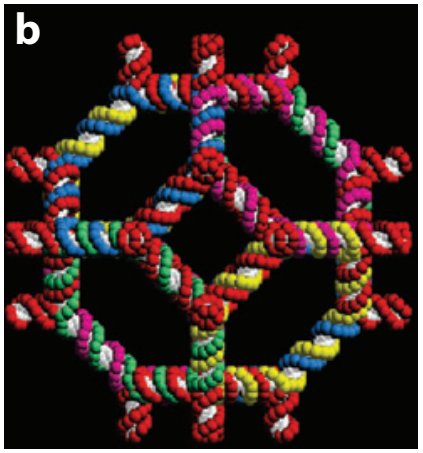
Synthesis of a DNA cube



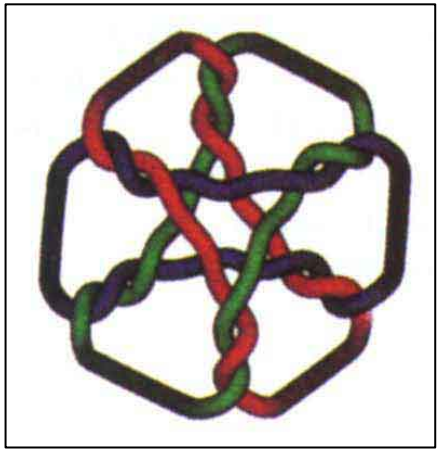
- The final species was only a cube 'topologically'. Geometrically it might have been a rhombohedral looking object.
- Characterizations were essentially done by gel shift arguments
- Seeman recalls , *"We went through a bunch of tricks to make the cube; it was basically a reconstitution of single-stranded species. It was a nightmare!"*

Chen and Seeman, Nature (1991), 350, 631.
. Paul Weiss, ACSNano (2008), 6, 1089

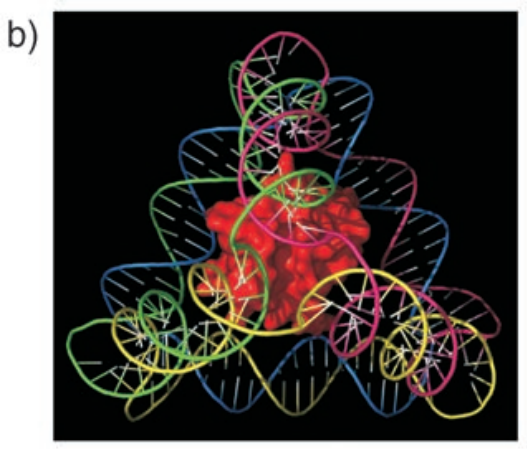
Other Geometries were realized



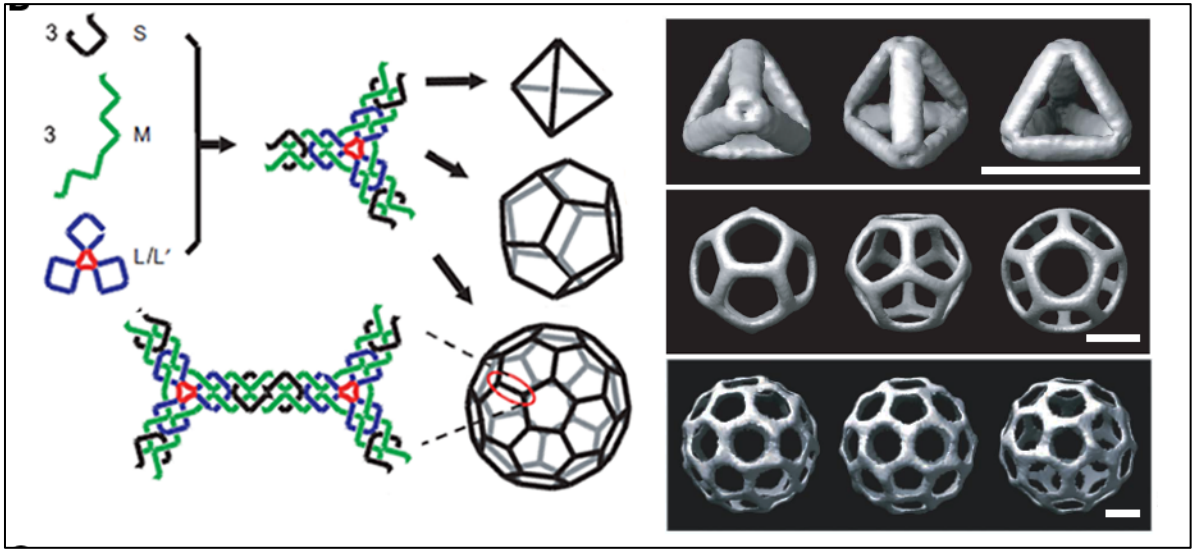
Truncated octahedron
(Seeman, 1994)



Borromean rings
(Seeman, 1997)



Protein encapsulation in a tetrahedron
(Turberfield, 2006)

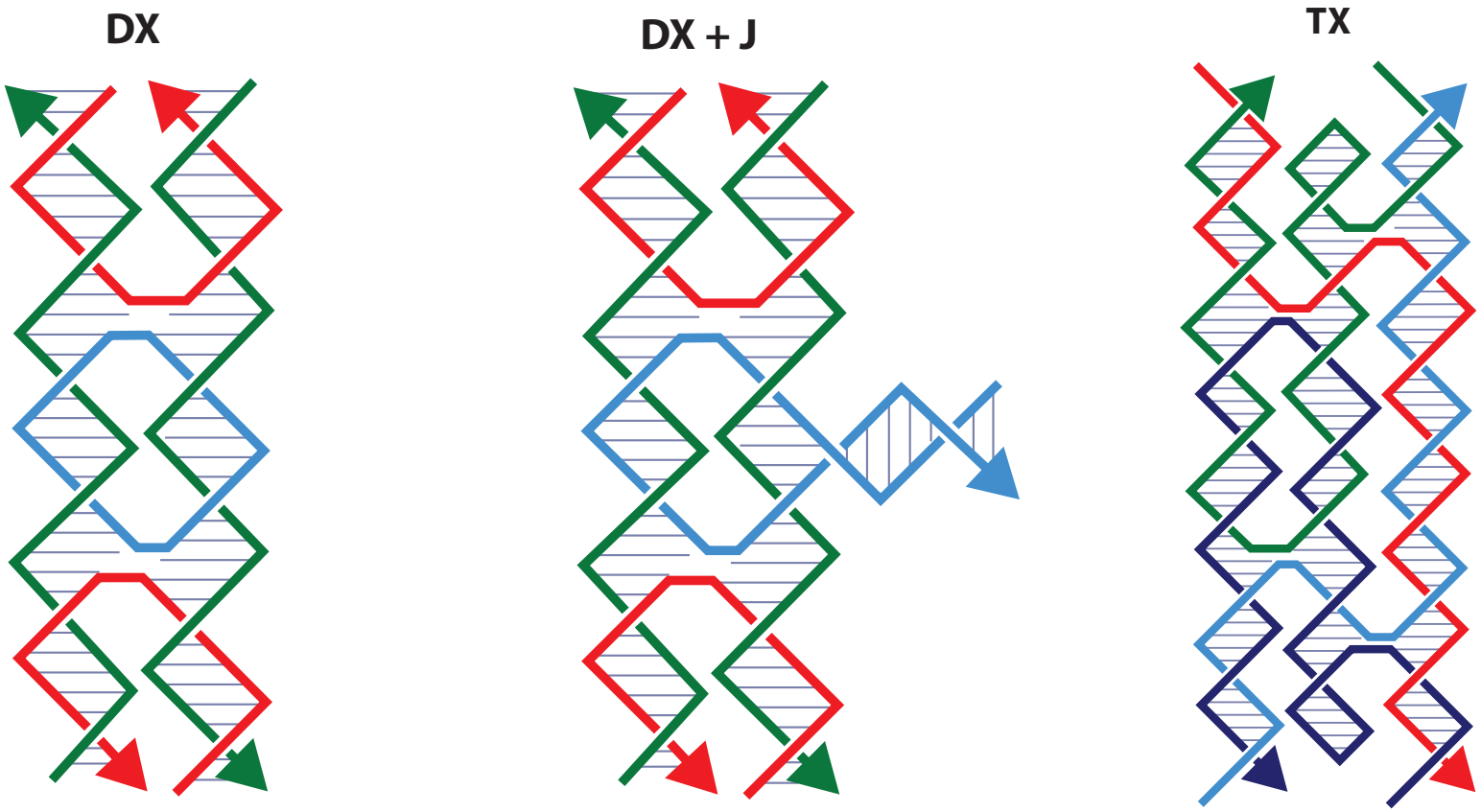


A General Self assembly method for 3-D polyhedra, *70-90% yield*
(Shih, 2009)

Zhang and Seeman, JACS (1994), 116, 1661.
Mao, Sun and Seeman, Nature (1997), 386, 137.
Erben et. al., ACIE (2006), 45, 7414.
Douglas et. al., Nature (2009), 459, 414.

