CH-413 Nanobiotechnology

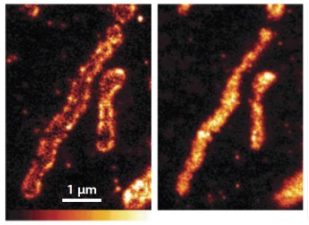
Self-assembly of nanoscale objects

Angela Steinauer

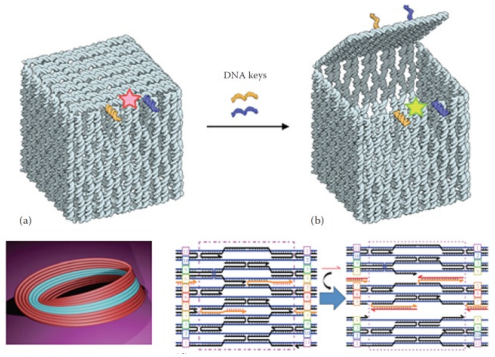
April 17, 2025

Engineering of micro- and nanoscale objects

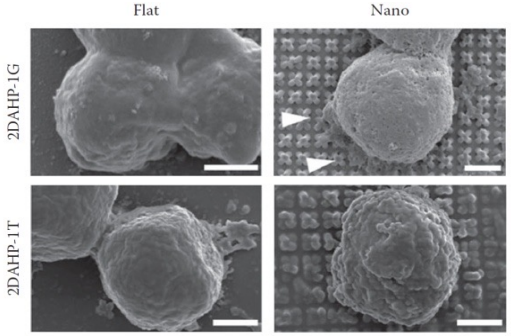
Watch molecular biology happen and **manipulate** the processes



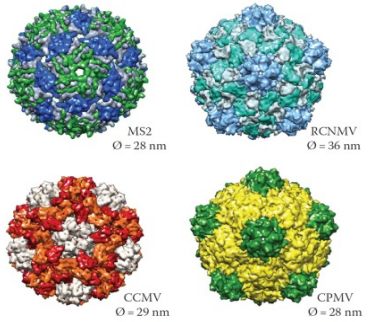
Craft new biomaterials



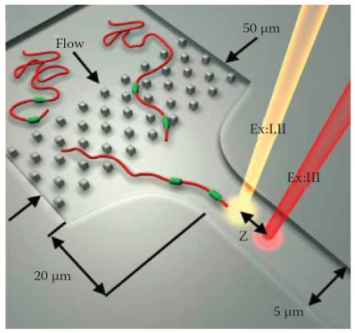
Grow cells and complex tissues *in vitro*



Target drugs to individual cancer cells



Diagnose diseases from single molecules or cells cells



What is self-assembly

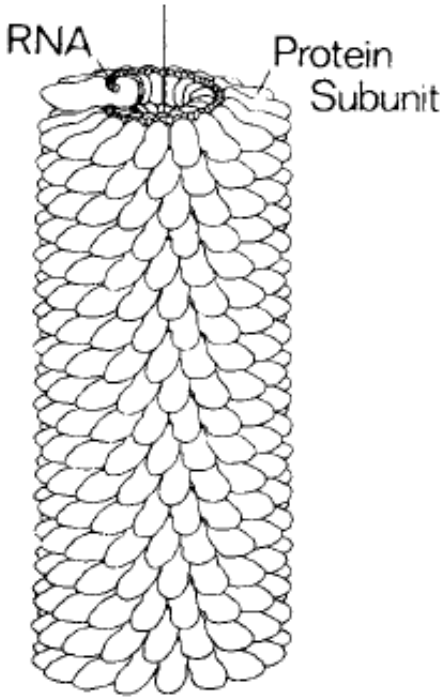
Definition of a self-assembly process

"**Spontaneous** association of molecules under **equilibrium conditions** into stable, **structurally well-defined** aggregates joined by **noncovalent bond**s."

(Whitesides, Mathias and Seto)

→ Molecules adjust their position to reach a **thermodynamic minimum**

→ Self-organization of complex systems, **basis of life**



Model of self-assembling virus

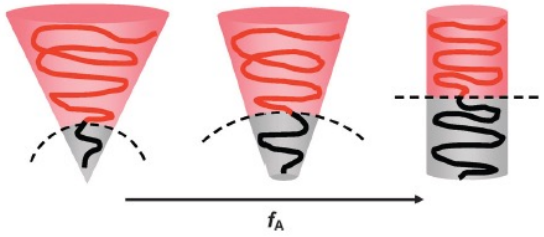
https://youtu.be/X-8MP7g8XOE

Self-assembly is nature's solution to the nanoengineering gap

Engineering of materials with 10-100 nm sized features:

- Difficult / inaccessible size scale: **no-man's land between synthetic chemistry and top-down fabrication**
- Larger than single molecules -> synthetic chemistry cannot help
- Too small for lithography

Self-assembly or controlled synthesis:



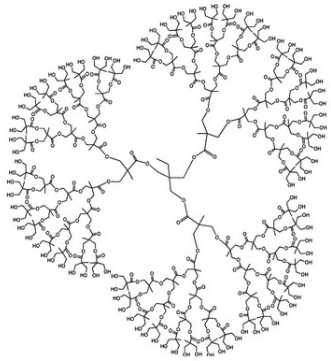
Mai & Eisenberg, Chem Soc Rev 2012

Block copolymers

Self-assembled monolayers

Inorganic nanoparticle (arrays)

Dendrimers



Feliu et al., Biomaterials 2012

However, all result in repetitive structures

Self-assembly is governed by free energy minimization

$$\Delta G = \Delta H - T\Delta S$$

ΔG = change in Gibbs free energy

ΔH = enthalpy change (heat absorbed or released)

ΔS = entropy change (degree of disorder)

T = absolute temperature

For self-assembly to occur spontaneously, ΔG must be negative.

Enthalpic contributions (ΔH)

Favorable enthalpy (ΔH < 0) arises from:

- **Non-covalent interactions**:
  - Hydrogen bonding
  - Electrostatics
  - van der Waals forces
  - π–π stacking
  - Metal coordination
- These interactions **release energy** when formed, contributing a **negative ΔH**.
- Example: DNA double helix formation is stabilized by **base pairing (hydrogen bonds)** and **base stacking (vdW/π–π)**.

Entropic contributions (ΔS)

Entropy tends to **oppose ordering**, because:

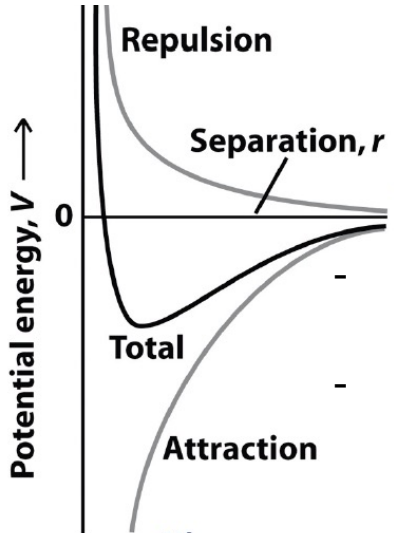
- **Assembly reduces the number of independent particles** (e.g., 100 monomers → 1 micelle = fewer microstates = lower entropy).
- **Translational and rotational entropy** are lost upon assembly.

However, in some cases **entropy can drive self-assembly**:

- **Hydrophobic effect**: Water molecules around nonpolar groups become more disordered when hydrophobes cluster → **entropy of water increases**.
- **Depletion forces / crowding**: Entropic forces can push components together to maximize space for other molecules.

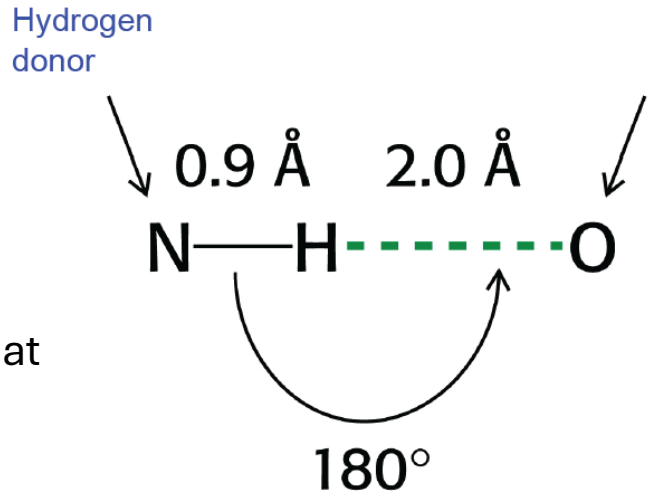
Forces governing molecular self-assembly

Van der Waals interaction



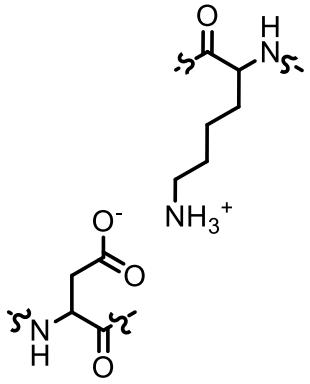
- Individually weak, cumulatively strong
- Molecules like to sit at the minimum of the Lennard-Jones potential

Hydrogen bonding



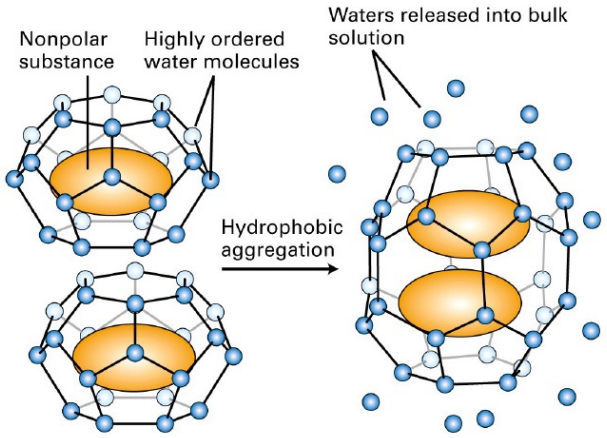
- Directional
- Hydrogen donor/acceptor interaction

Electrostatic interactions



- Coulomb attraction/repulsion between charged species
- Dampened in high salt, polar solvents

Hydrophobic effect



- **Entropic effect**
- Mainly due to disruption of water hydrogen bonding network

Forces governing molecular self-assembly

Force	Strength (kcal/mol)	Range	Directionality	System Examples
Hydrophobic	~0.6–3	Short	Low	Micelles, proteins
Electrostatic	~0.6–6	Long	Low	DNA–protein, colloids
Hydrogen Bonding	~1–6	Short	High	Base pairing, β-sheets
van der Waals	~0.1–0.6	Very short	None	Nanoparticles, SAMs
π–π Stacking	~1–3	Short	Moderate	Aromatic molecules, DNA stacking
Metal–Ligand Coordination	~6–60	Short	High	MOFs, metallocages

Balancing enthalpy and entropy

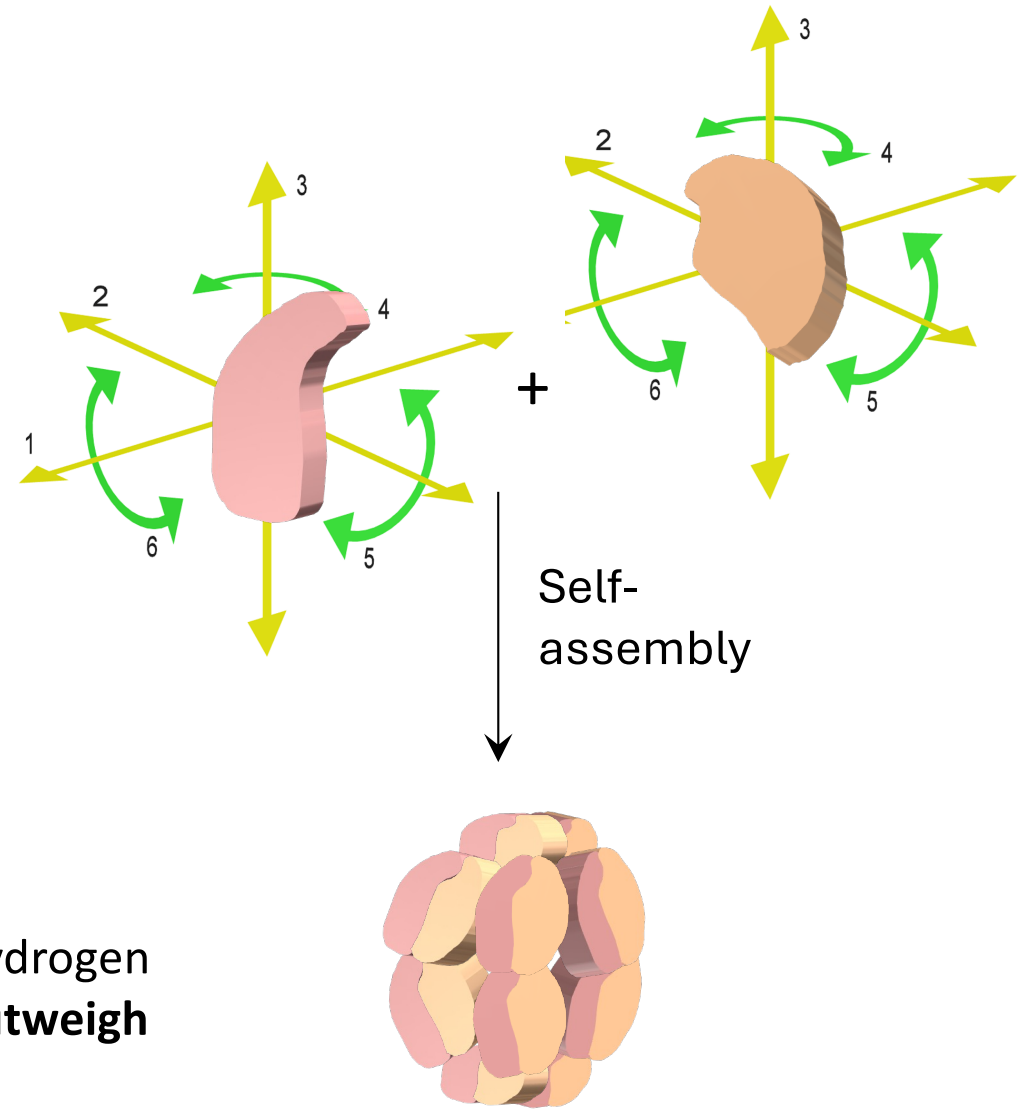
Monomer in solution:

