Assembling Materials with DNA as the Guide

Hanadi Sleiman
Department of Chemistry,
McGill University, Montreal, Canada
Challenges in Chemical Construction

Molecular Chemistry
Angstrom scale

Nanoscale?

How can we position components into any deliberately designed nanometer scale pattern, be it periodic or aperiodic?
Challenges in Chemical Construction

• How can we position components into any deliberately designed pattern?
• Can the pattern be externally manipulated?

Would lead to:
1. A fully functional artificial photosynthesis system
2. A multiprotein assembly to interrogate biological phenomena
3. A multimetallic/ enzyme catalytic "factory"
4. A capsule/nanotube to transport and deliver a drug selectively
5. A nanoparticle assembly for optimal biological detection
6. A nanoelectronic circuit board
Natural Self-Assembly

Cell

Ribosome

Tobacco mosaic virus

Photosynthesis

Hemoglobin
DNA: Programmable Template for Nanoscale Organization

- Highly Specific Molecular Recognition
- Dynamic Self-Assembly
- Shape persistence: rigid over 10 nm, persistence length 50nm
- DNA synthesis automated
- Enzymatic Control
- Biodegradable, Biocompatible

Lo, Metera, Sleiman, Curr. Opin. Chem. Biol., 2010, 14, 597
McLaughlin, Hamblin, Sleiman, Chem. Soc. Rev. 2011, 40, 5647
What do you know about DNA?

What properties does DNA have?
DNA Self-Assembly

Thymine

Adenine

Cytosine

Guanine

1 turn

~ 10 bp

~3.4 nm

Minor groove

Major groove

2 nm
DNA as a Template for Bottom-Up Assembly

- **Highly Specific Molecular Recognition:** programmable molecule
- **Shape persistence:** rigid and well-defined over long distances (Rigid rod over 2-3 helix turns ca. 10 nm, persistence length ca. 50 nm)
- **DNA synthesis:** automated, amplified by enzymes

Aldaye, Sleiman, Science, 2008, 321, 1795

**DNA:** promising template to pattern materials on the nanoscale
DNA: Programmable Template for Nanoscale Organization

- Highly Specific Molecular Recognition
- Dynamic Self-Assembly
- Shape persistence: rigid persistence length 50
- DNA synthesis automated
- Enzymatic Control
- Biodegradable, Biocon

Planar Bases
π Stack on top of each other
Synthesis of 5’-d[TAG]-3’

1. Attach on solid support
Synthesis of 5′-d[TAG]-3′

2. Deprotect DMT- group

\[
\text{Cl}_2\text{CHCOOH} \xrightarrow{\text{Cl}_2\text{CHCOOH}} \text{G}^{\text{Bu}} \text{Bu}\]

\[
\text{OMe} \quad \text{MeO}
\]

\[
\text{OMe} \quad \text{MeO}
\]
Synthesis of 5’-d[TAG]-3’

3. Couple the second nucleotide monomer
Synthesis of 5’-d[TAG]-3’

4. Oxidize P(III) tp P(V)

\[ I_2 \]
Synthesis of 5’-d[TAG]-3’

5. Deprotect 5’-DMT
Synthesis of 5'-d[TAG]-3'

6. Couple third nucleotide monomer

tetrazole

then iodine
Synthesis of 5'-d[TAG]-3'

7. Deprotect DMT
Synthesis of 5'-d[TAG]-3'

8. Full deprotection and cleavage with ammonia NH₄OH
Solid-Phase DNA Synthesis Cycle

- **Solid support bound nucleoside**
- **Cycle entry**
- **Detritylation** (\(\text{Cl}_2\text{CHCO}_2\text{H}\))
- **Oxidation** (\(\text{I}_2/\text{water}\))
- **Deprotection** (aq. \(\text{NH}_3\))
- **Coupling**
- **Capping** (\(\text{Ac}_2\text{O}/\text{py}\))

Beaucage & Caruthers
Structural DNA Nanotechnology

DNA Structure and Base Pairing Define:
Architecture, connectivity, programmability

Seeman, Nature 2003, 421, 427


Ned Seeman (NYU)
Structural DNA Nanotechnology

DNA Tile Assembly

Seeman, Nature 2003, 421, 427

Algorithmic DNA Assembly


DNA Origami

Turberfield, Angew. Chem. 2005, 3057

RNA Assembly

Aldaye, Sleiman, Science, 2008, 321, 1795
2D DNA Assemblies: Seeman’s Tiles

DNA branch points and sticky ends - no ability for long range order with these flexible tiles