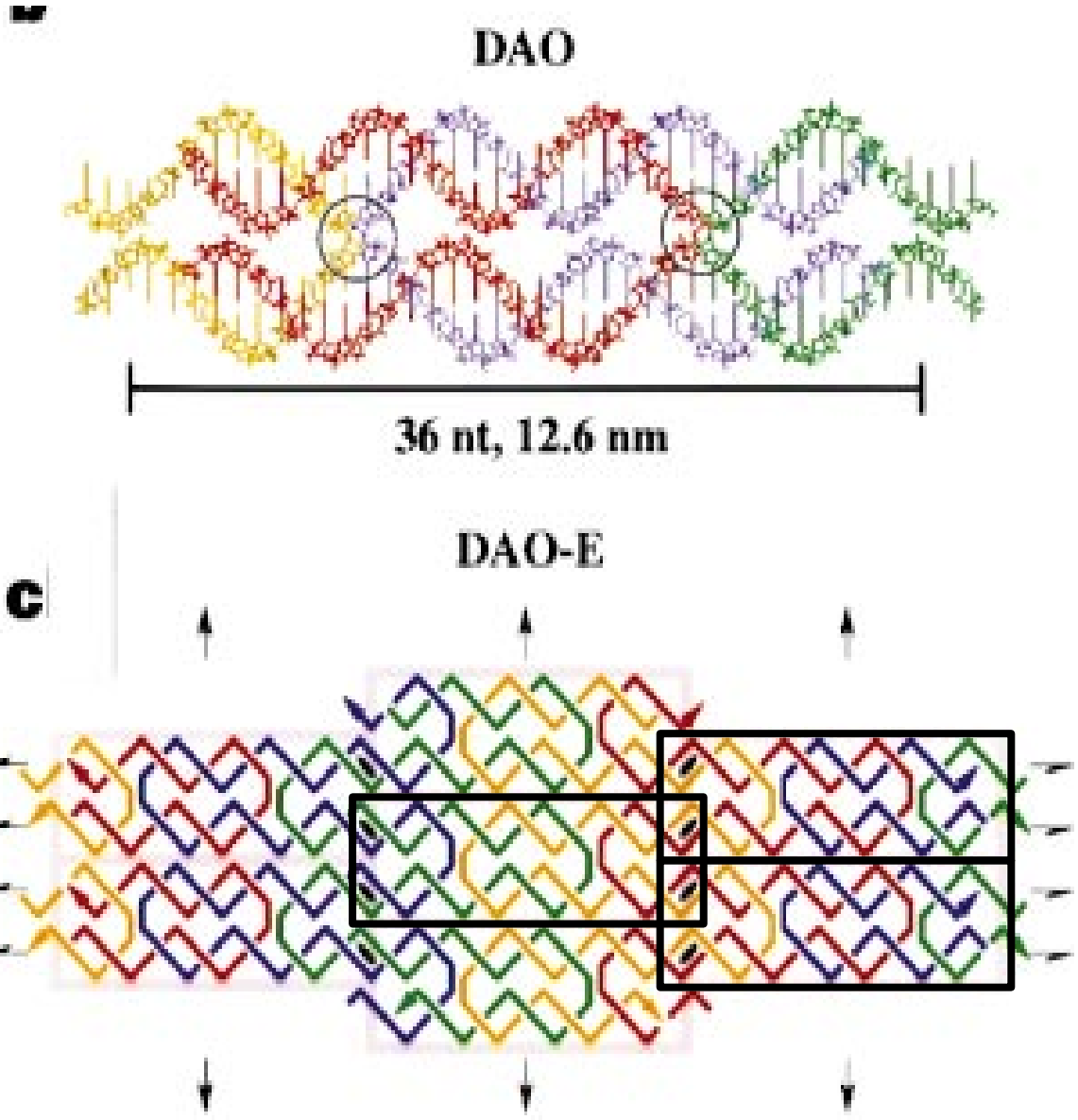
Building blocks – Design motifs

- 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.



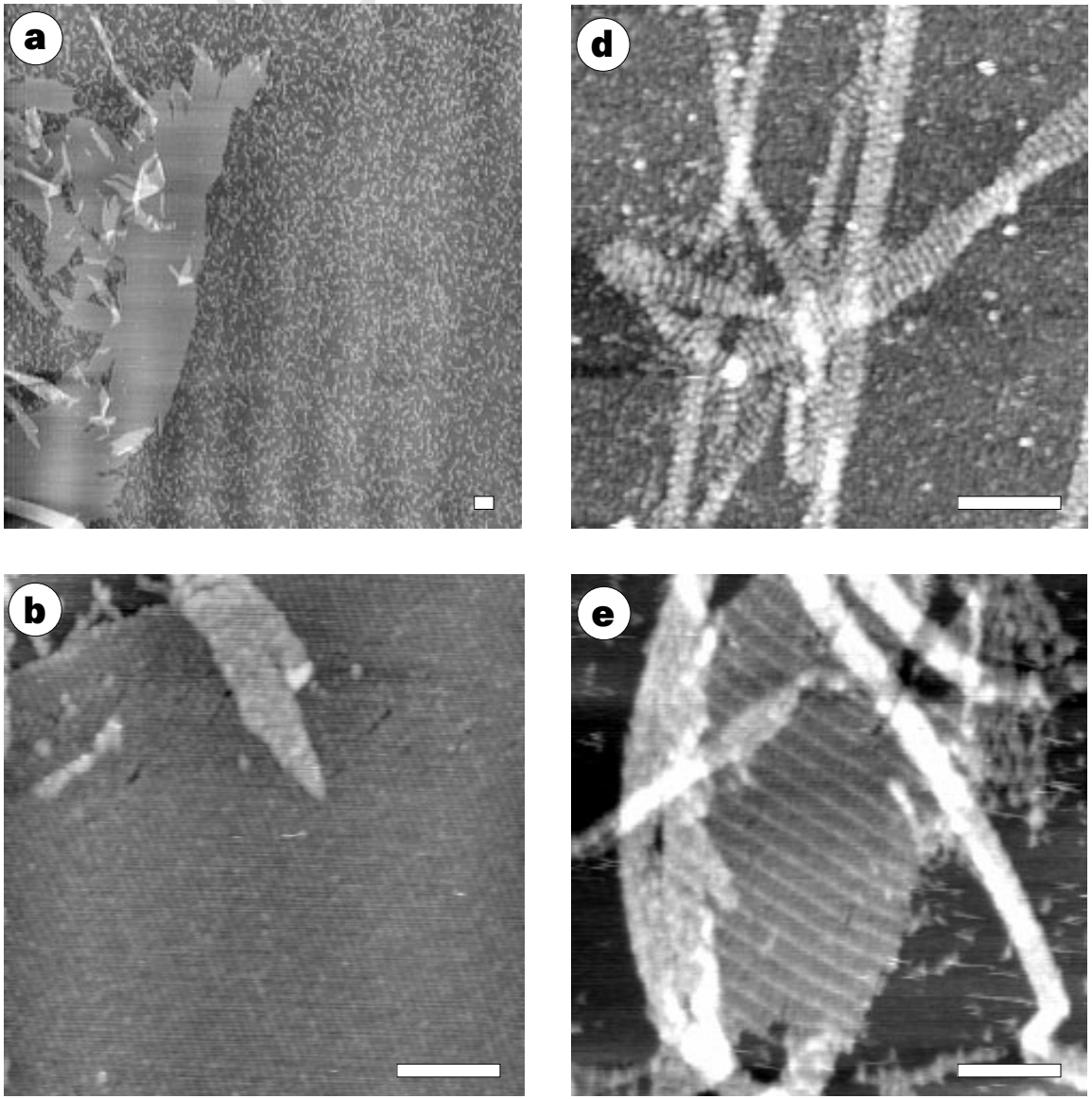
Seeman. Annu. Rev. Biochem. (2010), 79, 65.

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

Winfree, Liu, Wenzler and Seeman. Nature (1998), 394, 539.

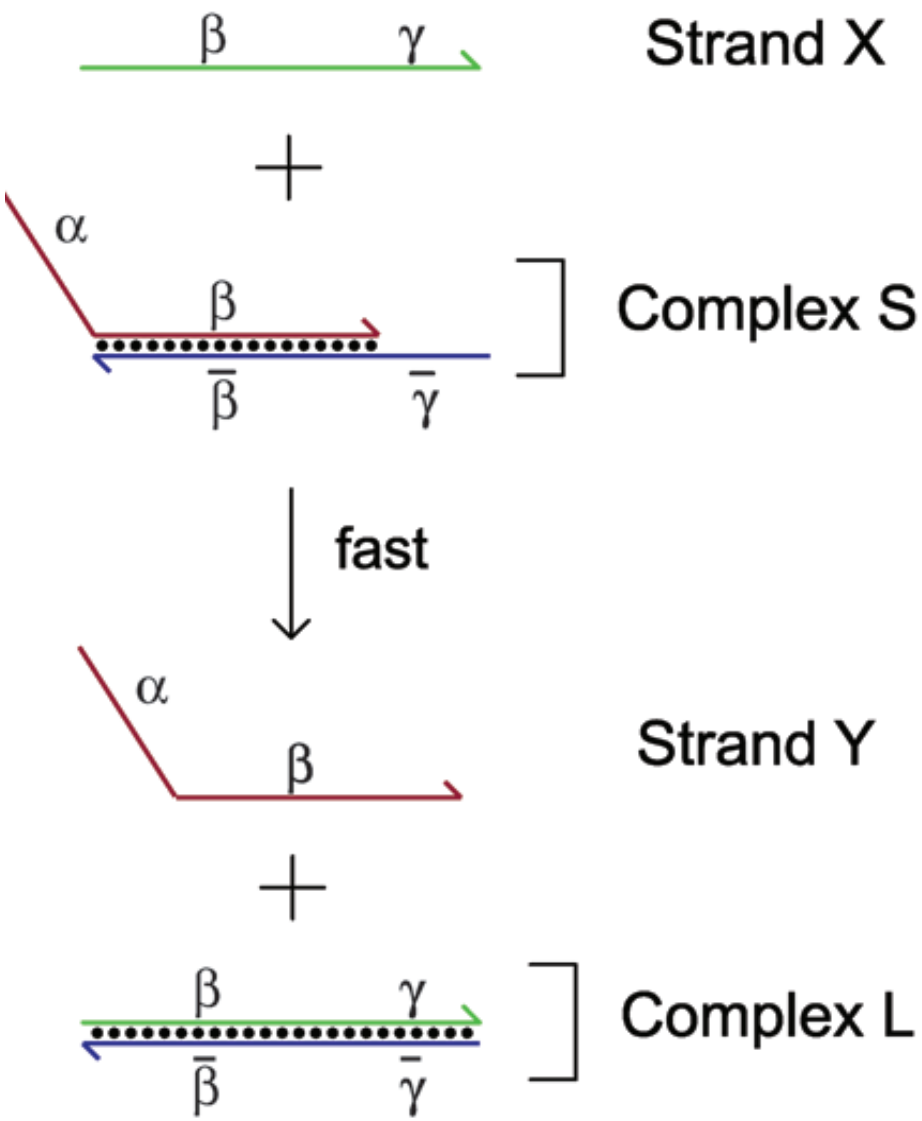


- AFM images of the self assembled array.
- Periodicity of tiling can be seen (e).
- The process is extremely simple:

"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."

Winfree, Liu, Wenzler and Seeman. Nature (1998), 394, 539.