Translational degrees of freedom: ability to move freely along different axes in the solution

Rotational degrees of freedom: ability to rotate around different axes

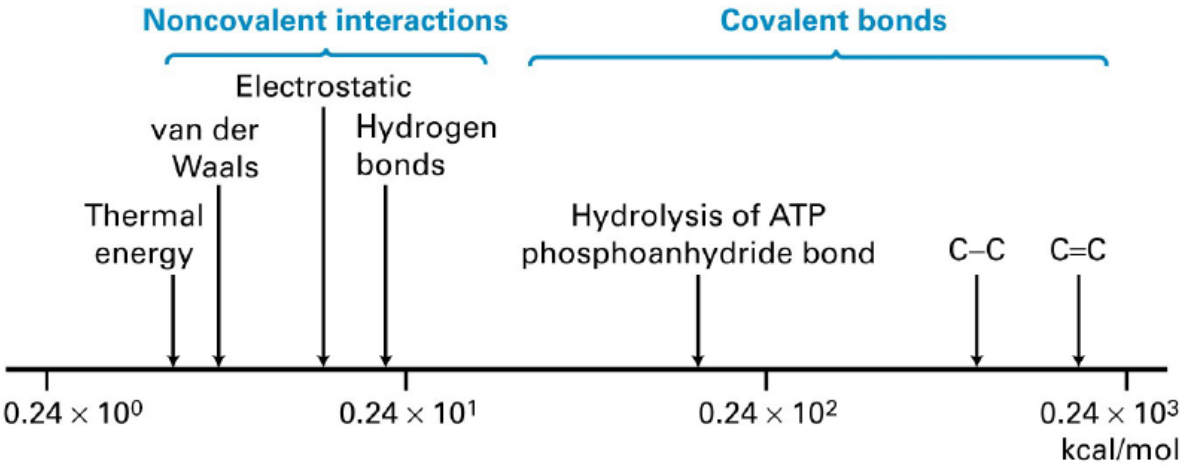
Upon assembly, monomers lose much of their translational and rotational freedom, **resulting in loss of entropy.**

Gain in enthalpy (from VdW forces, hydrogen bonds, ionic interactions etc.) **must outweigh entropic penalty.**



Forces governing molecular self-assembly

- Non-covalent bonds are weak:
  - 0.1 – 5 kcal/mol (vs. 40-100 kcal/mol for covalent bonds)
- **Many bonds** are required



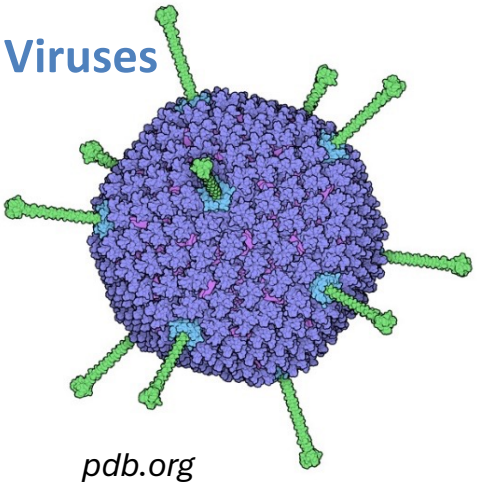
- Interactions between molecules must be **more favorable than solvent interactions**
- Must overwhelm **entropic** advantage of dissolving the complex

Biological self-assembly

- Many weak reversible interactions to obtain final structure → thermodynamic minimum
- Modular process through stable sub-assemblies
- Often small number of molecule types involved
- Positive cooperativity
- Complementarity in molecular shape through VdW and hydrophobic interactions

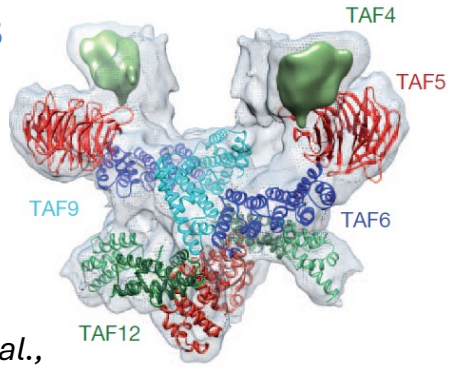
Self-assembly in nature

Viruses



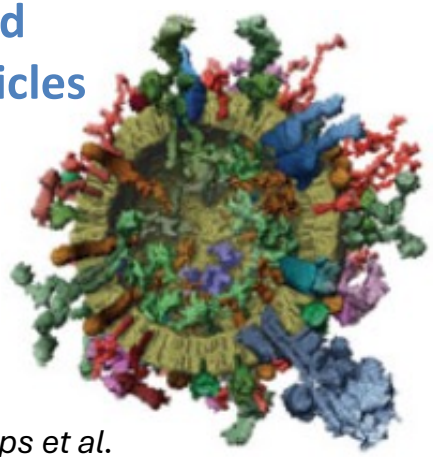
pdb.org

Protein complexes



Bieniossek et al., Nature 2013

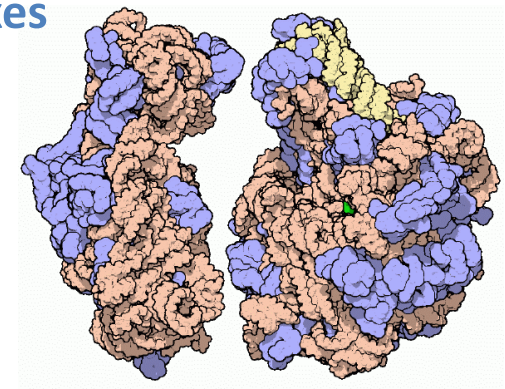
Lipid vesicles



Phillips et al. Nature 2009

Synaptic particle

Protein-RNA complexes



pdb.org

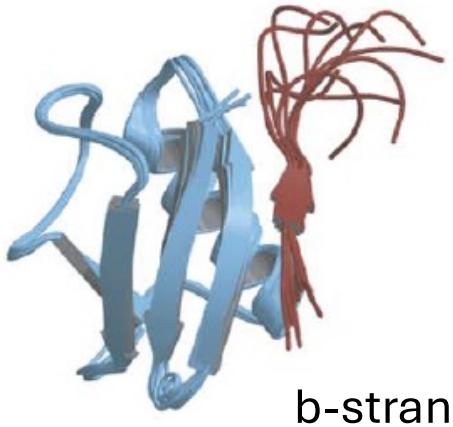
Ribosome

- Dimer- multimerization
- Homo- vs. heterooligomers
- RNA-based association
- Membrane-supported self-assembly
- **Cooperativity**: the modification of the conformation of the individual particles that increases the affinity for the other components
- **All-or-none transition** (e.g. nucleation-and-growth model for viruses)

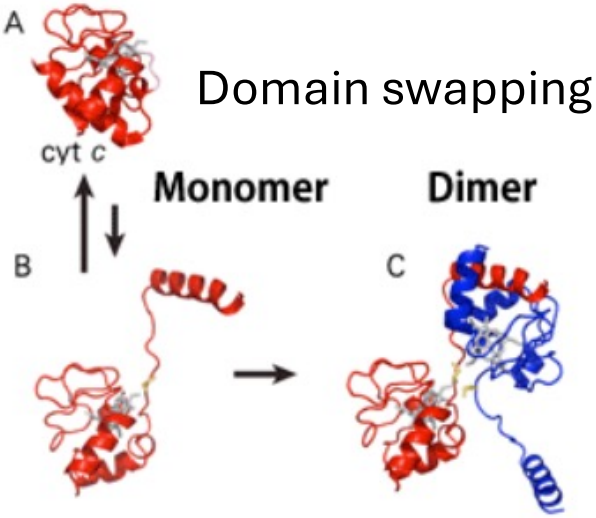
Protein motifs for self-assembly

Coiled coil
of a-helices

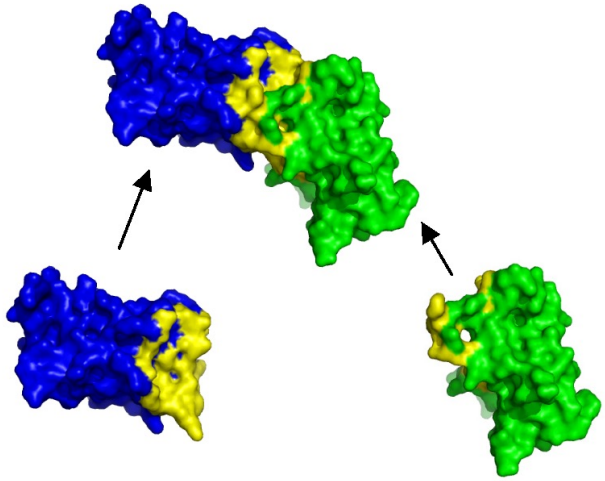
Helix bundle



b-strand
addition



Domain swapping



Large highly complementary
protein-protein interfaces

Engineered protein self-assembly

Aim:

The generation of new materials / particles with functionality based on proteins (mimicking nature).

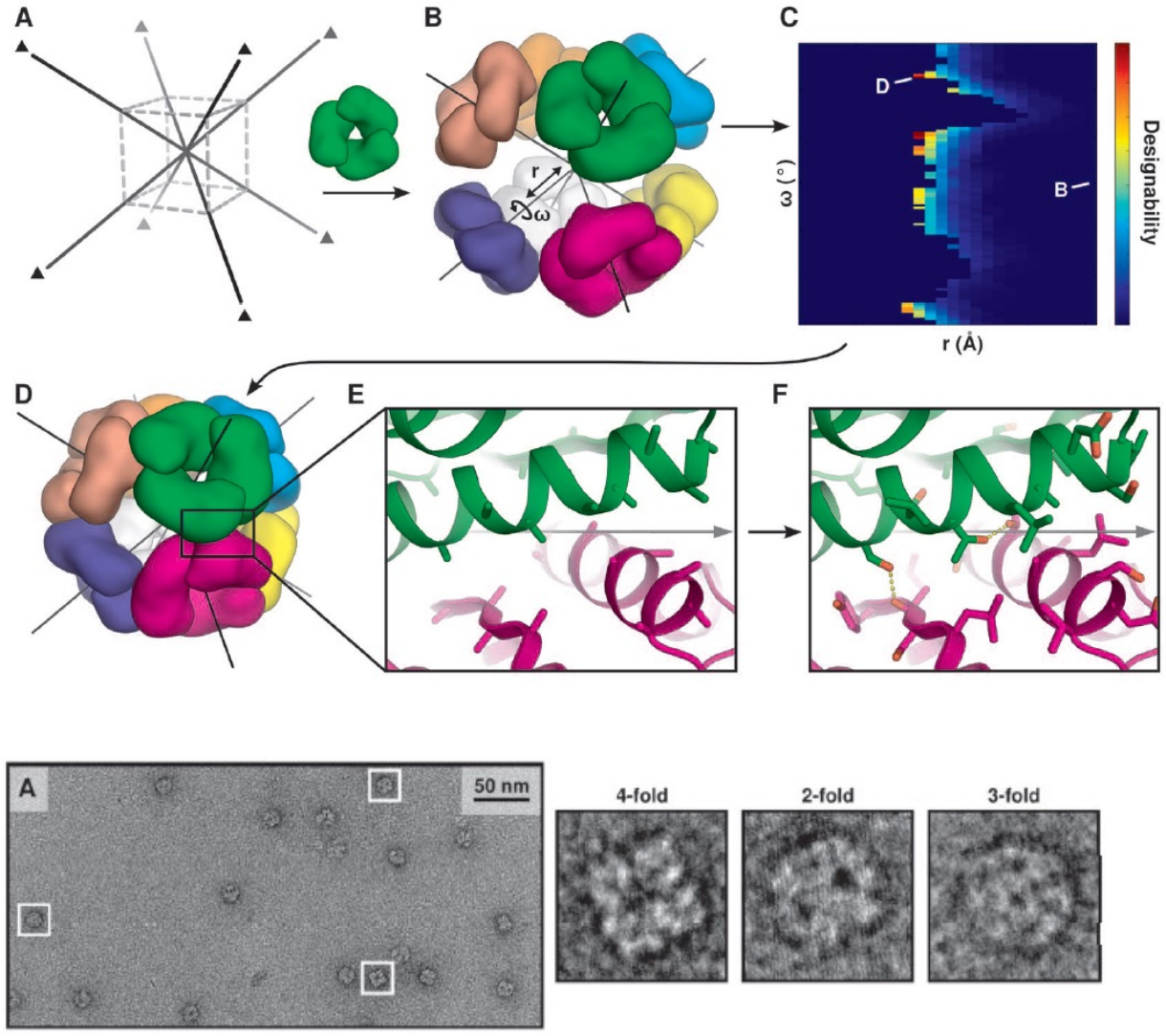
Problems:

- Engineering of a multitude of interactions
  - Protein folding
  - Protein association thermodynamics
  - Control of topography / stoichiometry

Methods:

- Directed evolution from a natural starting point
- Design from a natural starting point
- De-novo design