DNA Crossovers: One strand starts on one double helix and "crosses over" to a neighboring double helix

RIGID, "KNITTED" TILES THAT EXTEND AS 2D-SHEETS

Holliday crossover
junction
HJ
| crossover
DX junction
antiparallel
2 crossover
(reciprocal exchange)

Holliday junction
1 crossover
DX junction
tandem parallel
2 crossover
(reciprocal exchange)

Holliday junction
1 crossover

TX
3 crossover
3 strands
DNA Crossovers: One strand starts on one double helix and “crosses over” to a neighboring double helix

RIGID, “KNITTED” TILES THAT EXTEND AS 2D-SHEETS

Cross-shaped tile


Tree-point star tile

Fig. 2  Distances between crossovers define the angle between helices. Left: an integer number of half turns results in a 2D structure. Right: a non-integer number of half turns results in a 3 dimensional structure. In this case an angle of $120^\circ$ is achieved by a 7 base pair distance between crossovers.
DNA Crossovers: Nanotubes

Figure 2. The tool boxes of DNA tile examples. a) left: a DX DNA tile (adapted from ref. [8]); middle: a TX DNA tile (adapted from ref. [9]); right: a 12-helix DNA tile (adapted from ref. [14]). b) left: a three-helix bundle DNA tile (adapted from ref. [12]); right: a six-helix bundle DNA tile (adapted from ref. [13]). c) left: a parallelogram DNA tile composed of four four-arm junctions (adapted from ref. [28]); right: a DNA triangle tile composed of three four-arm junctions (adapted from ref. [31]). d) upper left: a cross-shaped tile (adapted from ref. [10]); upper middle: a triangular DNA tile formed from DX DNA molecules (adapted from ref. [30]); upper right: a 3-point-star DNA tile (adapted from ref. [11]); bottom: Atomic force microscopy (AFM) images showing the self-assembly of the tiles forming 2D periodic lattices with square (adapted from ref. [10]) and hexagonal (adapted from ref. [30] and ref. [11], respectively) cavities. All the DNA models were generated using the Strata program (www.strata.com).
Folding DNA to create nanoscale shapes and patterns

Paul W. K. Rothemund¹

*Nature* 2006, 440, 297
Great TED talk:

www.ted.com/talks/paul_rothemund_details_dna_folding.html
Fig. 6 Three dimensional DNA origami structures. (a) Two examples of the 3D structures created by Shih and co-workers in 2009.9 The structures were designed using the honeycomb lattice previously described. Adapted by permission from MacMillan Publishers Ltd: ref. 9, copyright 2009. (b) Visualized in 4D.
Fig. 8  Double helical DNA is bent to follow the rounded contours of the target object, held in place by rationally designed crossover networks. (a) Schematic representation of the nanoflask with dimensions indicated. (b) AFM images of the nanoflask. Scale bar is 75 nm. (c) TEM images of the nanoflask after random deposition on TEM grids. Scale bar is 50 nm. From ref. 20. Adapted with permission from AAAS.
3D- Origami
Three-Dimensional DNA Structures for Biological and Materials Applications

Hanadi Sleiman

Department of Chemistry, McGill University, Montreal, Canada
Drug Delivery

*Size and Shape* determine diffusion rates, cellular localization, toxicity and clearance.

*Presentation of targeting ligands* plays an important role in how they are recognized by cells.

*Molecule-responsive* hosts, that can ‘open up’ in the presence of specific molecules that are overexpressed in diseased cell.
Challenges in the Construction of 3D-Materials

- MOLECULAR CAGES
- 3D-FRAMEWORKS
- NANOTUBES
- POLYMERIC MICELLES

- LIPID ASSEMBLIES
- HYDROGELS
- DENDRIMERS

**Challenges in 3D-construction:**

- Can the size and shape be deliberately varied and controlled?
- Can we obtain monodisperse materials?
- Can we place different components on precise locations?
- Can we create ‘molecule-responsive’ cages that open and close on demand in the presence of specific molecules?