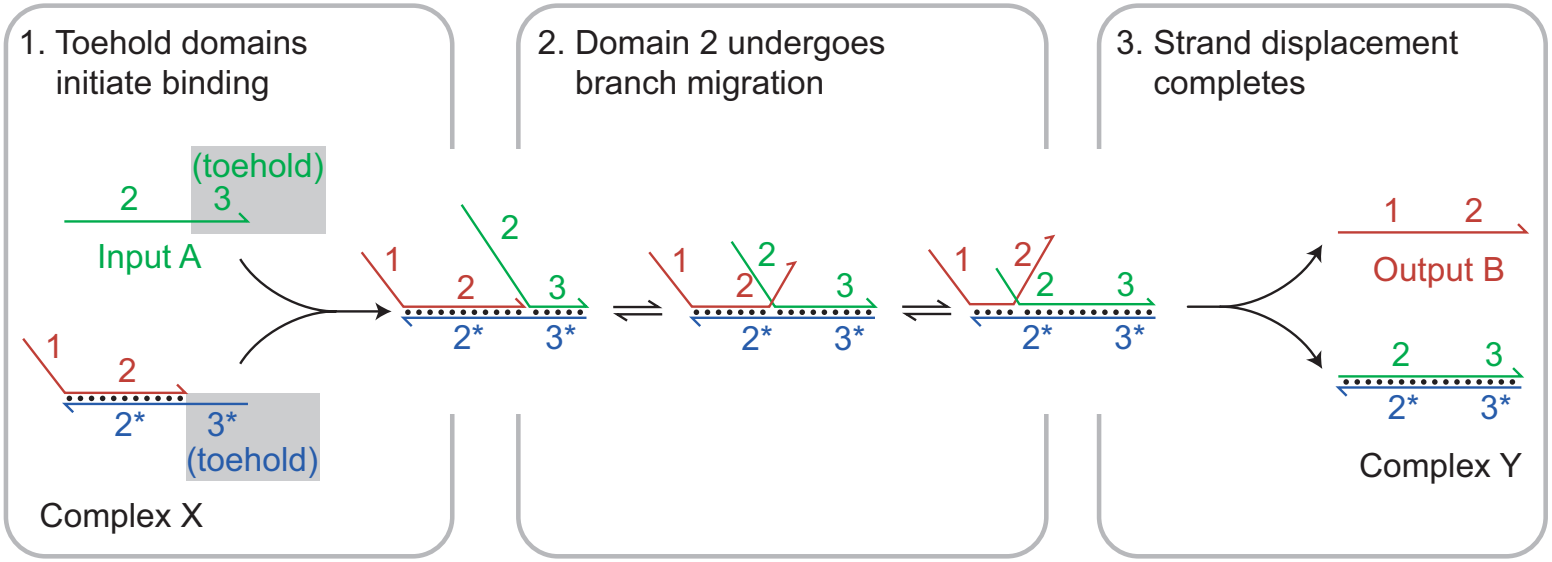
DNA strand Displacement

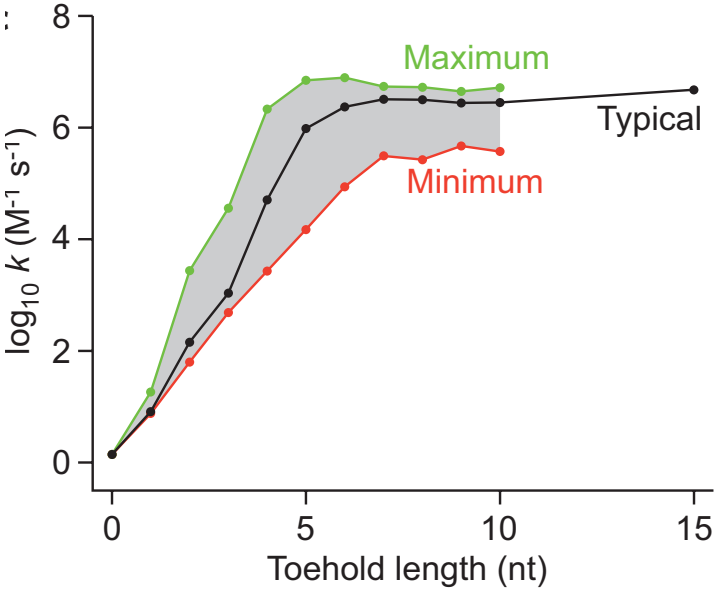


- Strand displacement is the process through which two strands with partial or full complementarity hybridize to each other, displacing one or more pre-hybridized strands in the process.
- The 'reaction' is generally rapid and quantitative since the process is highly thermodynamically favourable.
- The resulting new duplex (**L)** must have more continuous base pairs than the original one (**S).**

Hang and Winfree, JACS (2009), 131, 17303.

Use of 'toeholds' is critical for controlling the kinetics of strand displacement

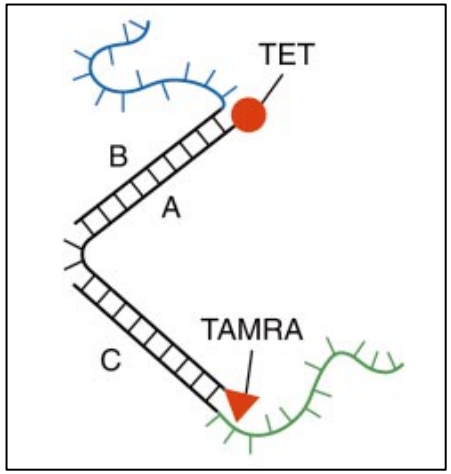




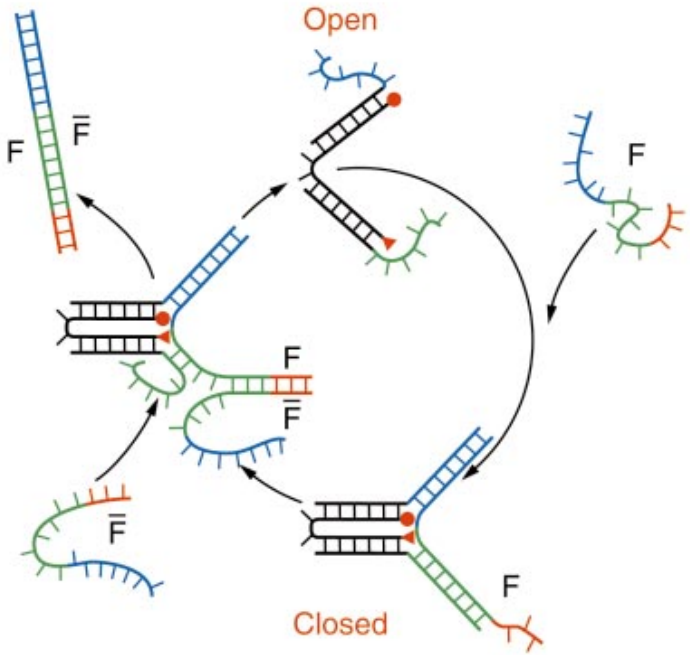
- Toehold mediated strand displacement results in rate constants upto 6 magnitudes higher for the forward reaction.
- In this case, the toehold domain (**3**) allows strand A to localize *specifically* on complex X. Subsequent equilibrium branch migration is followed by an irreversible displacement of **B**.

Hang and Seelig. Nature Chemistry (2011), 3, 103.

Dynamic DNA assemblies



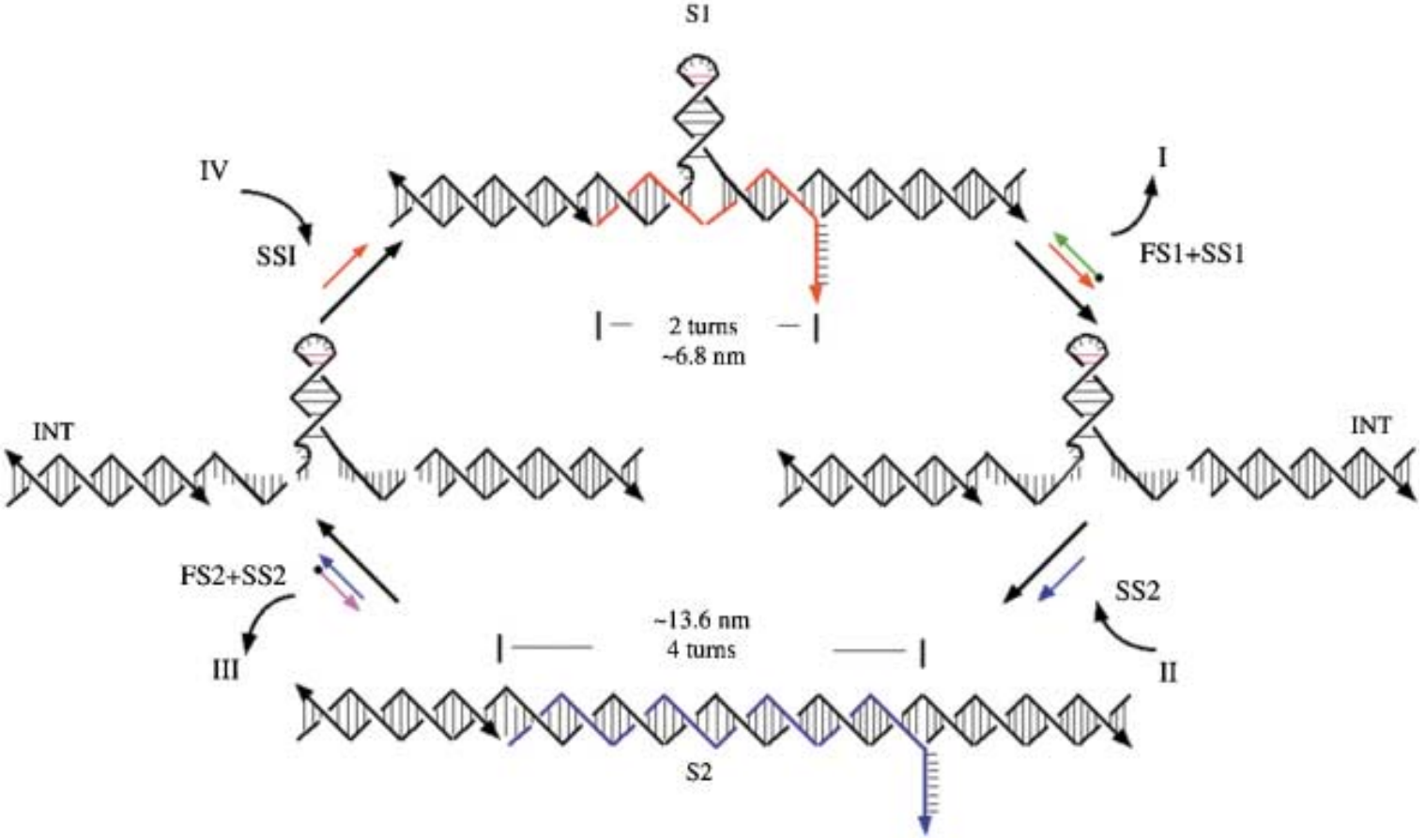
- **A DNA molecular tweezer fuelled by DNA (Turberfield, 2000)**
- The open, linear tweezer is shown on the left. Objective is to bring the two fluorophores TET and TAMRA together.



- 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.

Yurke et. al., Nature (2000), 406, 605.