De novo design: Programmed protein self-assembly

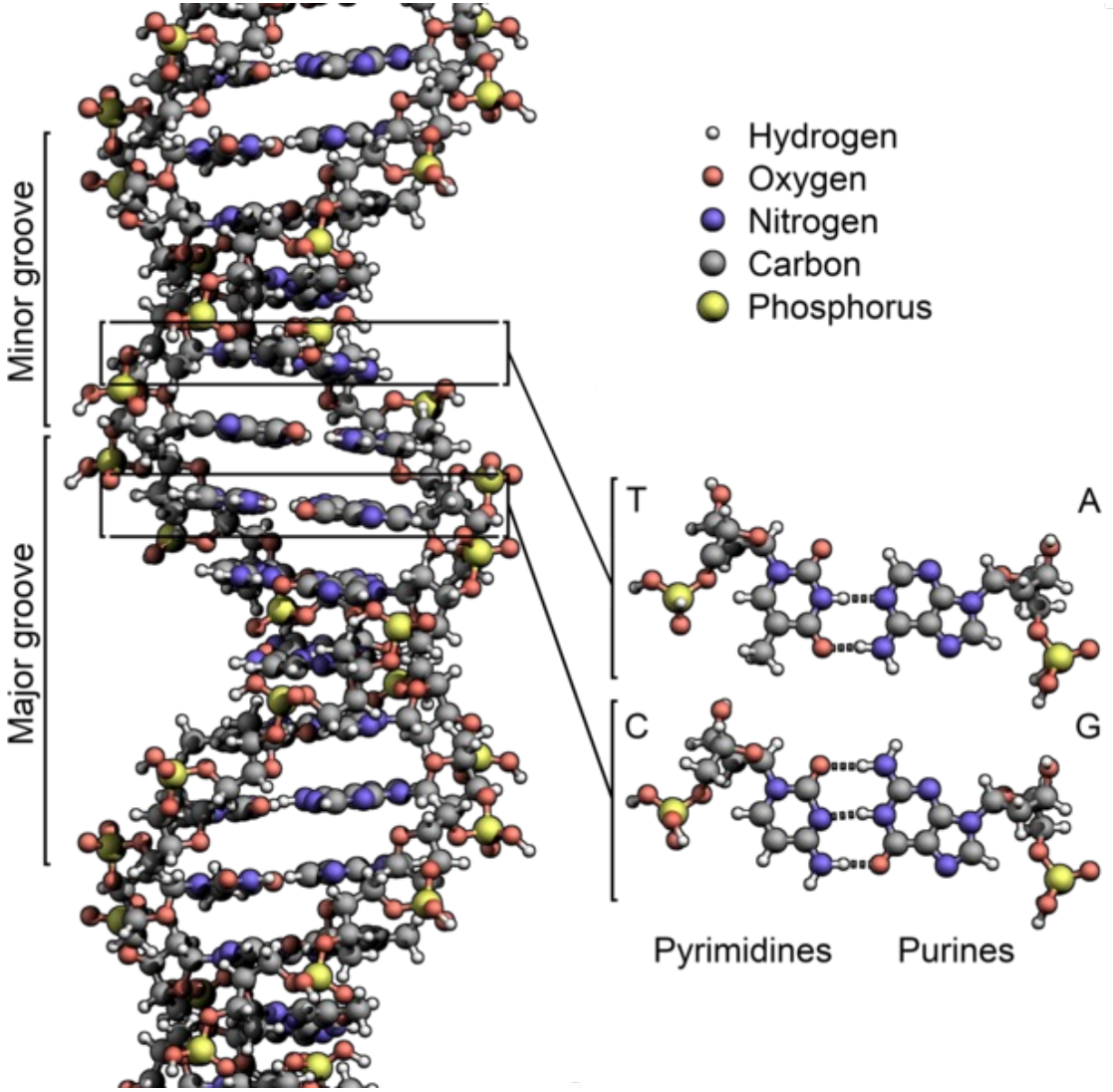


Computational *de novo* design

- Symmetrical docking, optimizing of degrees of freedom (RosettaDesign)
- Interface design:
  - Optimized shape complementarity, hydrophobic packing, hydrogen bonding, and electrostatics
- Naturally ocurring trimeric proteins as building blocks
- Reconsitution of objects with tetrahedral, octahedral, and icosahedral architecture

Baker lab: King et al., Science 2012

DNA as a nanomaterial

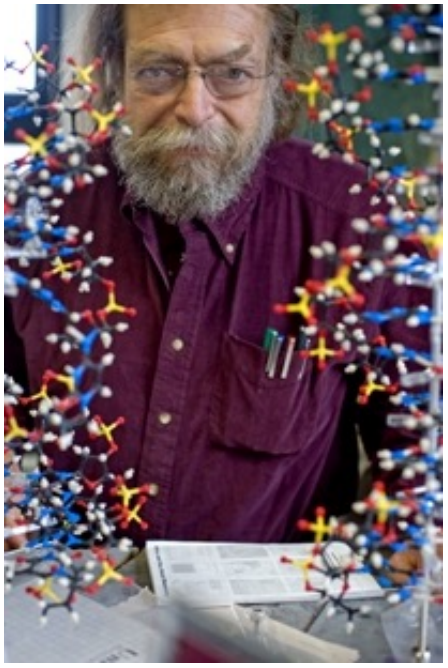


www.wikipedia.com

- **Basepairing**: information content
- **Non-repeating polymer** (unique DNA sequences)
- **Stiff** structure
- Chemically very **stable**
- Tolerant to **high temperatures** (thermal cycling possible)
- Defined programming of basepair sequence possible
- Chemical synthesis cheap and efficient

- **Programmable biomaterial!**

The foundations of DNA nanotechnology



Nadrian Seeman

X-ray crystallographer

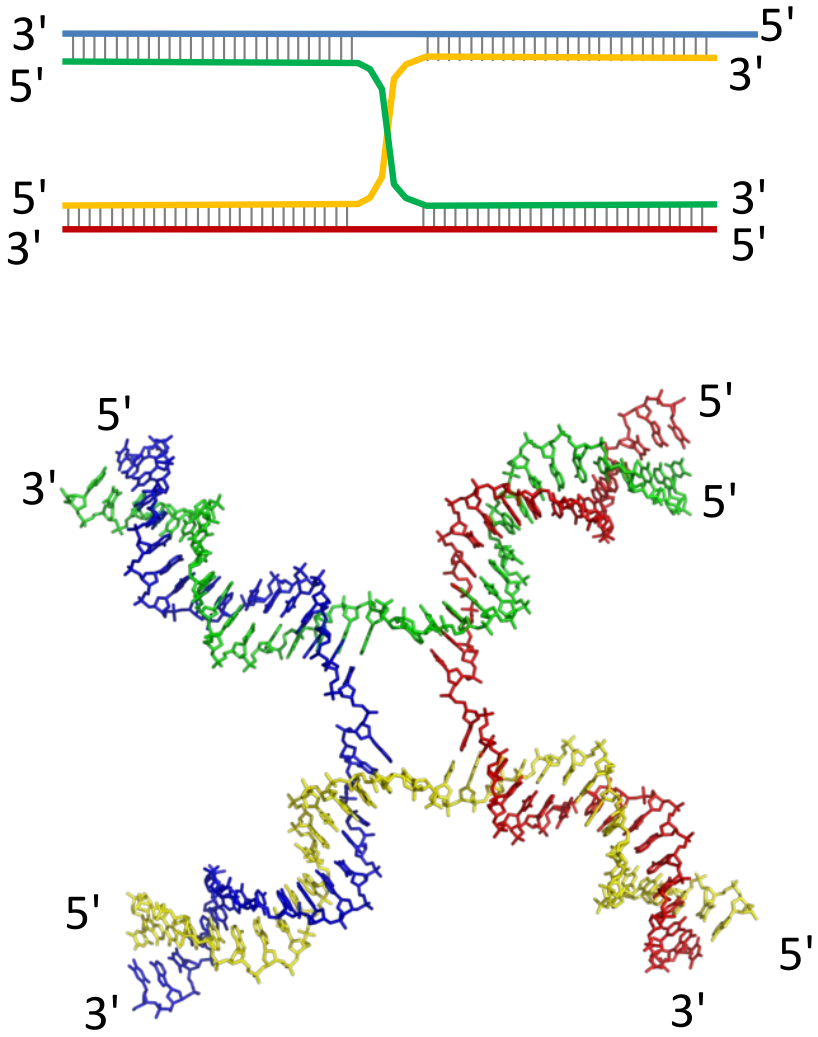
"One day I went over to the pub to think about what six-armed [Holliday] junctions might look like when I realized that they'd be just like the flying fish in Escher's woodcut *Depth* [...] And they're arranged like the molecules in a crystal."

the-scientist.com

M. C. Escher - *Depth*

→ DNA nanotechnology

The Holliday junction

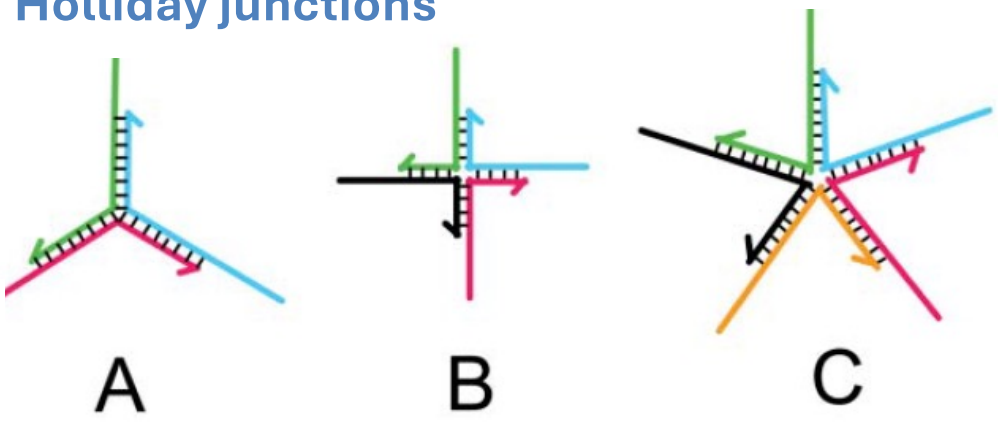


- Structure first described in the 1960s by Robin Holliday
- Forms during crossing-over / strand invasion process during meiosis
- Vital for genetic diversity: allows mixing of parental alleles!
- Occurs during homologous recombination processes
- Observed by microscopy in 1970s
- Can be resolved by cuts and religations into different products, resulting in strand exchange