- *drug delivery*
- *tissue growth*
- *templates for new materials*
- *control of reactivity of guests in confined space*
- *gas storage*
- *scaffolds for 3D-organization*
DNA: Programmable Template for 3D Organization

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Aldaye, Sleiman, Science, 2008, 321, 1795
Structural DNA Nanotechnology

DNA Tile Assembly
N. Seeman, C. Mao, H. Yan

Algorithmic DNA Assembly
E. Winfree

DNA Origami
Rothemund

Single-Stranded Tiles
P. Yin

3D-DNA Origami
K. Gothelf, W. Shih, H. Yan, T. Liedl, H. Dietz

A. Turberfield, Angew., Chem. 2005, 3057

Aldaye, Sleiman, Science, 2008, 321, 1795

DNA Base-Pairing
- Architectural control
- Connectivity
- Programmability

- Double stranded, rigid, DNA-dense
- Origami: hundreds of DNA strands in structure
Supramolecular DNA Nanotechnology:
Synthetic molecules mediate DNA assembly

- **Functional advantages:** luminescence, conduction, magnetic, catalytic, biological properties, increased stability
- **Structural diversity:** new methods, DNA-economic, orthogonal interactions
- Dynamic structures
- Error Correction


Supramolecular DNA Assembly

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- **Structural diversity:** new methods, DNA-economic, orthogonal interactions
- **Dynamic structures**
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Supramolecular DNA Nanotechnology:
Synthetic molecules mediate DNA assembly

- **Functional advantages:** luminescence, conduction, magnetic, catalytic, biological properties, increased stability
- **Structural diversity:** new methods, DNA-economic, orthogonal interactions
- **Dynamic structures**
- **Error Correction**


Branched DNA Complexes with Organic and Transition Metal Vertices

Vargas-Baca, Mitra, Sleiman, Angew. Chem. 2001, 40, 4629
Mitra, Di Cesare, Sleiman, Angew. Chem. 2004, 43, 5804
F. Aldaye, H. Sleiman, Angew. Chem., 2006, 45, 2204 ("Hot Paper")
Functional Advantages: Rigid Organic Linkers Direct DNA Self-Assembly and Significantly Stabilize DNA Duplexes

Control of Stability

Temperature (°C)

J. Am. Chem. Soc. 2012, 134, 14382

Andrea Greschner
Functional Advantages: Metal-DNA junctions show remarkable stability, new geometries and charge conductance

Angew. Chem. 2001, 40, 4629
Angew. Chem. 2004, 43, 5804
Angew. Chem. 2008, 47, 2443
Angew. Chem. 2009, 48, 9919
Angew. Chem. 2011, 50, 4620

Yang, Sleiman,
*Nature Chem.*, 2009, 1, 390

Hua Yang

Dauphin-Ducharme, Rosati,
Mauzeroll, Sleiman, Submitted
Branched DNA Complexes with Organic and Transition Metal Vertices

Synthesis of 2D-DNA Templates


Faisal Aldaye
Synthesis of 2D-DNA Templates


SOLID SUPPORT

T4 DNA Ligase

5'-phosphate  3'-OH

Ligated Strand
Dynamic DNA Templates for Gold Nanoparticle Assemblies

J. Am. Chem. Soc. 2007, 129, 4130
Bioconj. Chem. 2010, 21, 1413
Angew. Chem. 2006, 45, 2204
Modular Access to Structurally Switchable 3D-DNA Cages

Three-Dimensional DNA Cages

Modular Access to Structurally Switchable 3D-DNA Cages


McLaughlin, Hamblin, Sleiman, ChemComm. 2011, 47, 8925
Diversity

Heteroprism

Biprism


McLaughlin, Hamblin, Sleiman, ChemComm. 2011, 47, 8925


Heteroprism

Biprism
Structural Oscillation in Real-Time with Added Agents

Modular Access to Structurally Switchable 3D-DNA Cages

*Nature 2007, 450, 323*

Nanochemistry

**Gene boxes**

Faisal Aldaye and Hanadi Sleiman of McGill University in Montreal, Canada, have now developed a versatile way to make various DNA polyhedral nanostructures at a stroke.

*Nature Materials 2008, 7, 102*

the symposium. For Hanadi Sleiman (McGill University), DNA is a structural molecule — a building block that, together with rigid organic molecules at the vertices, can be used to make 3D polyhedral cages.