A Dynamic 2-D array

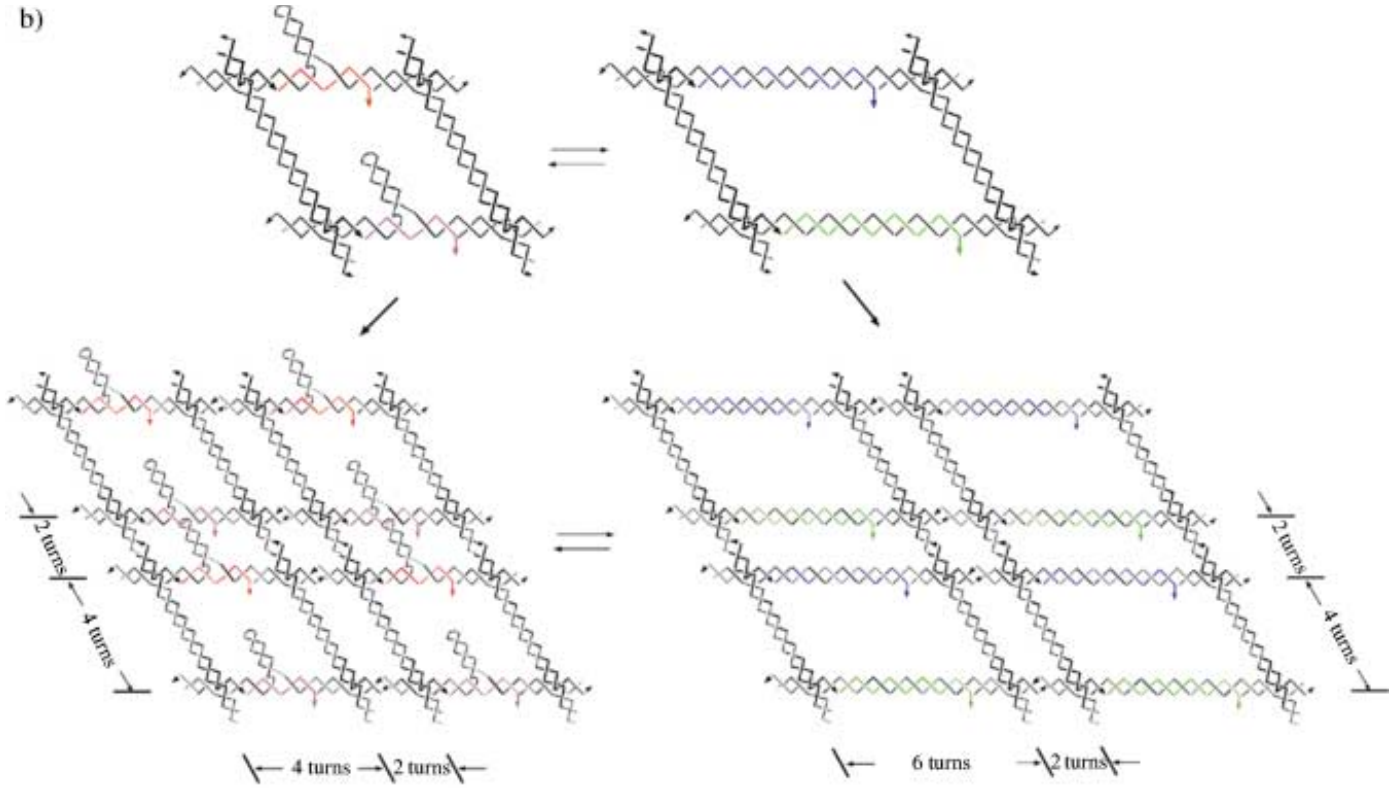


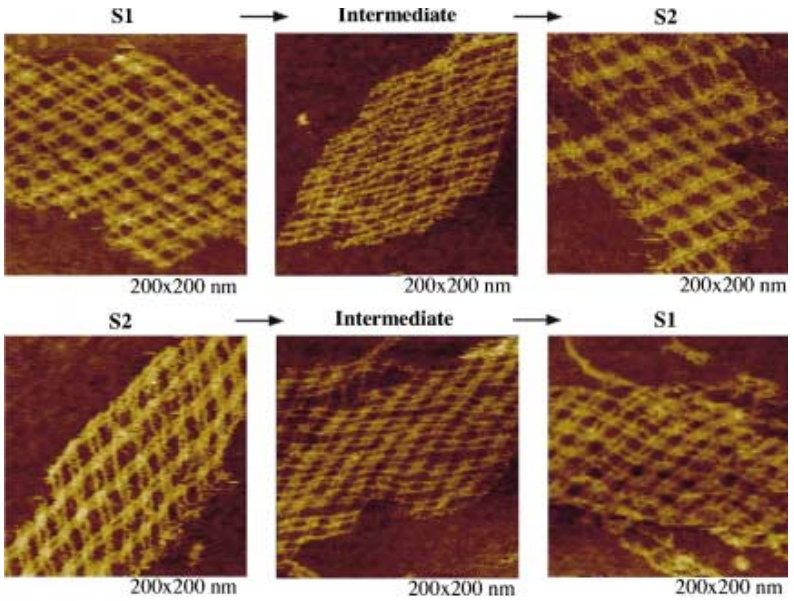
- The idea is to use a modified DX tile that can alter its dimension by strand displacement (Hao Yan, 2003).

- The orange and blue strands in the tile can be interchanged, thus changing the length of the tile

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

Dynamic DNA array

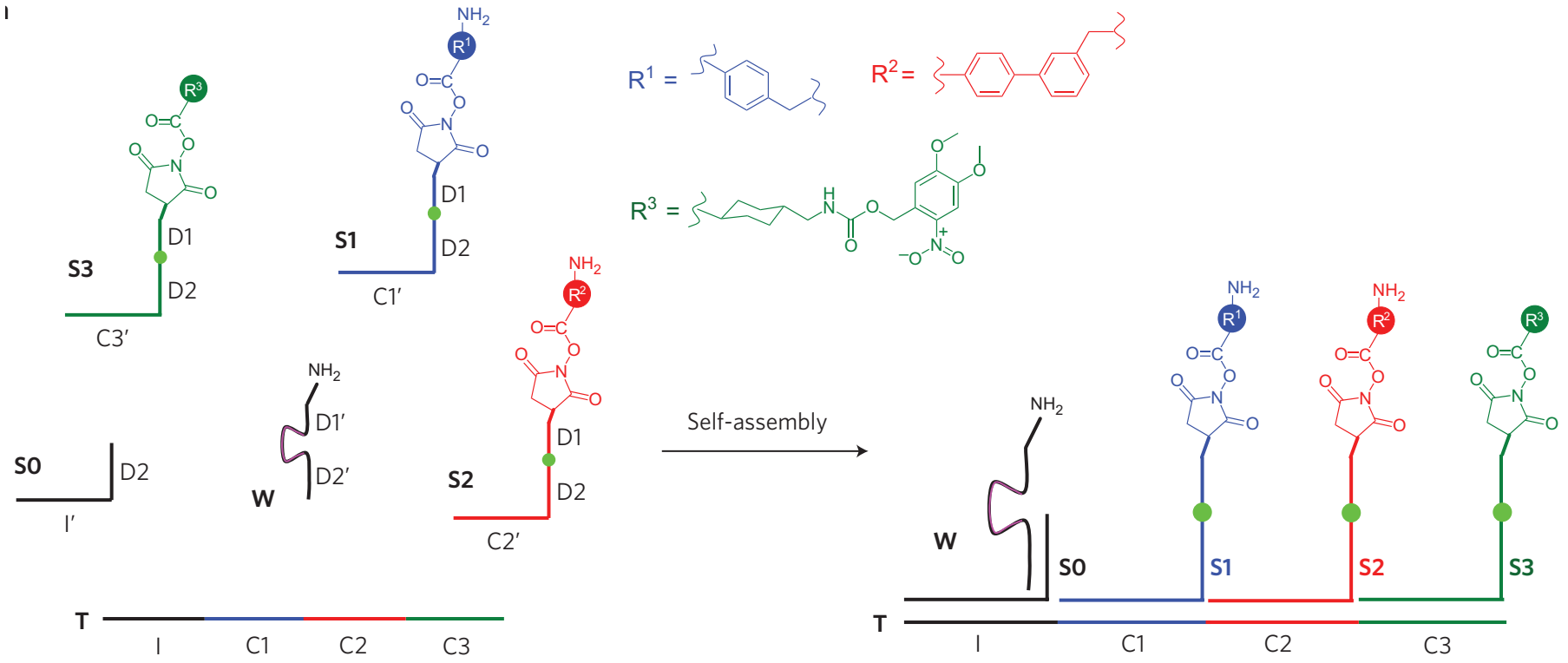




- Strand displacement reaction on the entire array transforms individual tiles from S1 to S2.
- The dimensional changes at the tile level translates to a collective 'breathing' of the array.

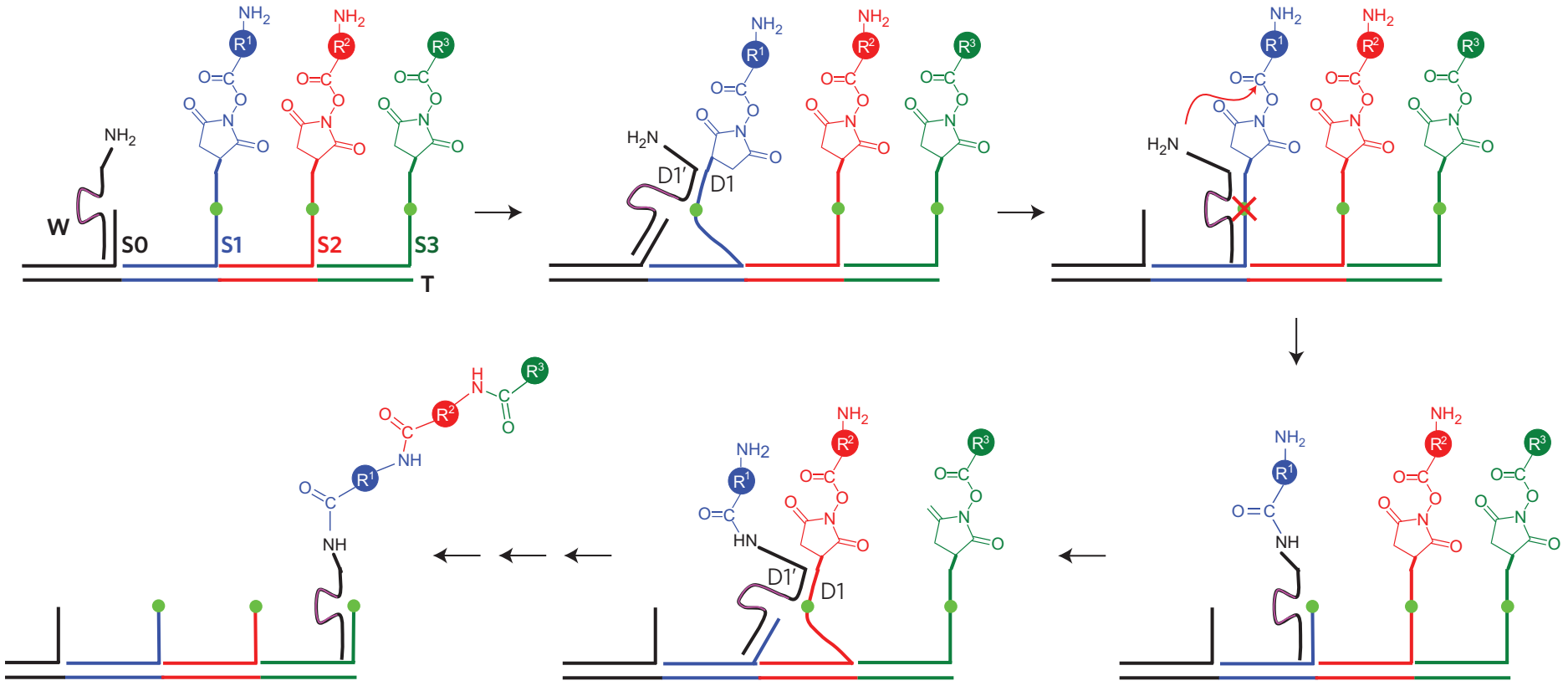
Feng et.al., ACIE (2003), 42, 4342.