- **This crossover forms the basis of most DNA nanostructures**

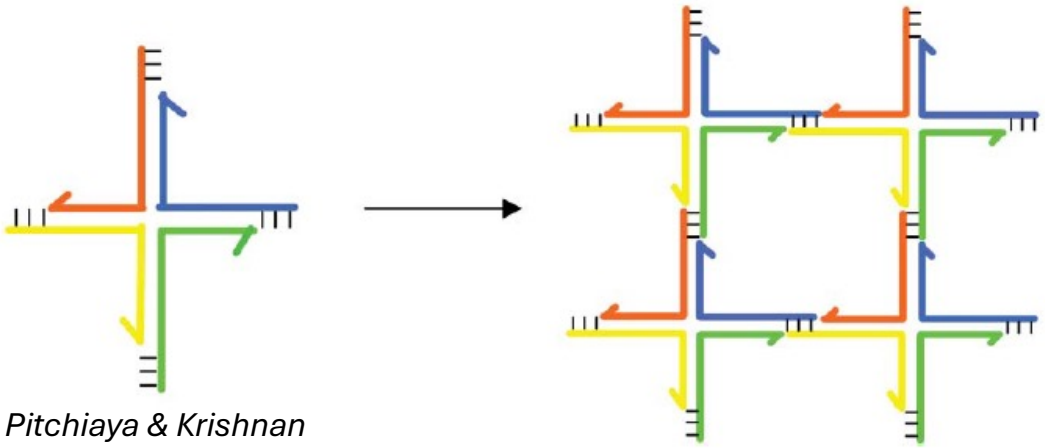
Self-assembly with Holliday-junctions

Topologies of multi-armed Holliday junctions



- Multi-armed DNA constructs are possible

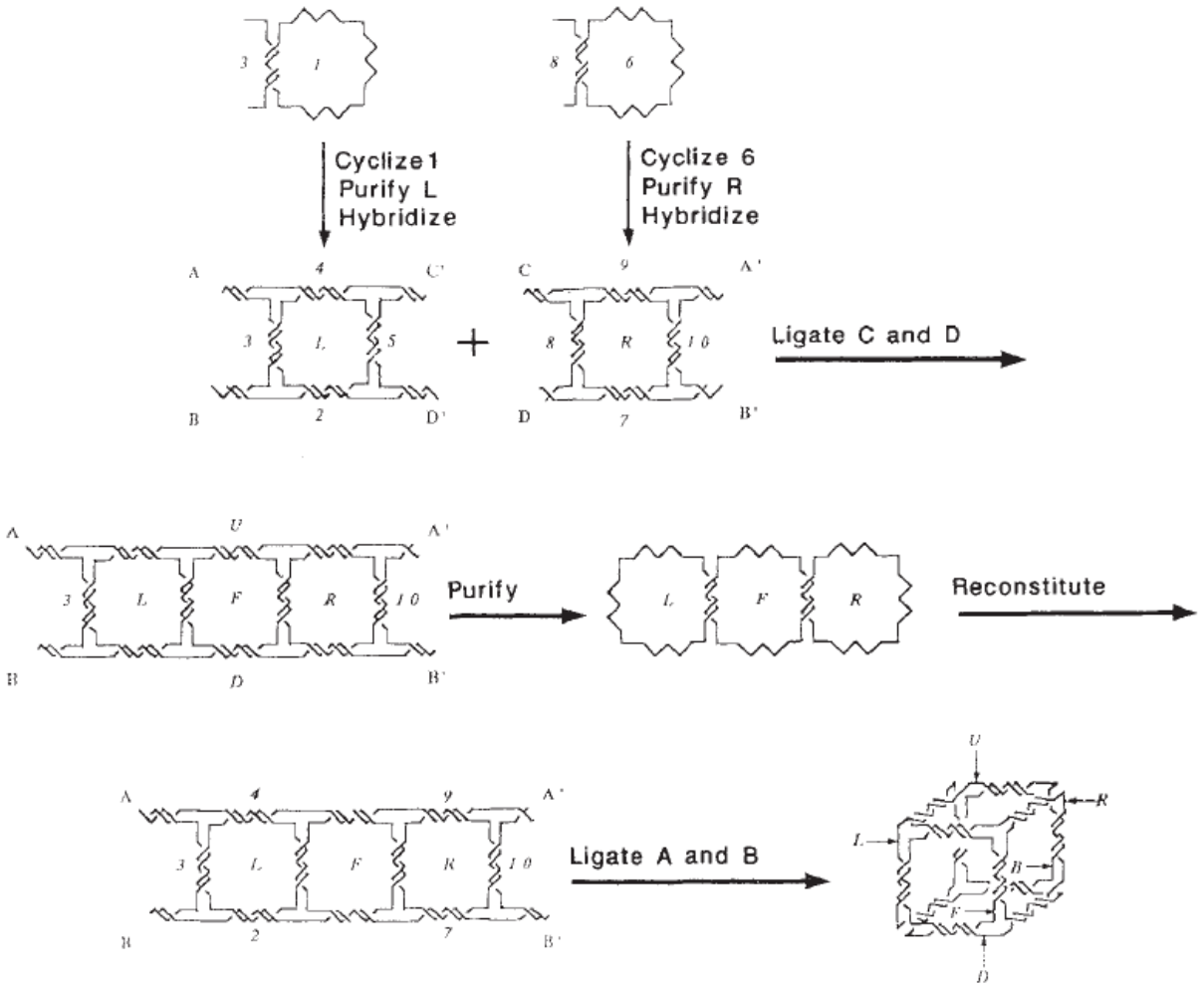
2D-array formation



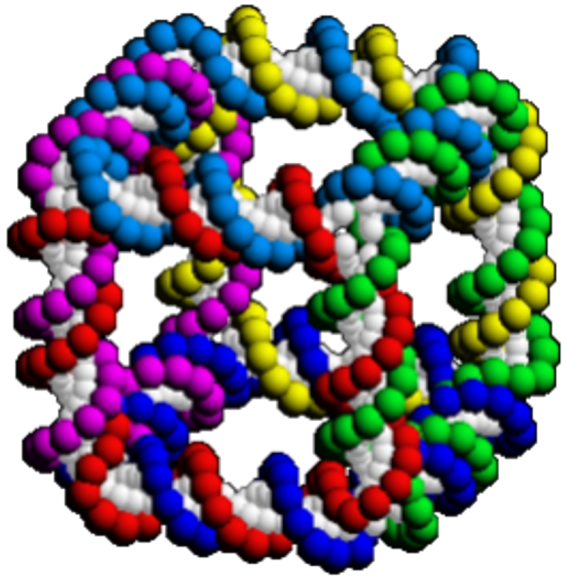
Pitchiaya & Krishnan ChemSocRev 2006

- Sticky ends allow self assembly into 2D or 3D elements

DNA cube: First rationally designed DNA polyhedron structure

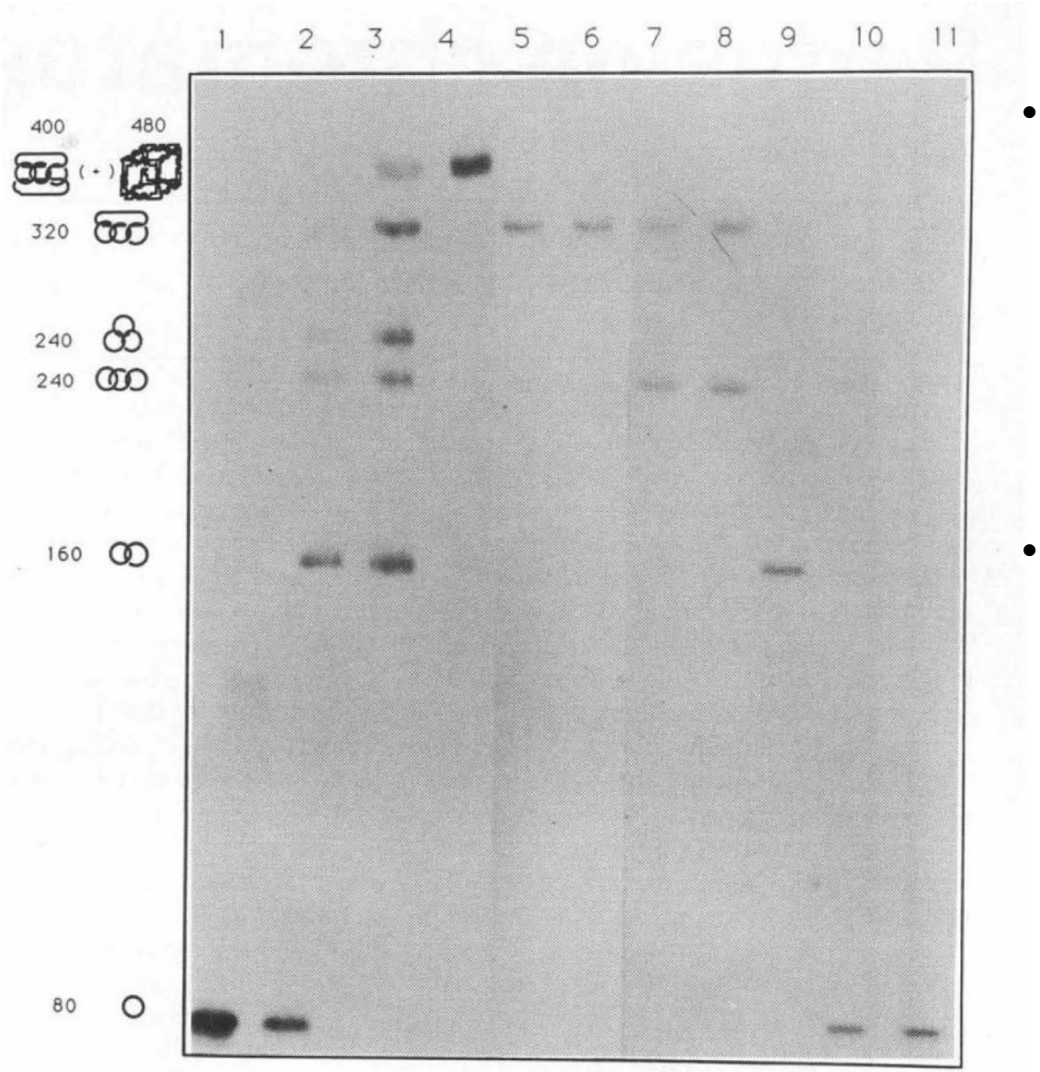


Chen & Seeman, Nature 1991



- Very small object!
- 12 edges of equal length
- Multistep synthesis
- Low yield (<10%)
- **First demonstration of DNA as an architectural material**

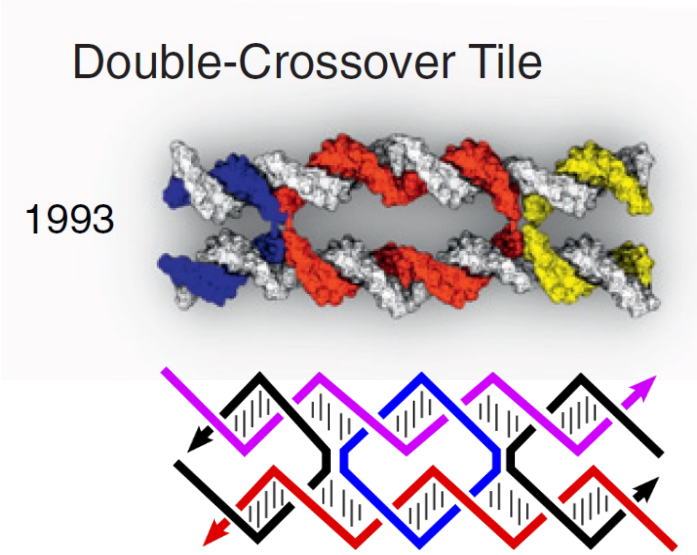
DNA cube: products of the final ligation step



Chen & Seeman, Nature 1991

- Autoradiodiagram of denaturing gel
  - Lane 1: cyclic 80-mer marker
  - Lane 2: markers for intermediate products (up to four cycles)
  - Lane 3: Products of final ligation (80-mer lost during manipulation)
  - Lane 4: purified product
- Digestion analysis
  - Lanes 5 and 6: FR and LF digestions produce 4-cycle product
  - Lanes 7 and 8: double digests for BL and RB, and BL and LF, respectively, produce double belt
  - Lane 9: BL and RD digest produces 2-circle catenane
  - Lanes 10 and 11: Digest for BL, RB, FR, and LF produces single 80-mer circle

Flat double-crossover tile allows construction of more complex objects



Fu & Seeman
Biochemistry 1993

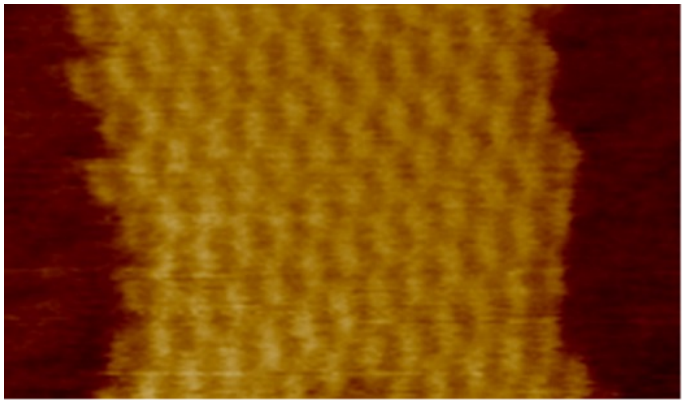
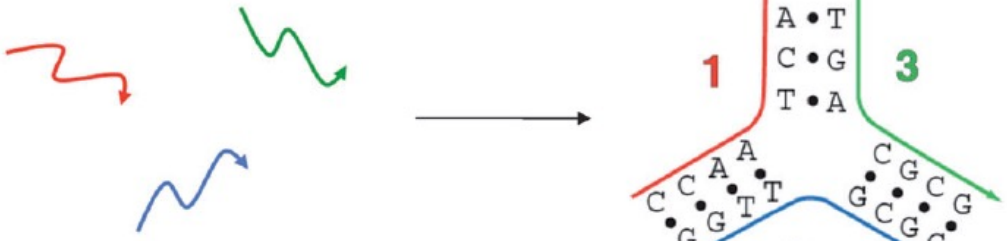


Image source: Wikipedia

- **Two parallel DNA double helices linked by crossover**
- Many conformation / topologies possible
- They differ in mechanical and chemical stability (protection of the junction sited in the interior)
- All topologies were mapped and the most stable one was determined -> some prone to aggregation
- Winfree et al. produced large tile arrays from tiles with sticky ends
- Even DNA-based computation possible (tile is a molecular logic gate, specific sequences represent binary values (0 or 1)

DNA nanostructures: Origami approach

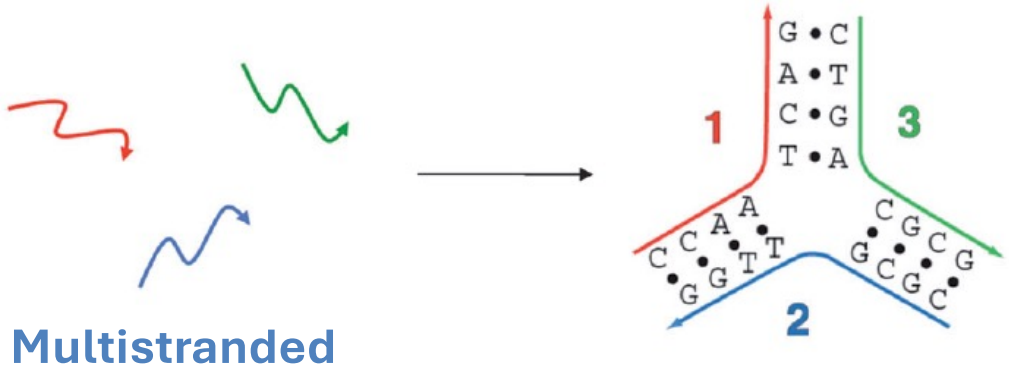


Multistranded

Problem:

- Stochiometry
- Entropy
- Only simple shapes

DNA nanostructures: Origami approach



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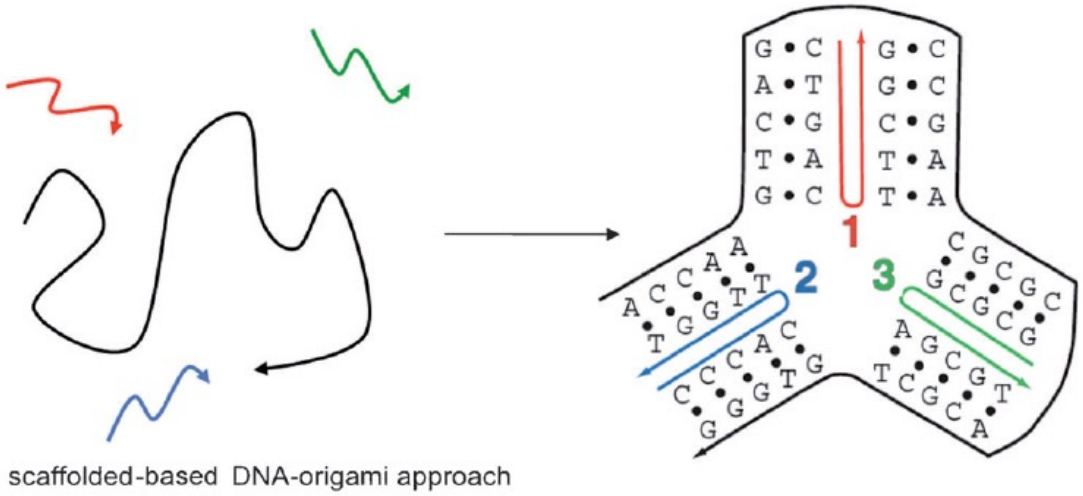


Origami:

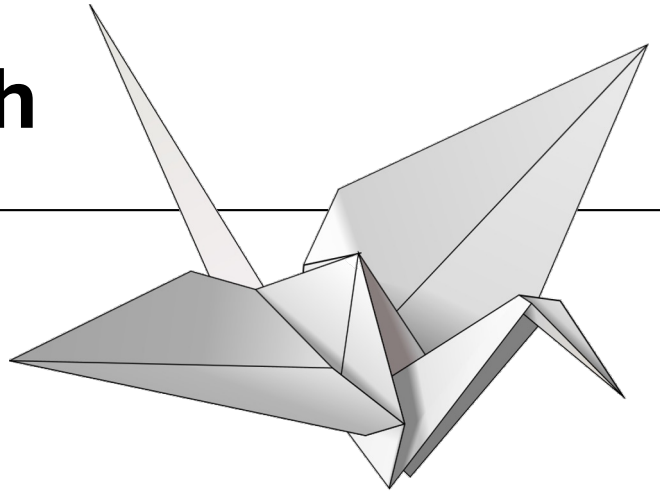
- Scaffolds and staples
- One guiding strand → entropic advantage