*ACS NANO 2008, 2, 4.*

by Sleiman from McGill University. These cage-like structures used internal loops in the vertices in the polyhedral structures to create a system where multiple cage geometries and cage size could readily be switched, as demon-

McLaughlin, Hamblin, Sleiman, ChemComm. 2011, 47, 8925
**Structural Challenges: for example, Drug Delivery**

**Size and Shape** determine diffusion rates, cellular localization, toxicity and clearance

(Discher et al, Nature Nano 2007, 2, 2249)

**Presentation of targeting ligands** on these vehicles plays an important role in how they are recognized by cells.

(Mooney et al, NanoLett 2007, 7, 161)

**Molecule-responsive** hosts, that can ‘open up’ in the presence of specific molecules, have been more difficult to construct.

Yet, the precise control of structure in synthetic 3D-hosts has been difficult
Time-lapse video microscopy clips of shape-dependent phagocytosis by macrophage.

Yoo J, Mitragotri S PNAS 2010;107:11205-11210
DNA Nanotubes of Tunable Geometry and Single- or Double-Stranded Character

Aldaye, Lo, Karam, McLaughlin, Cosa, Sleiman
Nature Nanotech., 2009, 4, 349
DNA Nanotubes of Tunable Geometry and Single- or Double-Stranded Character

Peggy Lo, F. Aldaye
DNA Nanotubes of Tunable Geometry and Single- or Double-Stranded Character

Aldaye, Lo, Karam, McLaughlin, Cosa, Sleiman
Nature Nanotech., 2009, 4, 349
Control of Geometry: CUBIC DNA NANOTUBES

DNA Nanotubes of Programmable Geometry and Permeability

- Delivery of therapeutics
- Templates for nanowires
- Positioning of devices
- Tracks for molecular motors
- Interconnects with tunable persistence length

Loading and Selective Release of Cargo in DNA Nanotubes

Peggy Lo
Can we encapsulate guests inside the DNA Nanotubes?

P. Lo, P. Karam, G. Cosa, H. Sleiman,
Nature Chemistry,
2010, 2, 319.
Encapsulation of Gold Nanoparticles

Distance between gold nanoparticles (nm)

Aggregation of AuNPs

(i) LS-nt + 15 nm AuNP
(ii) (iii)
“Sieving” Ability of the Nanotubes: only the correct size encapsulated
Yurke, Turberfield, Mills, Simmel, Neumann, Nature 2000, 406, 605
Can we selectively release these guests?
Can we selectively release these guests?
Can we selectively release these guests?
Kinetics of Nanoparticle Release

Guiding strand approach to limit the growth of nanotubes

All aboard the DNA nanotube

15 March 2010

Cargo-carrying DNA nanotubes that can rapidly release their load on demand have been reported for the first time by Canadian researchers. The self-assembling nanotubes could help to tailor the delivery of biologically active molecules within 3D DNA structures and then release them. Hanadi Sleiman and Cosa’s groups at McGill University in Montreal have now done just that.

Size selective DNA nanotubes deliver on time

Cordella Sealy

A team from McGill University in Canada has used DNA to construct nanotubes that can selectively encapsulate and release Au nanoparticles of different sizes at precise locations along their length [P. Kho, et al., Nature Chemistry (2010), doi:10.1038/nchem.275].

The nanotubes are built up from triangular building blocks with different side lengths, in this case 7 nm and 15 nm. The building blocks are linked together to form triangular nanotubes of varying cavity dimensions along their length. When Au nanoparticles with a core size that are added to the nanotube components, heated to 70 °C and then cooled slowly, the nanotubes form with nanoparticles inside many other systems that rely on more complex chemistry as prior light activation.

"What would be interesting would be to design nanotubes such that they release their cargo when they are near a diseased cell, for example a cancer cell," she says. "If the ‘closing strands’ of the link nanotubes are designed to bind to cancer-specific proteins, they will disassemble when near a cancer cell, releasing any guest to the site."

This kind of spontaneous but controlled release of particles could also be used to produce a matterable optical change, so that the system could also form the basis of a biosensor.

(Faculty of 1000 Biology)
Delivery of Therapeutic Drugs in the Tumor Environment

Cancer-specific cell surface receptor

Aptamer bound to receptor

Toxin

Cancer Cell
Opportunity: Drug Delivery with DNA Nanostructures?

- Can we simplify their design?
- Are DNA cages susceptible to nuclease degradation?
- Are they taken up by cells?
- Do they show therapeutic activity?
- Can they encapsulate small molecules?