DNA walkers



- **Autonomous multistep Organic Synthesis with a DNA walker. (David Liu, 2010)**
- Synthesis of a tripeptide 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.

He and Liu, Nature Nanotechnology (2010), 5, 778.

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.

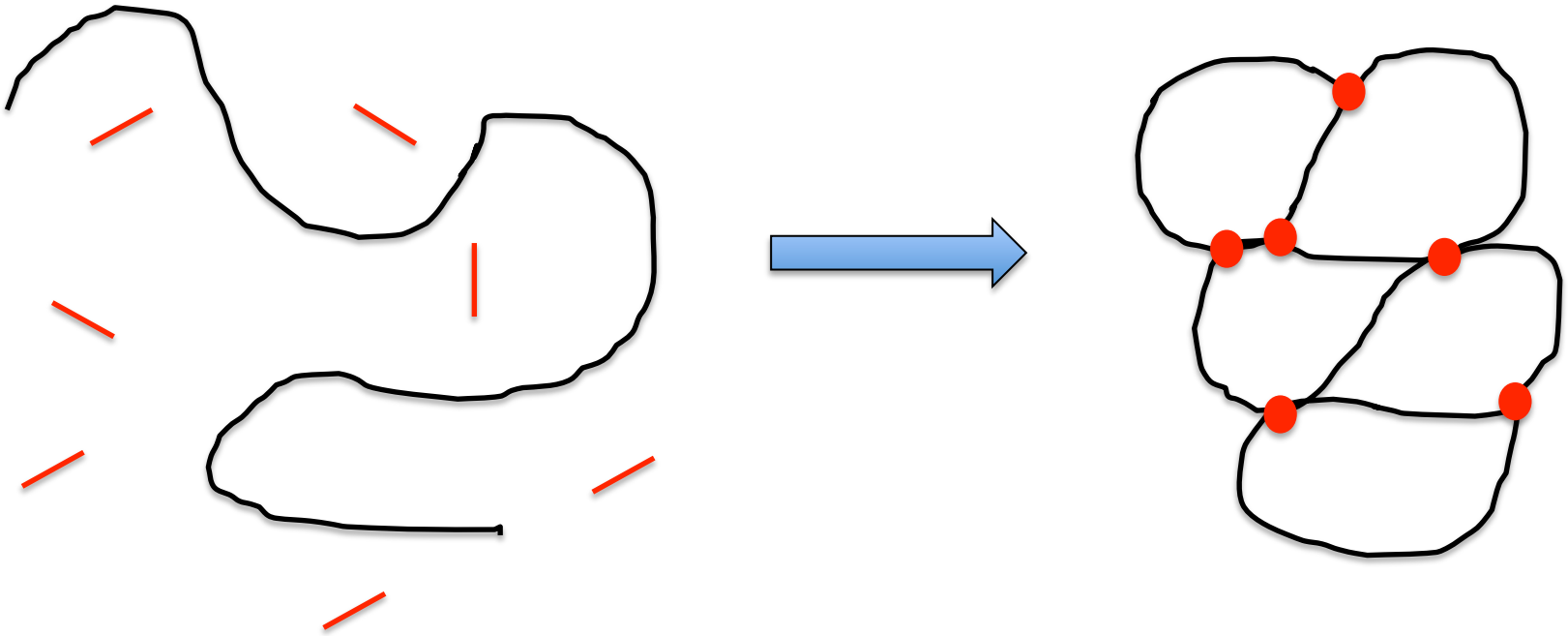
By this time, DNA had established itself as a superior building block. However, as in the case of classical supramolecular designs, new architectures depended on ingenious use of DNA hybridization and imagination. Larger self assembled structures were limited by the repetitive nature of their building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

The field was to be transformed by Paul Rothemund's revolutionary publication in 2006.

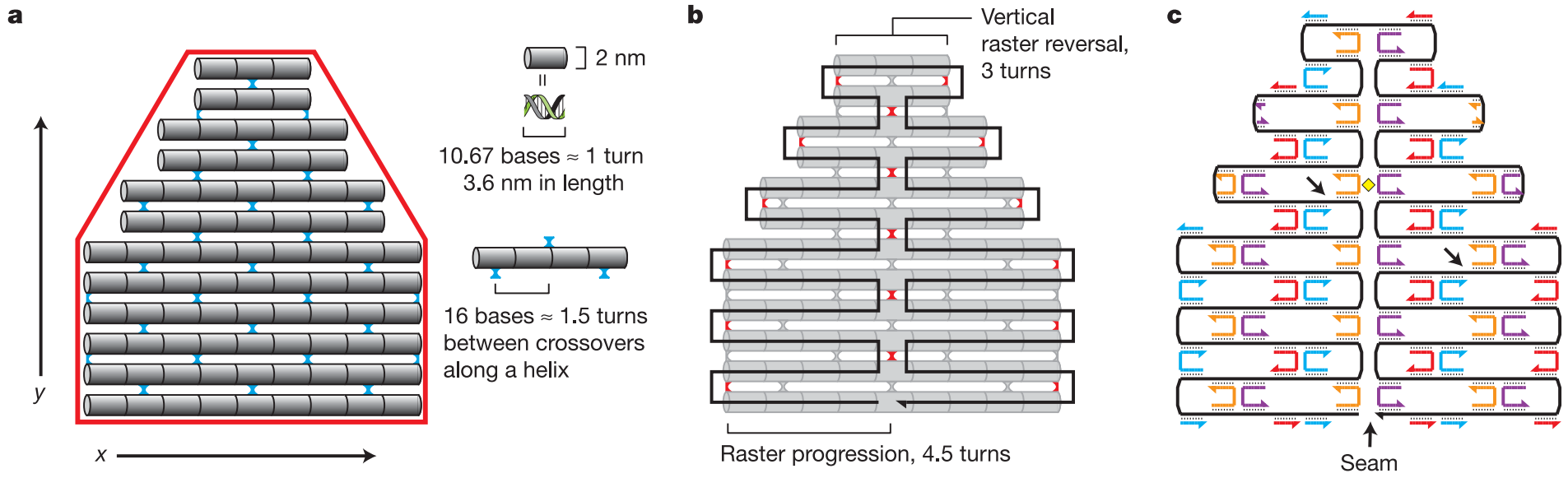
DNA Origami

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):

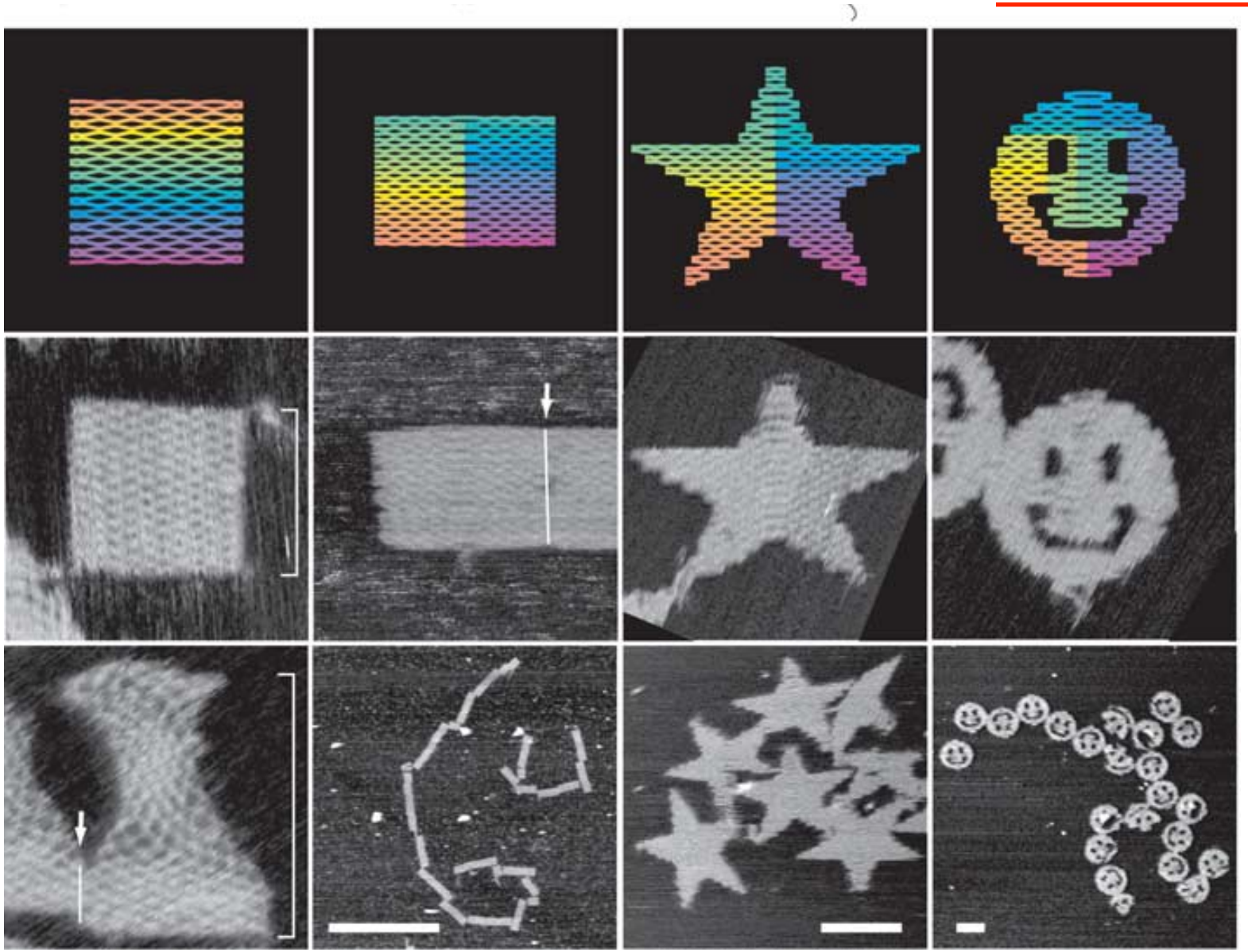


DNA Origami



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.