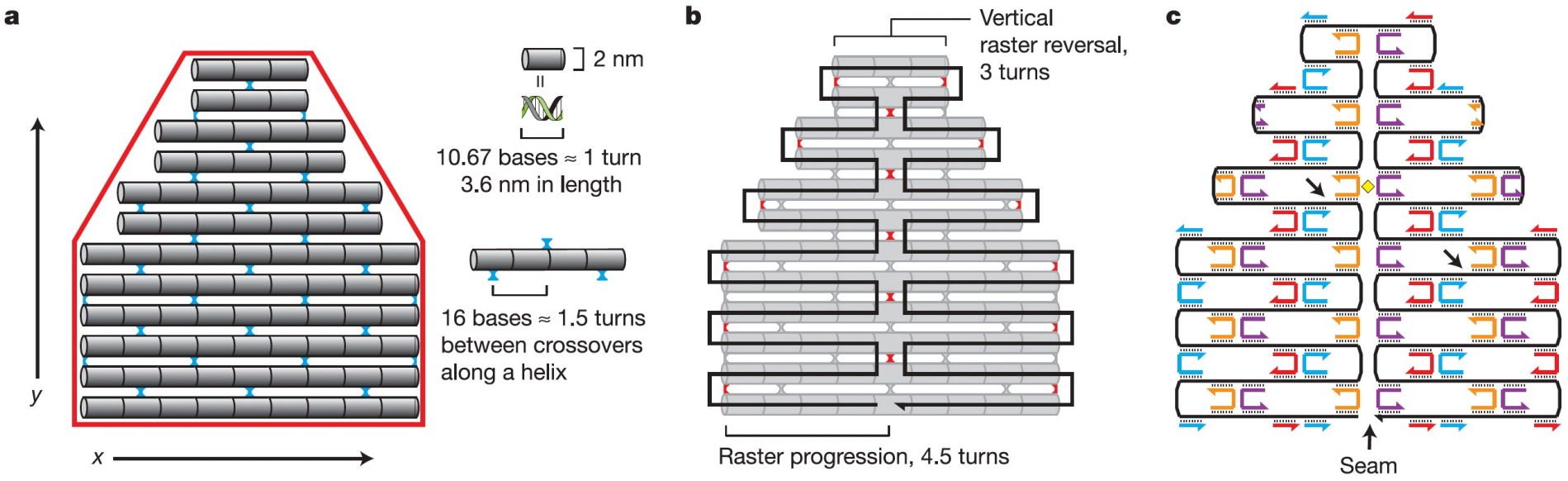
Scaffolded DNA origami approach

Barbara Sacc & Christof M. Niemeyer
Angew Chem 2012

DNA origami approach

Paul Rothemund, Nature 2006

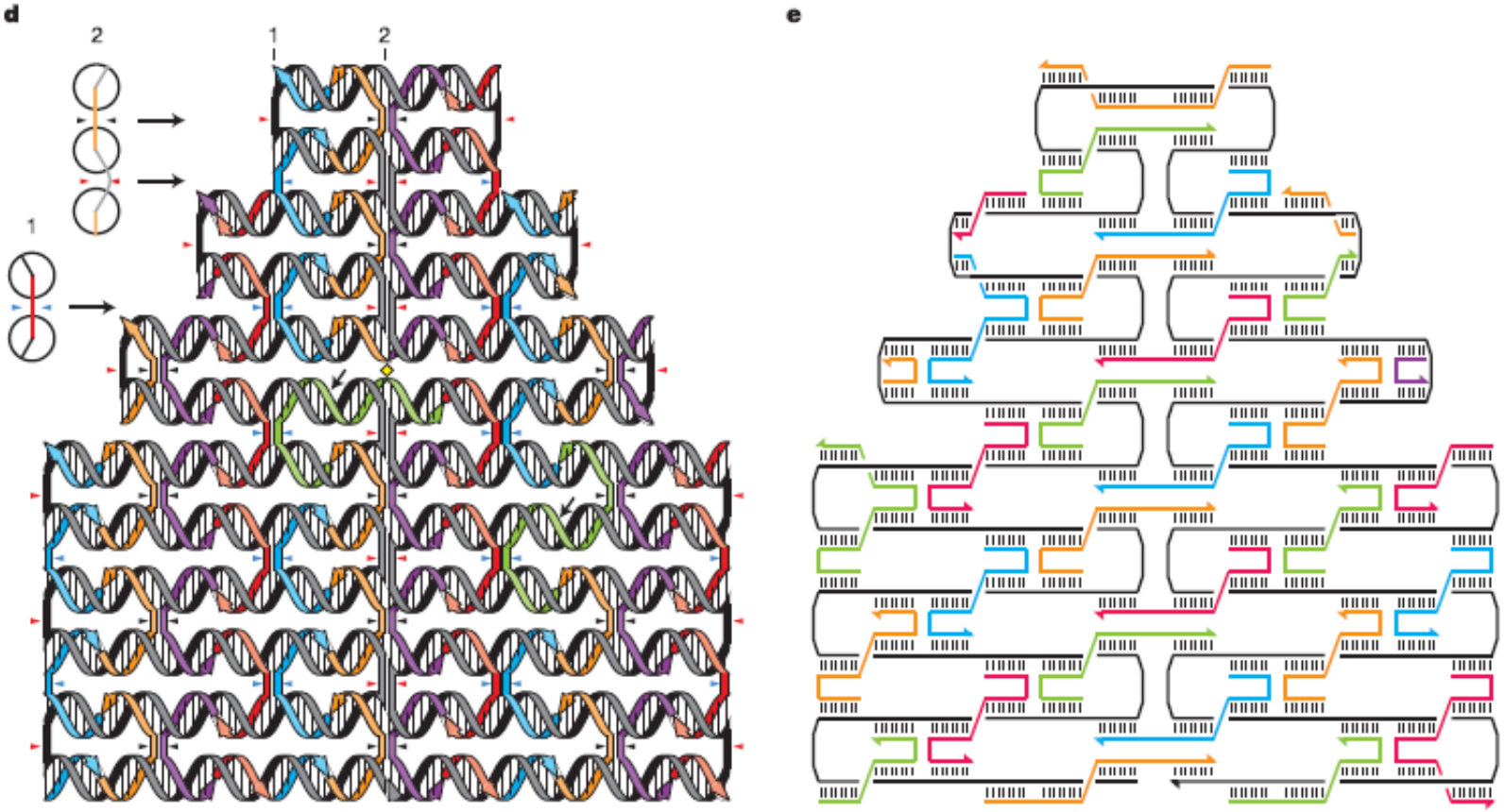




Staples bind two helices and are 16-mers

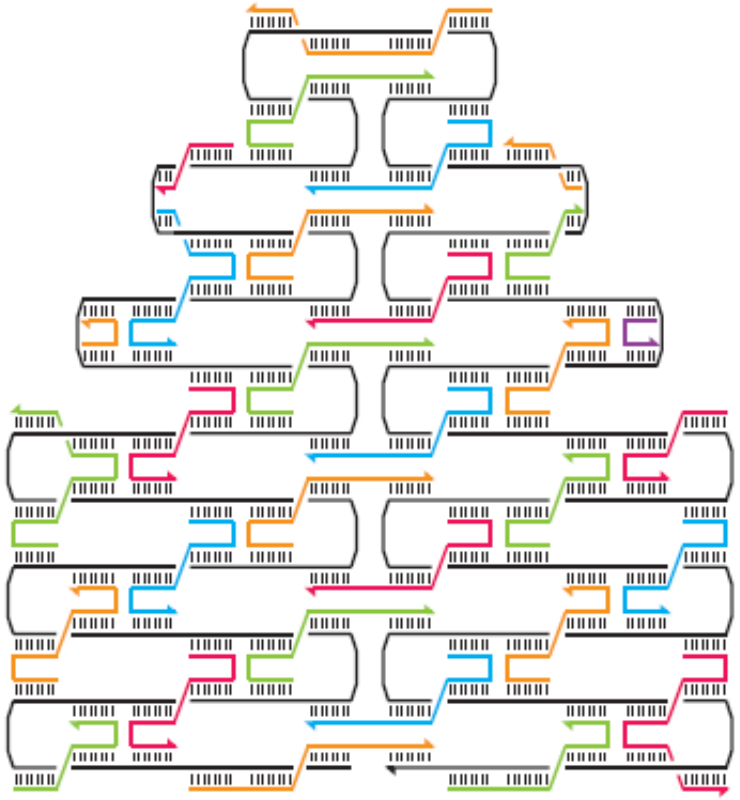
DNA origami approach

Paul Rothemund, Nature 2006

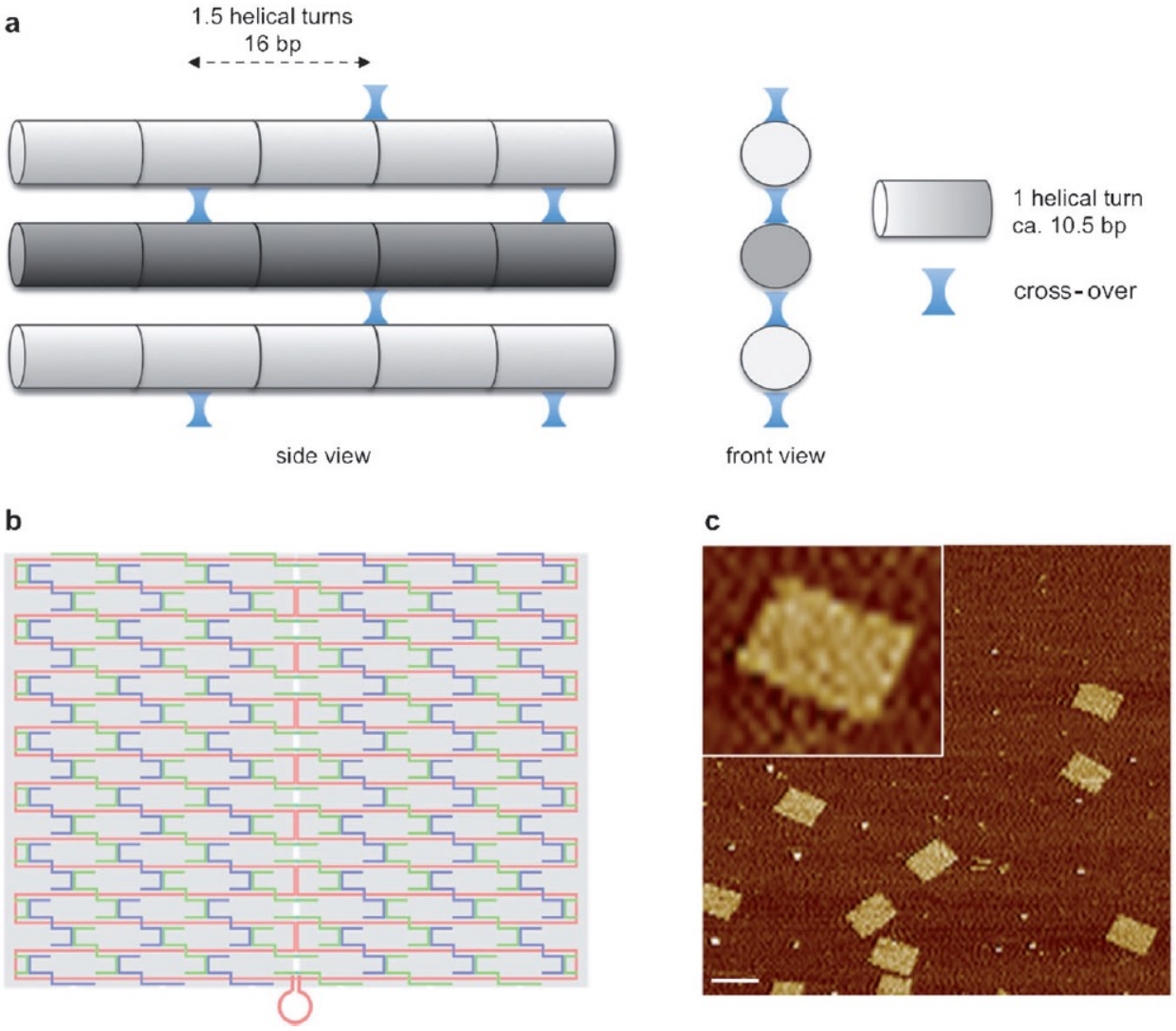


DNA folding procedure

- 7.25 kilobase long **M13mp18** genome (circular)
- Folding with aid of ca. 200 staple-strand
- Array of antiparallel helices through periodic cross-overs
- Self-assembly process:
  - One pot, requries counterions (Mg^{2+}, Na^{+})
  - Heat to 90 °C
  - Cool to room temperature



General origami approach



Design of appropriate staple strands:

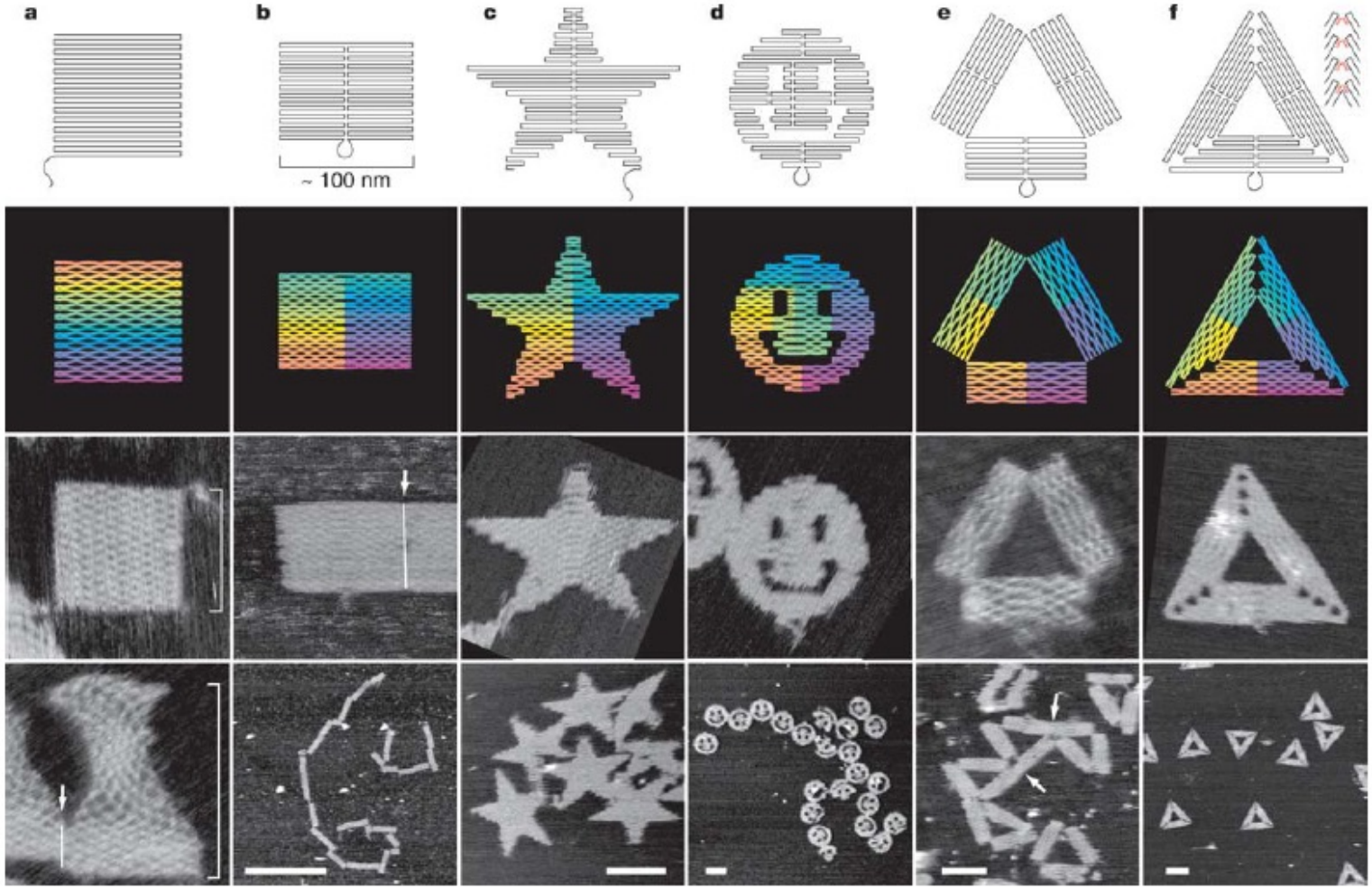
Arrangement of DNA double helices

High yield, homogenous distribution of particles

10^{14} similar structures achievable in one assembly

DNA origami: Arbitrary structures achievable

Paul Rothemund, Nature 2006



Paul Rothemund: DNA origami

- https://www.youtube.com/watch?v=WhGG__boRxU&list=WL&index=2&t=595s&ab_channel=TED

