3D Origami: Sculpting and Bending Tubes of DNA

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What is this talk about?
Research out of Shih Lab

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Origami Refresher

M13mp18 viral genome 7249 bases long

+250 helper strands in Mg++ buffer

Anneal

Anneal

100 nm
Design of 2D Origami

- Scaffold crossover
- Staple crossover
Design Rules for the Honeycomb Lattice

- Potential crossovers every 7 bases
  - 7 bases = \( \frac{2}{3} \) turns (240° or -120°)
  - 14 bases = 1 \( \frac{1}{3} \) \( \text{rd} \) turns (480° or 120°)
  - 21 bases = 2 turns (720° or 0°)
- Entire origami made up of 7 base cylinder
- Scaffold crosses over at position 2 or 5
- We make staple crossover at every potential crossover point
  - Except when the scaffold crossover is 5 bases away
  - Maintains uniform cross over density
- Cut staples such that length = (18,49)
  - Mean = (30, 42)
Crossover Rules
Crossover Rules
Crossover Rules
Design Examples: Monolith
Design Examples: Square Nut
Design Examples: Slotted Cross
Design Examples:
Design Examples: Slotted Cross
Design Examples: Slotted Cross
Oligos

• Seven different scaffolds prepared in the lab
  • p7308, p7560, p7704, p8064, p8100, p8364, pEGFP

• Reverse phase cartridge purified staples
  • DMT protecting group retained at the 5'-end upon the completion of the last cycle of synthesis
  • Synthesized oligos are transferred to a resin that can bind to this protecting group
  • Impurities are washed away
  • DNA is cleaved off the resin
  • Low-cost enrichment of full-length product
  • A substantial reduction in yield
Experiments

- DNA: 10 nM scaffold + 50 nM staples
- Buffer: 5mM Tris + 1mM EDTA (pH 7.9 at 20 °C)
- Salt: 16mM MgCl₂
- Annealing schedule:
  - 80 °C – 60 °C : 80 mins
  - 60 °C – 24 °C : 173 hrs
Gel Runs and TEM

- 2% Agarose
- Running Buffer: 45mM Tris borate + 1mM EDTA (pH 8.3 at 20 °C) and 11mM MgCl₂
- 4 hrs at 70 V, ice cold bath
- DNA extracted from excised band
- Uranyl formate negative stain for TEM
Results
Factors Affecting Yield

- Duration of thermal ramp
- Divalent cation concentration
- Monovalent cation concentration
Gel Data
<table>
<thead>
<tr>
<th>Factor</th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermal Ramp</td>
<td>Slow migration Poorly formed Structures</td>
<td>Fast migration Well formed Structures</td>
</tr>
<tr>
<td>Divalent Cations</td>
<td>Slow migration Poorly formed Non Aggregate Structures</td>
<td>Fast migration Well formed Aggregate Structures</td>
</tr>
<tr>
<td>Monovalent Cations</td>
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<td>Fast migration Poorly formed Non Aggregate Structures</td>
</tr>
</tbody>
</table>
More Gel Data

- Genie Bottle (pEGFP)
- Genie Bottle (p7308)
- Six-helix bundle (pEGFP)
- Six-helix bundle (p7560)
Typical Conditions

• Duration of thermal ramp: 173 hrs
• Divalent cation concentration: 16 mM Mg
• Monovalent cation concentration: 5 mM Na
Twisting and Bending
Bending, No Twisting
More Exotic Stuff
Claim: Clear stripes indicate well formed structures.
Yield Analysis

* are not included in the yield calculation since the stripes are not clear
• Yield ~ 50% at radius of curvature 10 nm

• Yield decreases as radius of curvature decreases

• Low yield for multimeric object such as gears, sometimes less than 10%
Conclusion

• 3D extension of origami

• Implemented using the honeycomb lattice

• Sculpt away unnecessary parts of the lattice

• Change the number of bases per turn to twist or bend the honeycomb

• Long annealing schedule

• Carefully controlled cationic concentration

• Average to low yields