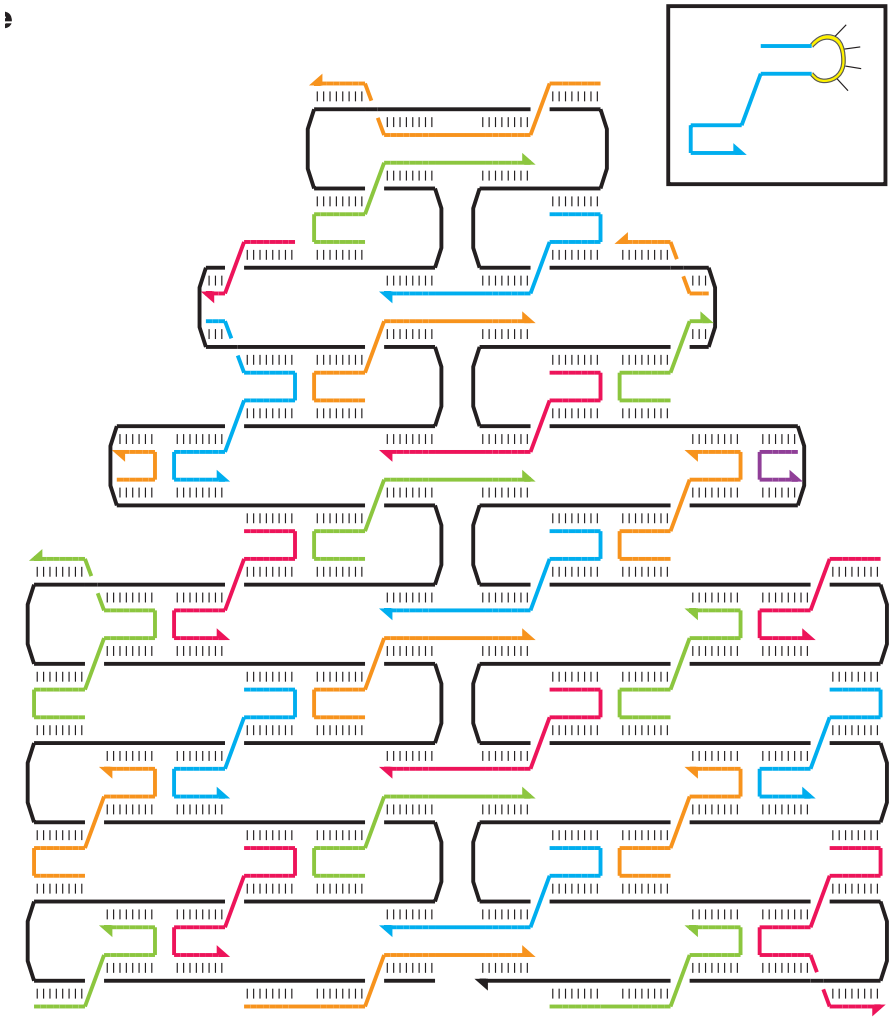
Paul Rothemund, Nature (2006), 440, 297.



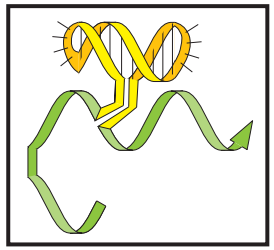
Any desired shape can be accessed. Yields are greater than 70%

Paul Rothemund, Nature (2006), 440, 297.

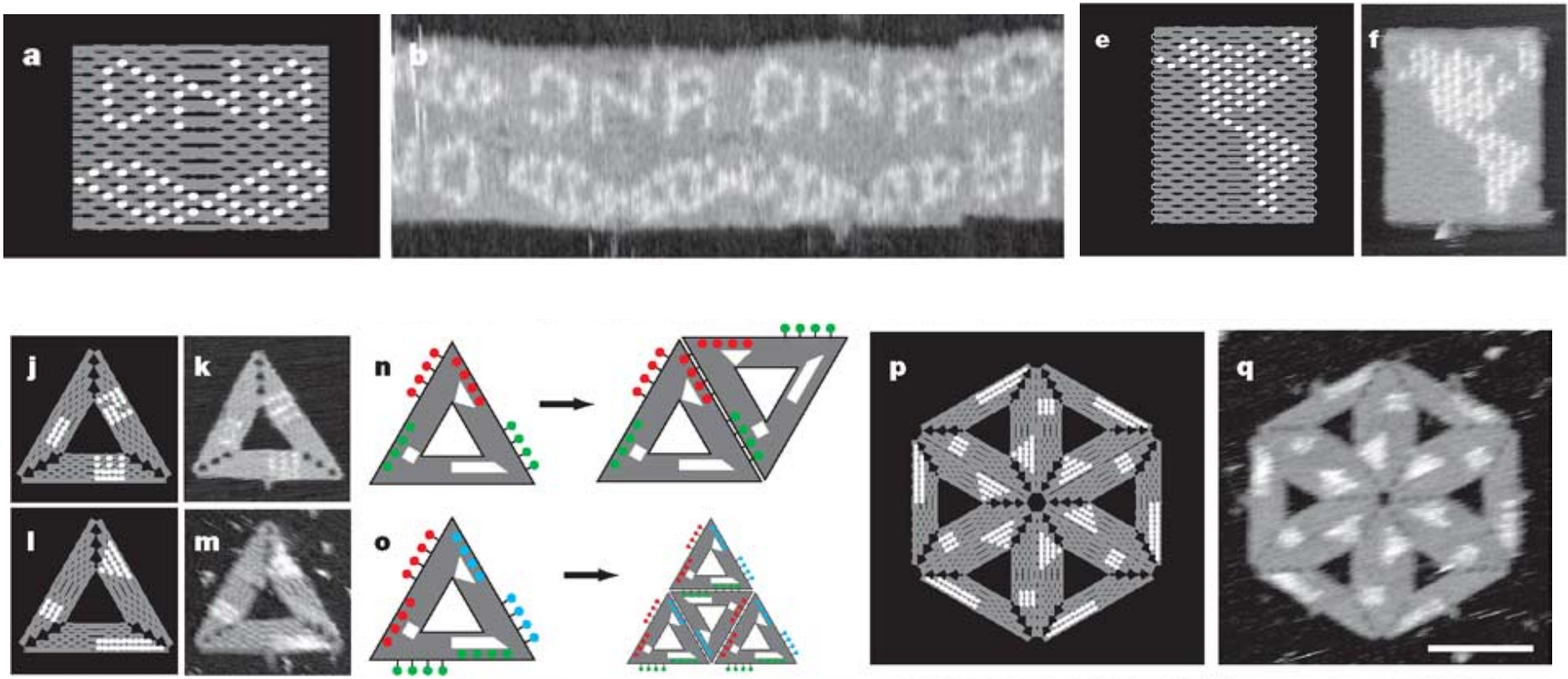
Patterning with staple strands:
Staple strands specify location on the scaffold. Thus, the shape can be regiospecifically marked by modifying the necessary staple strand.



Paul Rothemund, Nature (2006), 440, 297.



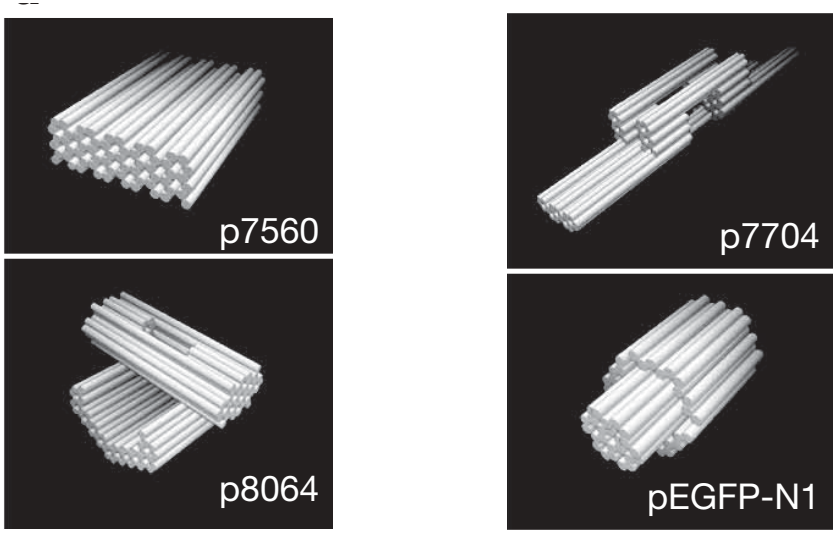
Patterning with staple strands:
Markers on staple strands are seen as distinct topological features on the AFM.



Paul Rothemund, Nature (2006), 440, 297.

3-D DNA Origami

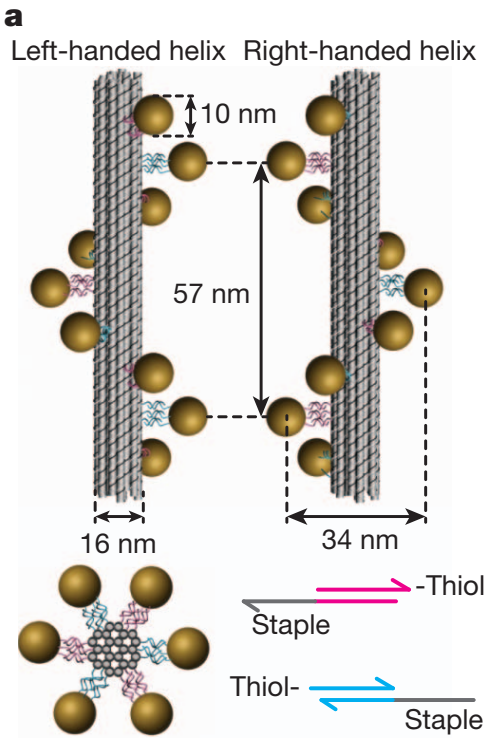
- The folding of a scaffold strand was soon extended to attain 3-D architectures.
- Shih and coworkers developed 'caDNAno' an open source software for design of DNA architectures. The software designs all staple strands based on the desired shape.

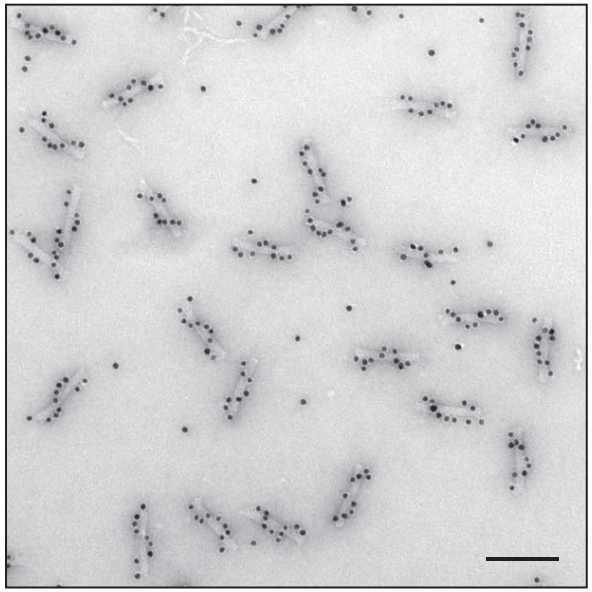


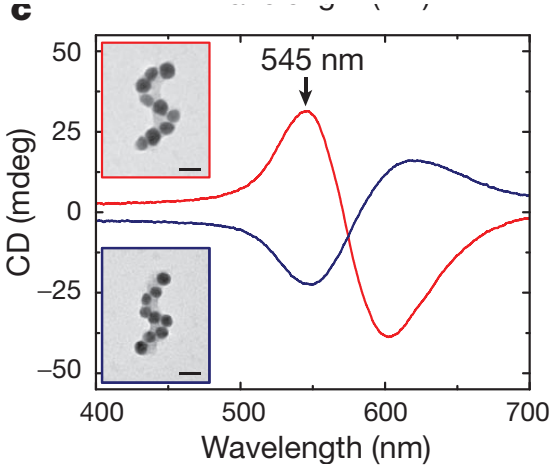
"With caDNAno, an individual with no prior knowledge of programming or DNA structure can complete a short tutorial and then be capable of generating sequences within a day for building a new shape comparable in complexity to the examples demonstrated here."