- 5:09

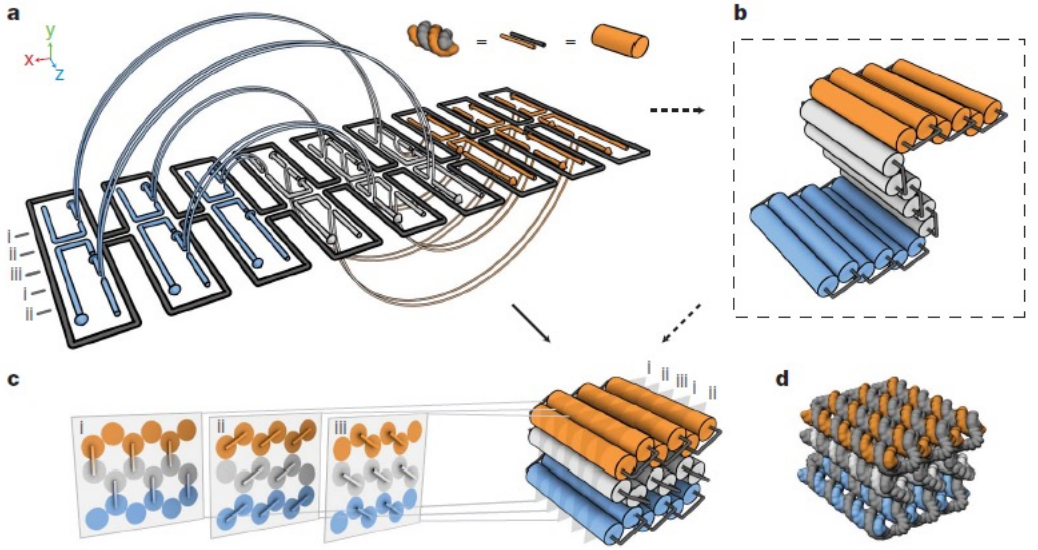
Exercise

You have designed a DNA origami structure to form a specific 3D shape using a scaffold strand derived from the M13 bacteriophage and 200 staple strands. After running your assembly experiment, you observe that the yield of correctly formed structures is significantly lower than expected. Instead of the desired shape, you find a mixture of incomplete and misfolded structures when analyzed by atomic force microscopy (AFM).

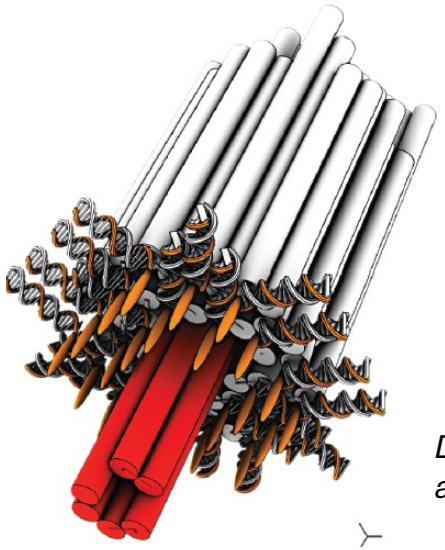
Discuss the potential reasons for the low yield and incorrect formation of the DNA origami structures. Propose a systematic troubleshooting plan to identify and address the issues.

Extension to 3D structures

- Single layer origami: not stable for shear stress
- More rigid 3D objects are required
- Strategy: packing multiple helices into space-filling structure



Shih lab: Douglas et al. Nature 2009



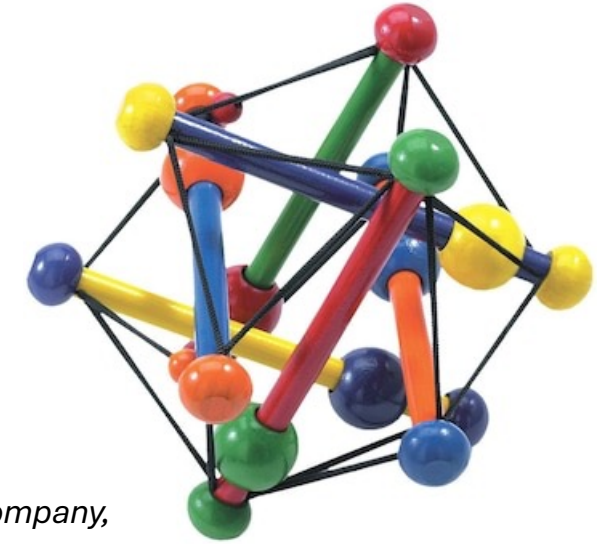
Diez & Simmel lab: Langecker et al. Science 2012

Tensegrity rules

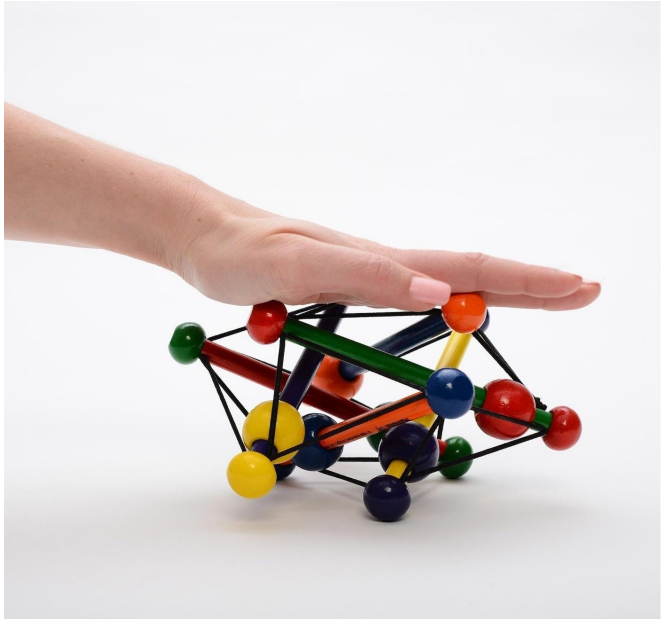
Tensegrity: "The property of a skeletal structure having continuous tension members (such as wires) and discontinuous compression members (such as metal tubes) so that each member performs efficiently in producing a rigid form."

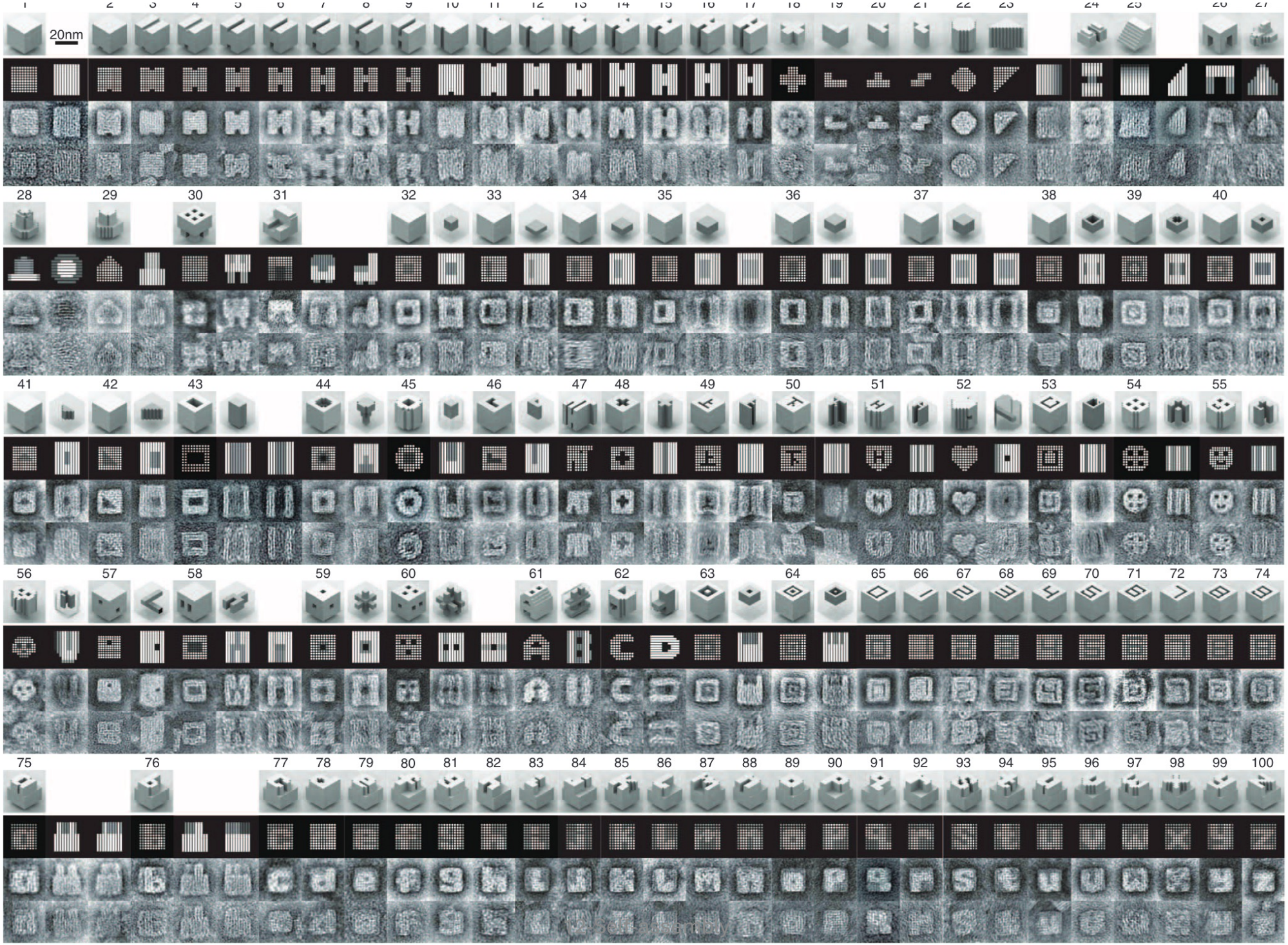
(Merriam Webster dictionary)

- Stiff sequences (struts) → push outward
- Flexible linkers (tendons) → pull inward



Manhattan Toy Company, Skwish Classic





Ke et al, Science 2012

Use of DNA origami

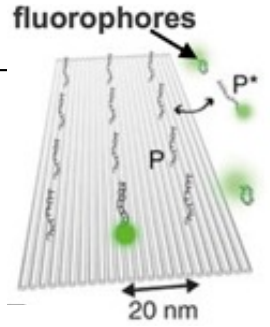
Prepared by computational design

Analyzed by single molecule imaging (AFM, EM)

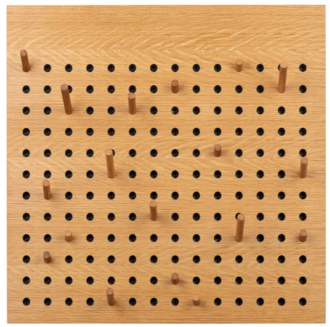
Uses:

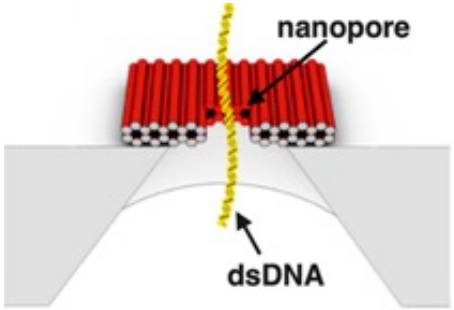
- Molecular pegboards to arrange arbitrary objects in 2/3D space, for arrayed objects such as:
  - Nucleic acids
  - Small molecules
  - Proteins
  - Nanoparticles

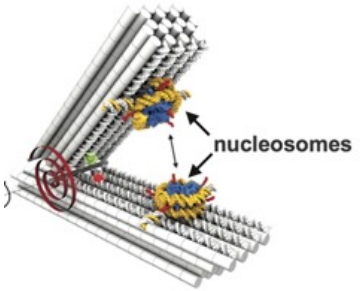
- Functional devices:
  - Nano-calipers: force probes
  - Pores and channels
  - Encapsulation of function
  - DNA encoding of function (aptamers)

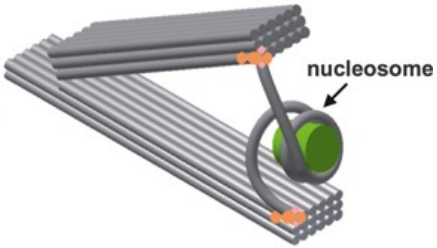


Pegboard:



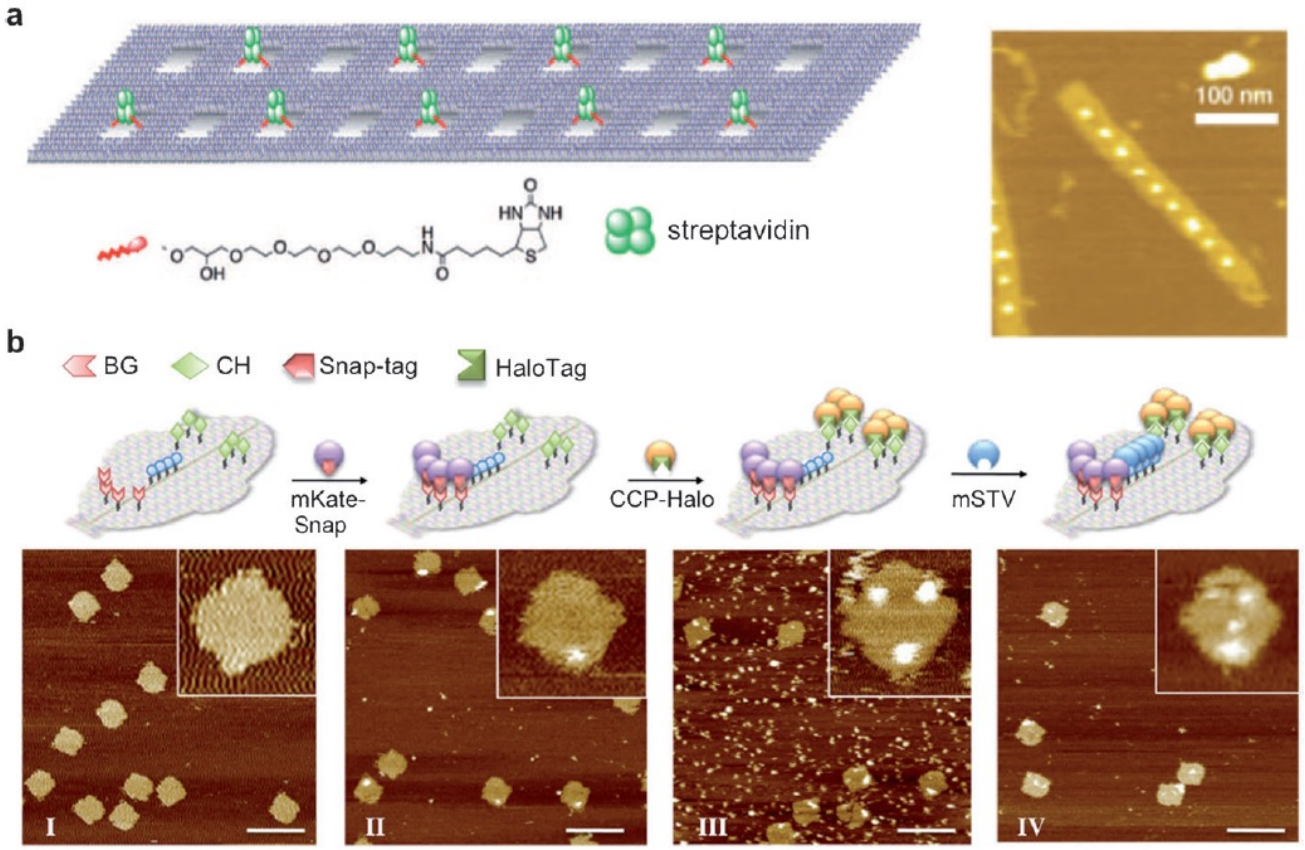






DNA origami devices for molecular-scale precision measurements, 2017
Carlos E. Castro,Hendrik Dietz and Björn Högberg

Functionalization of DNA objects



Barbara Sacca & Christof M. Niemeyer
Angew Chem 2012

- Bulky motifs, dumbbell hairpins
- Hybridization of DNA tagged components to terminal extensions
- Biotinylated DNA insertion

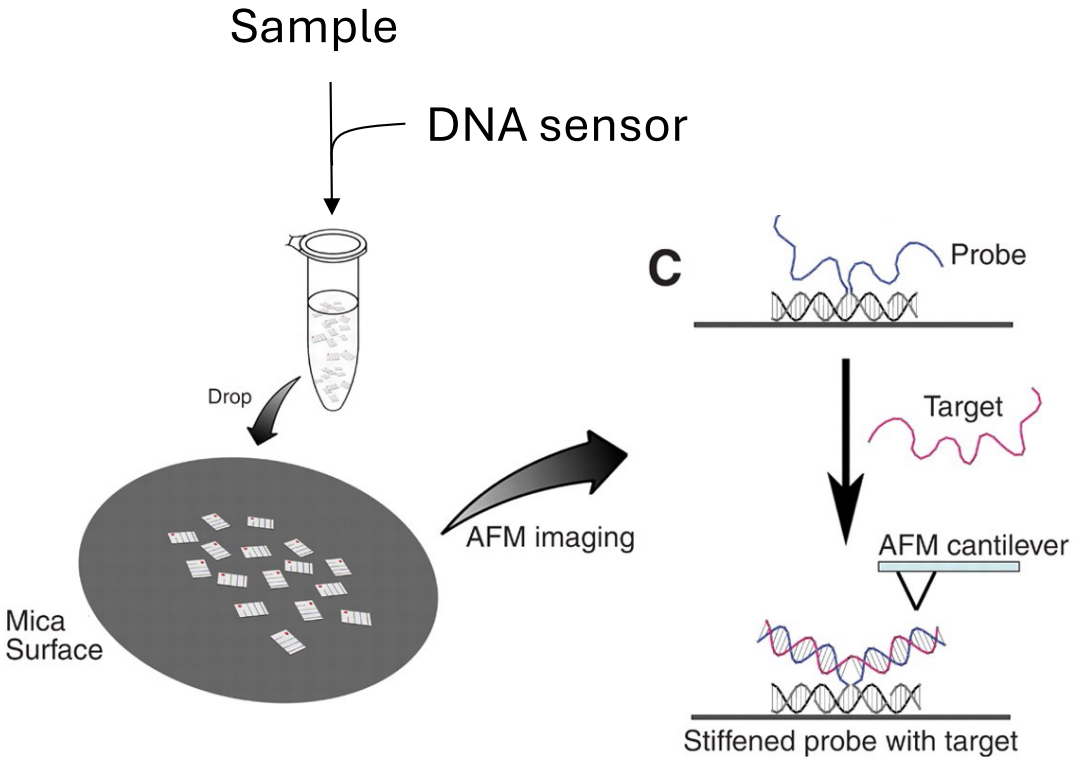
Case study: DNA origami sensors

Aim:

Generation of a DNA microarray on the nanoscale

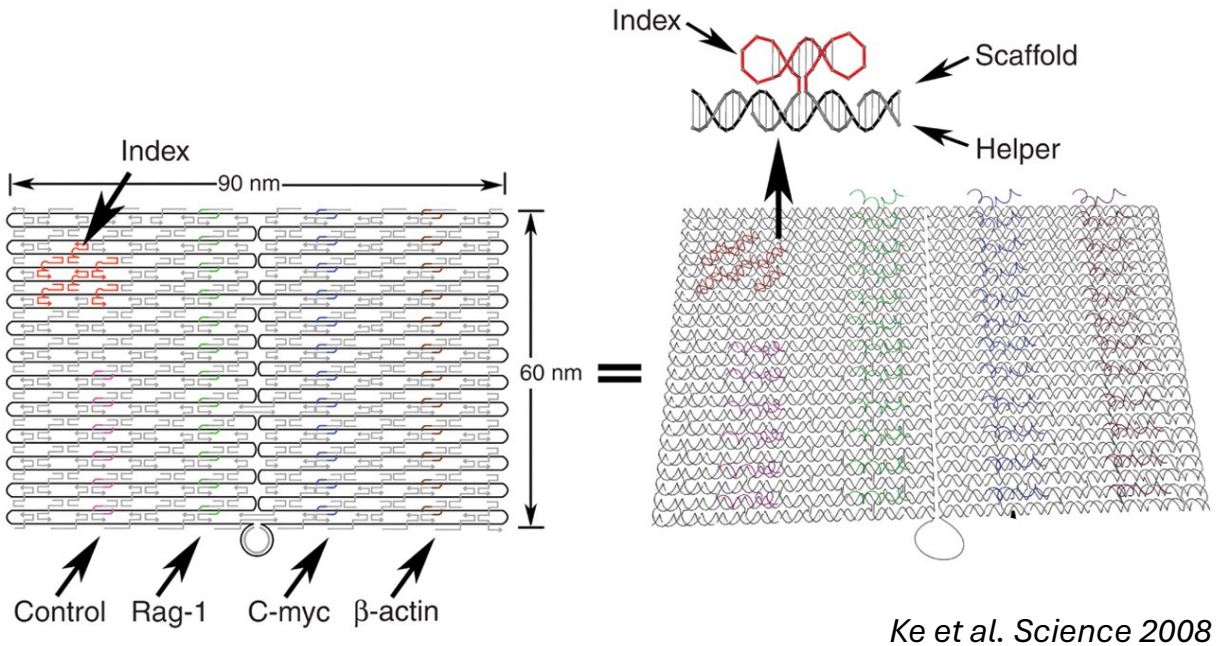
Multiplexed detection of several gene products on a single molecule level

Spatial arrangement on a DNA origami tile → readout by AFM



Ke et al. Science 2008

Sensor design: DNA origami tile with index



Ke et al. Science 2008

DNA tile: circular M13 viral DNA, held together with 200 helper strands

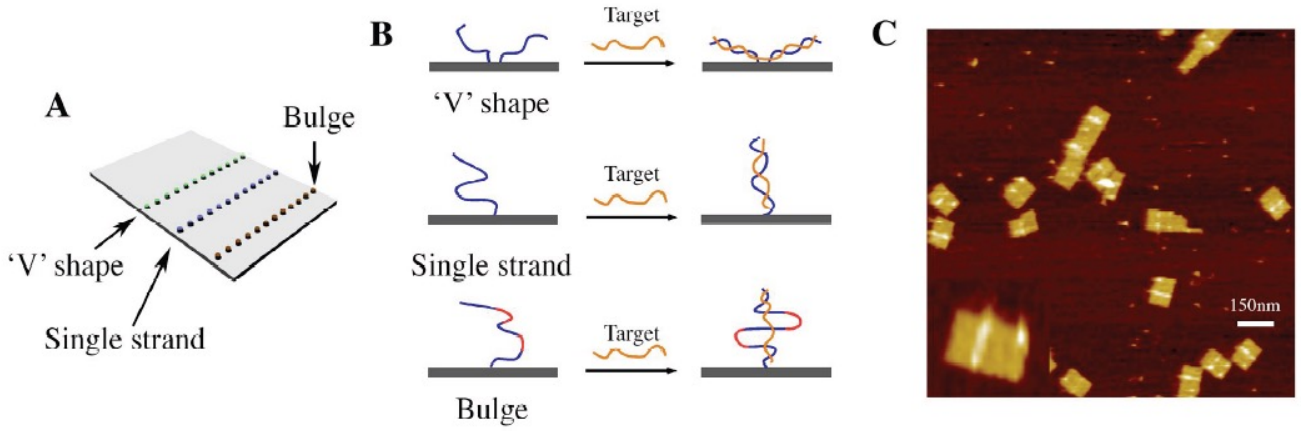
DNA dumbbell, asymmetrically placed for index readout