- size and shape deliberately controlled
- monodisperse materials
- different ligands on precise locations
- multivalency
- ‘molecule-responsive’ cages that open and close on demand in the presence of specific molecules
Previous Approaches in DNA Nanotechnology

**DNA Tile Assembly**
- Seeman, Nature 2003, 421, 427

**Algorithmic DNA Assembly**

**DNA Origami**
- Turberfield, Angew., Chem. 2005, 3057

**RNA Assembly**


- Double stranded, rigid, DNA-dense
- Origami: hundreds of DNA strands in structure

Can we create ‘DNA-economic’ Structures?
The rung is obtained from 5 unmodified DNA strands.
Simplified Design and Rapid Room Temperature Assembly

Variable Binding Region

Constant Core

A single substitution yields a unit with new addressability:

Hamblin, Hariri, Carneiro, Lau, Cosa, Sleiman, ACS Nano, 2013, 7, 3022
‘DNA-Minimal’ Cages: A cube from 4 strands

McLaughlin, Hamblin, Sleiman,
‘DNA-Minimal’ Cages

McLaughlin, Hamblin, Sleiman,
Modularity of the Approach


McLaughlin, Hamblin, Sleiman, ChemComm. 2011, 47, 8925
Simple modifications at the 5’/3’ ends and 3D topology significantly improve nuclease resistance of the DNA prisms in fetal bovine serum (FBS).

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Conway, McLaughlin, Sleiman, Chem. Comm, 2013, 49, 1172
Can 3D-DNA Structures Enter Cells?

Johans Fakhoury
DNA cubes are taken up by a variety of cancer cells

(HeLa, LnCap, GM847, WI-38, MRC-5)

Merge

GFP
+Cy3
+Cy5

GFP
+Cy3
+Cy5
Zoomed

Johans Fakhoury
DNA Nanotubes enter HeLa cells efficiently

Delivery of Therapeutic Drugs in the Tumor Environment

Cancer Cell

Aptamer bound to receptor

Toxin
• Two binding events of a prostate cancer marker cause the cube to unzip from 3D to 2D
  - The unfolding is mediated by strategically placed nicks at the corners of the cube
• Robust uptake in Chronic Lymphocytic Leukemia cells that are drug resistant

Challenge: increase endosomal escape, in order to bypass the need for transfection
Opportunity: Patterning Lipids and Polymers on DNA


Core-Shell Biohybrid Materials
(increased circulation time, decreased immunogenicity, increased cell penetration, targeting ligands)
Block Copolymer Self-Assembly: Isotropic Shapes

Protein Assembly: Anisotropic and Highly Specific

α-helix

coiled-coil
Dendritic Lipid-DNA Conjugates

Attachment of lipids to DNA Cube
DNA-amphiphiles can aid in membrane anchoring and cell uptake
Properties of resulting hybrid structures can be controlled by structure, number and position
Four Lipids on DNA Cube: “Handshake” to form Dimer

a) C-A$_4$ $\rightarrow$ C-A$_4$-D2 $\rightarrow$ (C-A$_4$-D2)$_2$

b) C-A$_2$-DNA $\rightarrow$ (C-A$_4$-D2)$_2$

C) Individual constructs

b) C-A$_1$ $\rightarrow$ C-A$_2$-D1

d) % Interact. vs $R$ (nm) for (C-A$_4$-D2)$_2$

9.3 nm
Eight Lipids on DNA Cube: “Handshake” Inside the Cube!

e: C-A₄B₄ → C-A₄B₄-D2 → C-A₄B₄-D2

f: C-A₄B₄-DNA → C-A₄B₄-D2

g: % intensity vs. R(nm) for C-A₄B₄-DNA (7.7nm), C-A₄B₄-D2 (6.2nm)
Encapsulation of Small Molecules and Release

- These structures can be loaded with a hydrophobic cargo.
- Through strand displacement the hydrophobic cargo can be released.
**Encapsulation of Small Molecules and Release**

**Other small molecule cargo:**

Loading capacity:

Loading capacity of the small molecules was found to be between 5 and 10 molecules per cubic micelle depending on the guest and D-DNA used.
Patterning Lipids on DNA: A “Handshake” of the Lipid Chains

Intermolecular assembly → Directed assembly

Intramolecular assembly → Encapsulation