Douglas et. al., Nature (2009), 459, 414.

DNA based self Assembly of Chiral plasmonic nanostructures





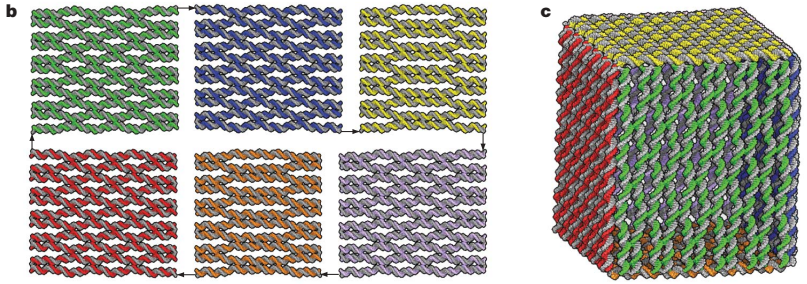


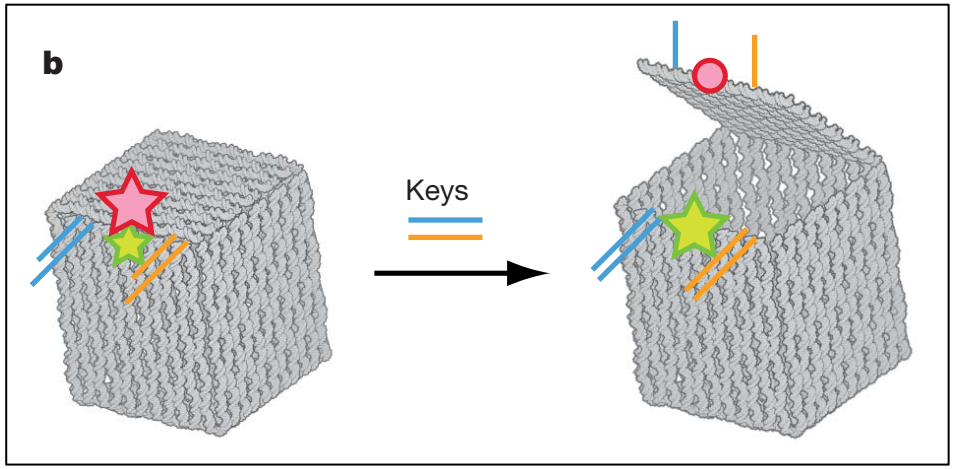
a) Functionalized staple strands on the cylindrical backbone allow precise attachment of gold nanoparticles.
b) Left and right handed helices seen in TEM.
c) CD for the chiral nanostructures

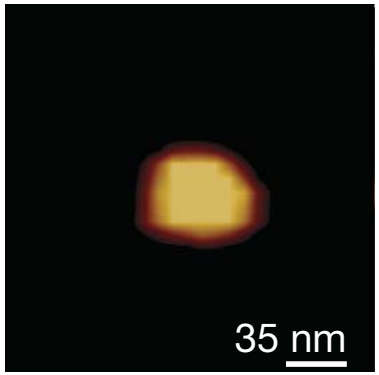
Kuzyk et. al., Nature (2012), 483, 311.

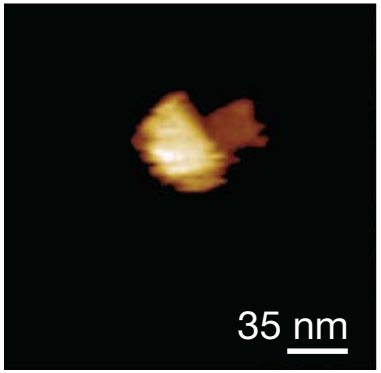
Dynamic Origami Architectures

Self Assembly of a nanoscale DNA box with a controllable lid







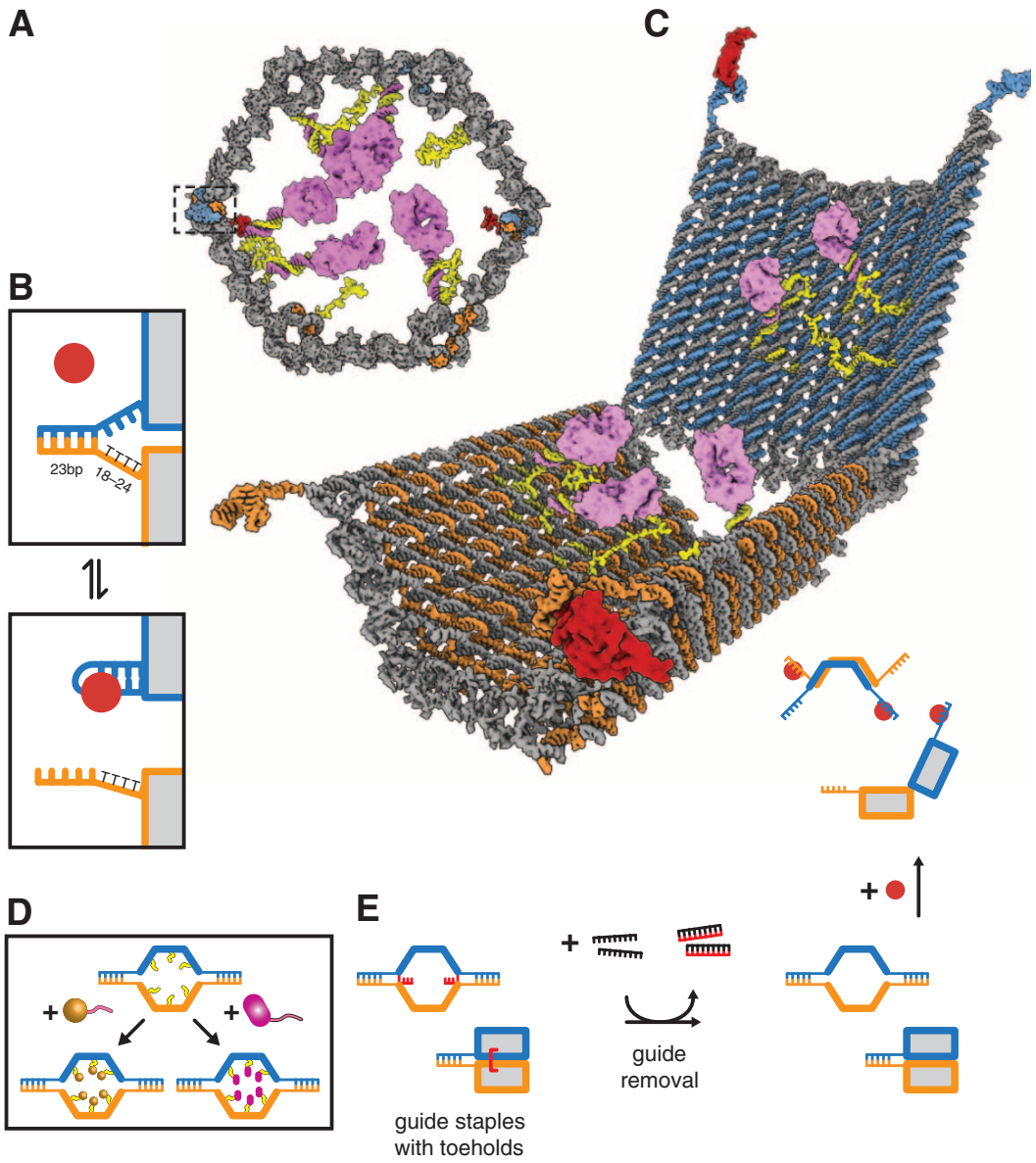


- A single scaffold folded into a cube and lid.
- Lid could be opened by strand displacement of the 'lock' strands with 'key' strands.
- AFM images of an individual box; closed and open.

Andersen et. al., Nature (2009), 459, 73.