Four test strips for detection of RNA

12 copies of the probe strand, separated by 5 nm

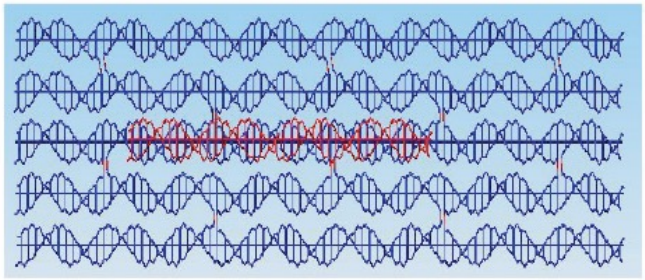
Lines separated by 20 nm

Design of the probe architecture

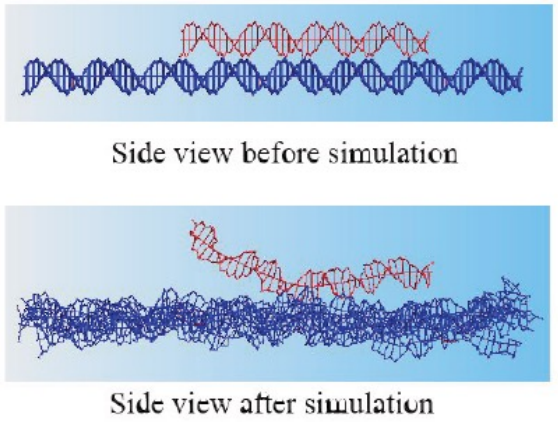


Testing different detection methods

V-shape results in best signal by AFM



Top view before simulation



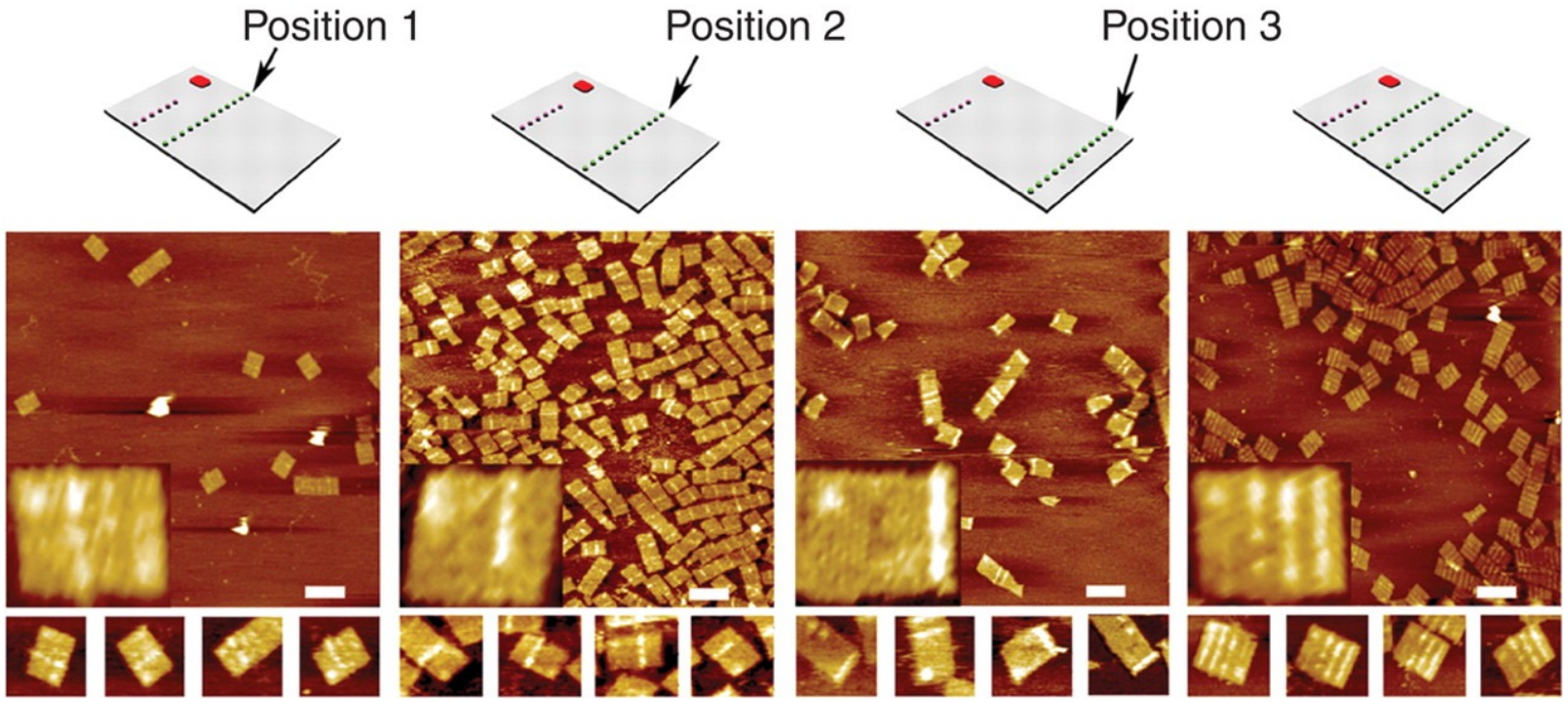
Side view before simulation

Side view after simulation

Molecular dynamics simulations exhibit V-shape of bound DNA-RNA hybrid strands

Ke et al. Science 2008

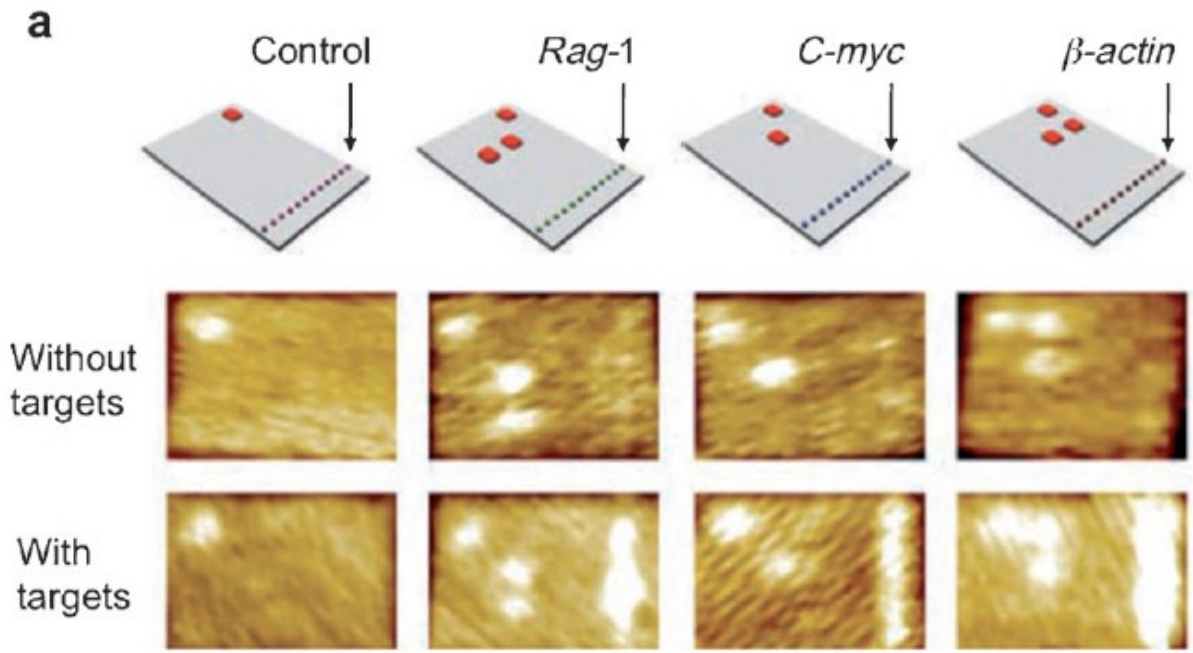
Probe position and binding efficiency



Ke et al. Science 2008

- All probes are for RNA of Rag-1 protein, 10 nM of tiles mixed with 600 nM of target DNA
- Interestingly, **probe position influences binding efficiency**
- Edge position results in best binding, why?

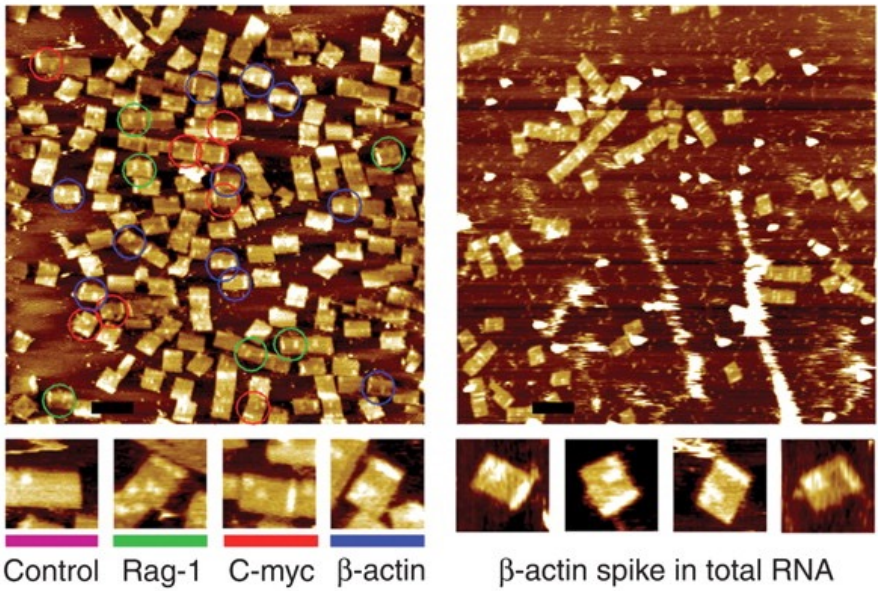
DNA origami sensors

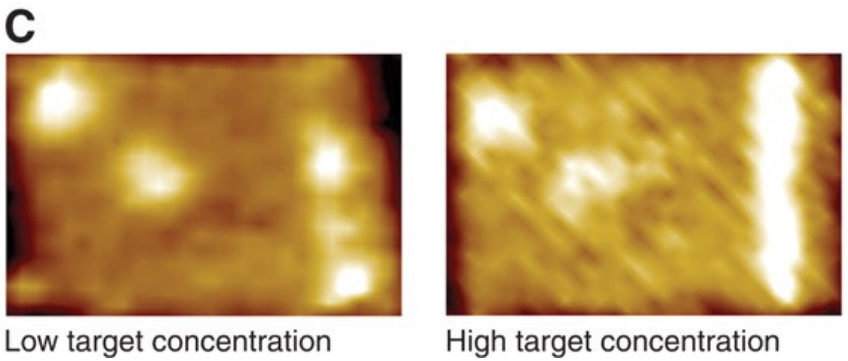


- Only the edge positions are employed for sensing
- Barcode in the index region is used for tile discrimination
- AFM images can show distinct pattern for all tiles

Ke, S. Lindsay, Y. Chang, Y. Liu, H. Yan, Science 2008, 319, 180

Tiles function as high specificity sensors





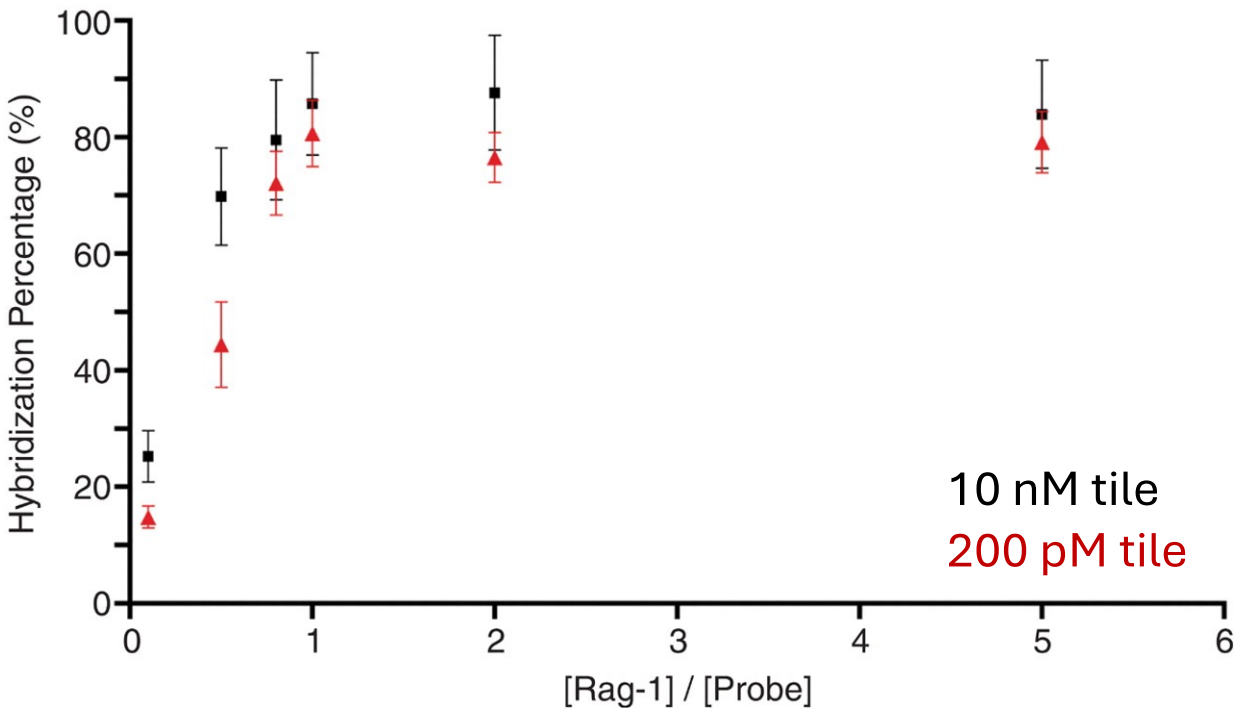
Four tiles (for the four model RNAs) are readily discriminated by their barcodes in a mixture

Spike-in of β-actin RNA into high background of cellular RNA: specific detection

No cross-reactivity observed (only β-actin tiles show binding)

Ke et al. Science 2008

Binding is highly specific and stoichiometric



Ke et al. Science 2008

- Nearly linear increase at [target]/[probe] < 1 observed
- Saturation at [target]/[probe] > 1
- Non-Michaelis-Menten binding
- Detection is only limited by concentration of tiles
- Every target molecule is bound at low concentrations.
- This is due to the very high energy of the binding (-50 kcal/mol)

Conclusion:
These sensor tiles can detect RNA at single molecule resolution

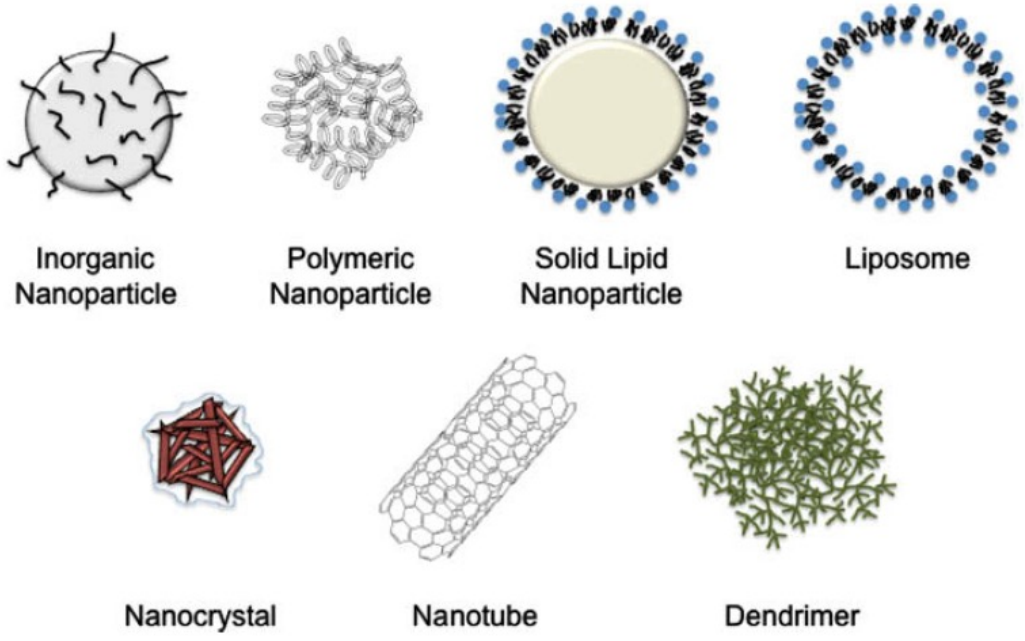
→ Challenge: difficult to estimate the Kd of this reaction

Medical Applications: Drug Encapsulation and Controlled Delivery

Possible applications:

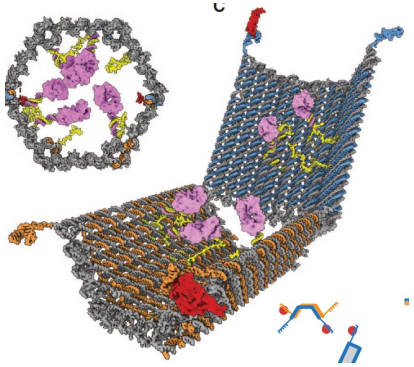
- Encapsulation of drug molecules
- Retention in cells, improved pharmacokinetics (slow release, long plasma lifetimes)
- Exact targeting of toxic molecules (reduction of side effects)

Nanoparticles employed in drug delivery



Faraji, Bioorg. Med. Chem. 2009

Douglas et al. Science 2012



Activity

- Discuss: what are the limitations of DNA origami structures?

Connections

- https://connections.swellgarfo.com/game/-Ny0-36i13UCj5NWiwnk