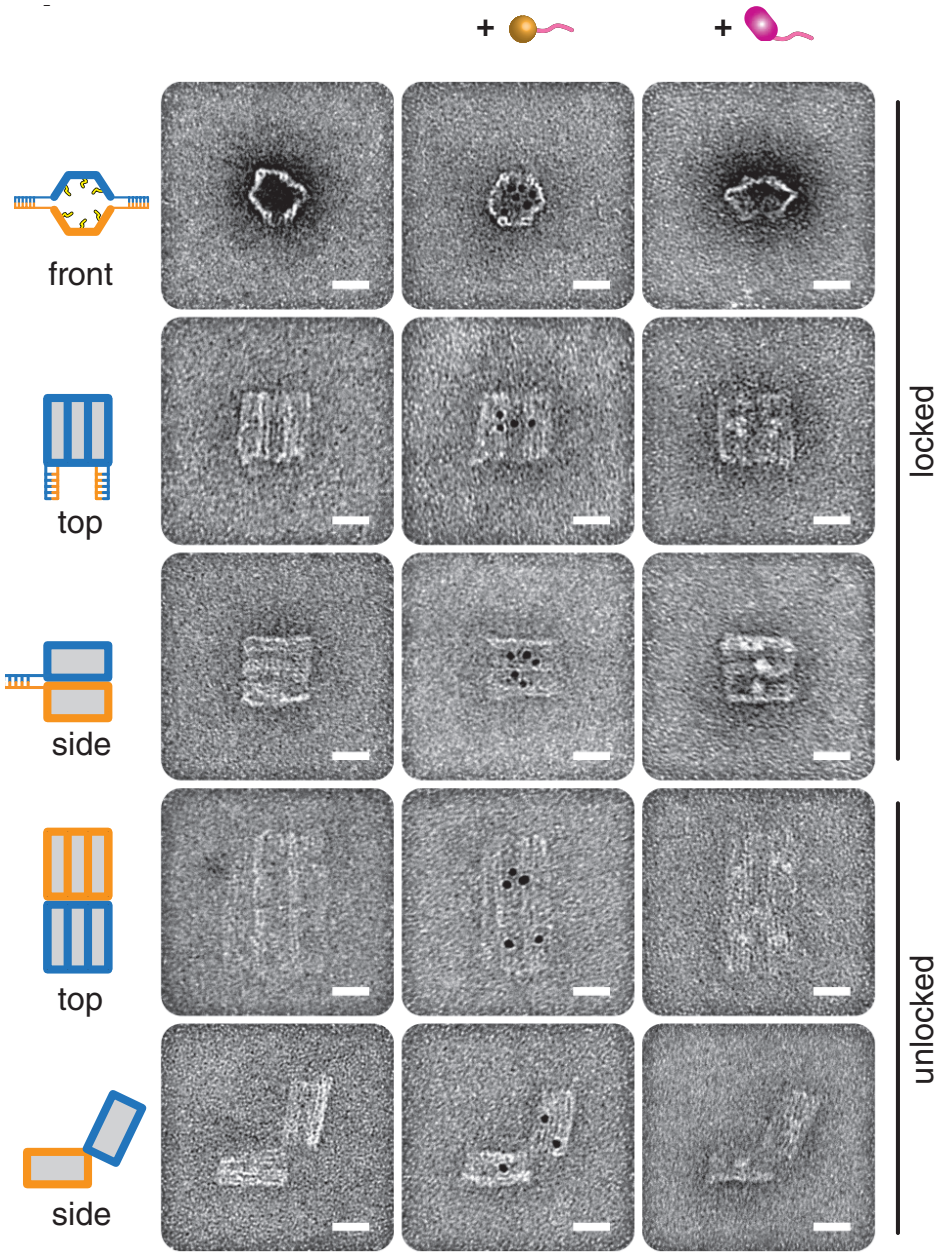
DNA Nanorobot

A logic gated nanorobot for targeted transport of molecular payloads.



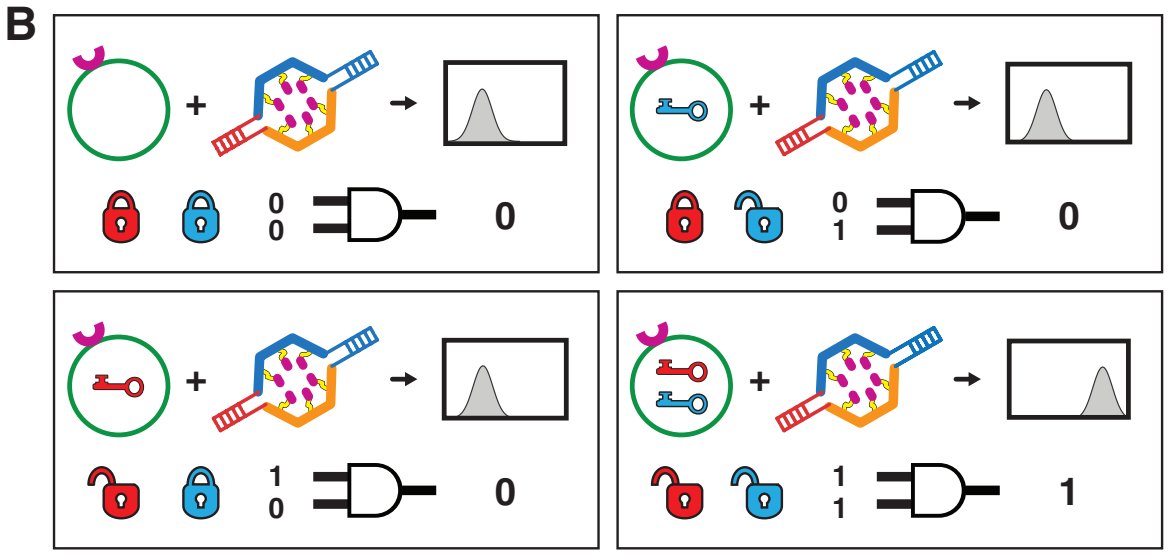
- A ‘mousetrap’ container design that can carry cargo inside (in this case, antibodies).
- The container is locked by aptamer keys to a target protein.
- The protein will bind to the aptamer and open the container, releasing stored cargo.

Douglas, Bachelet and Church, Science (2012), 335, 831.

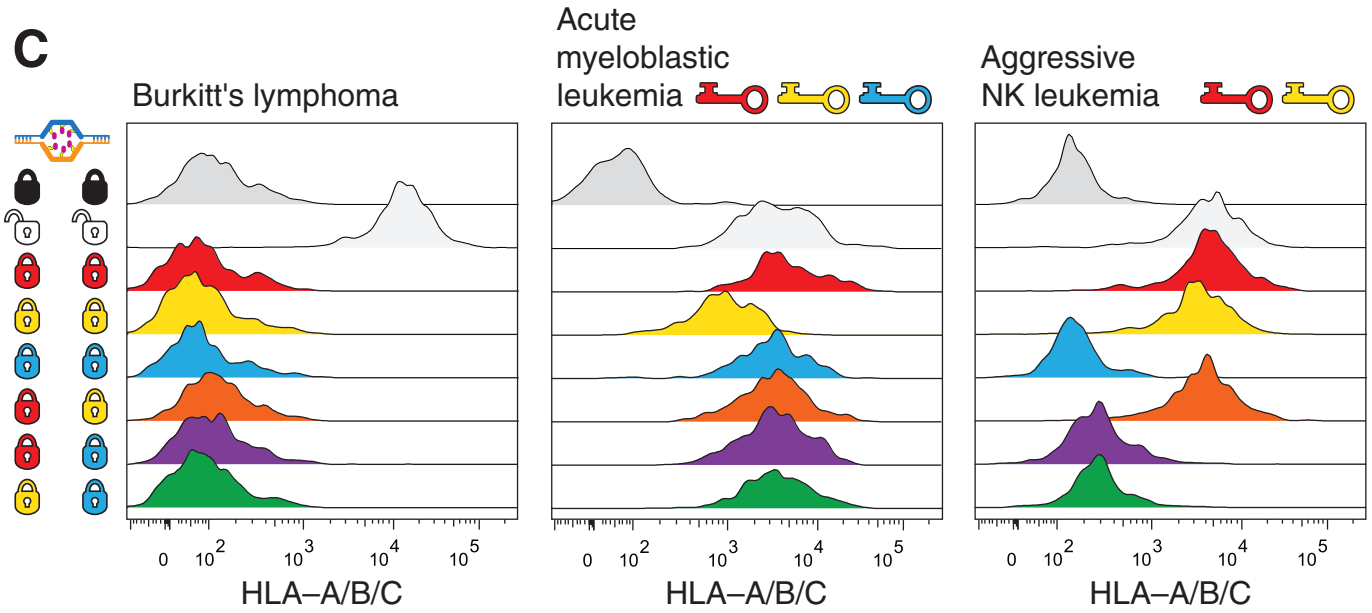


Individual states captured by AFM

Douglas, Bachelet and Church, Science (2012), 335, 831.



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

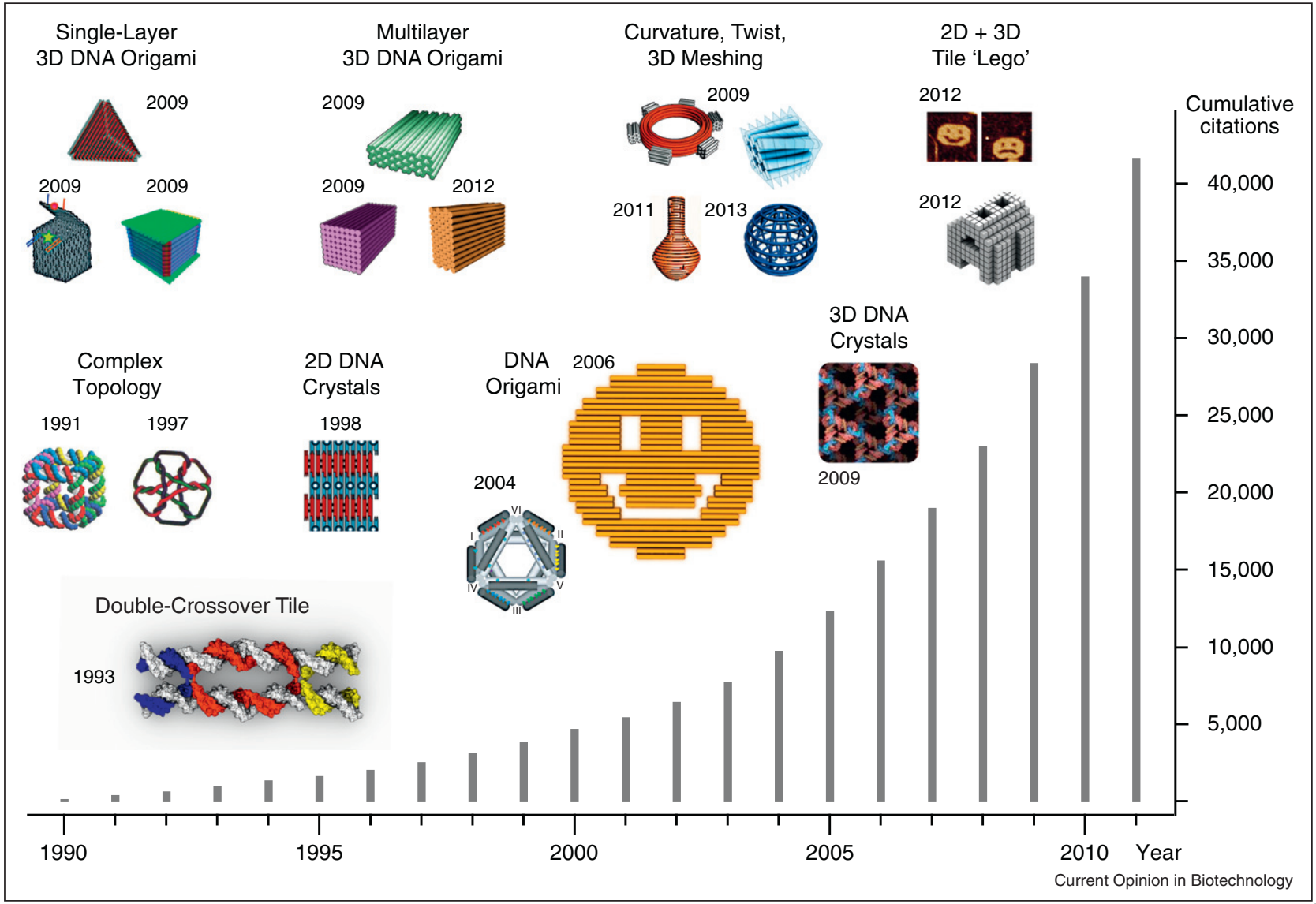


Douglas, Bachelet and Church, Science (2012), 335, 831.

Summary

- 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



Linko and Dietz. Curr. Opin. In Biotech. (2013), 24, 1.

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 ber pase 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.*

Pinheiro et.al., Nature Nanotechnology (2011), 6, 763.
Amir et.al., Nature Nanotechnology (2014), 9, 353.

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.