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3D Origami: Sculpting and Bending Tubes of DNA

Duke Biomolecular Journal Club

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Harish Chandran



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What is this talk about?



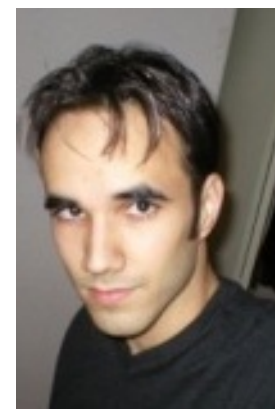
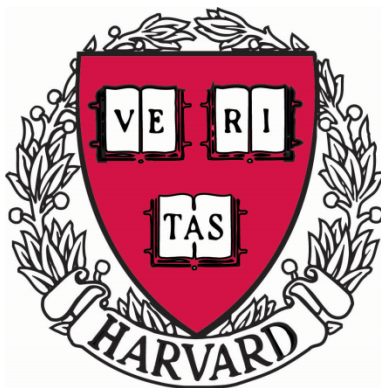
3D DNA Origami

HD / SMD 2009

Research out of Shih Lab



William Shih



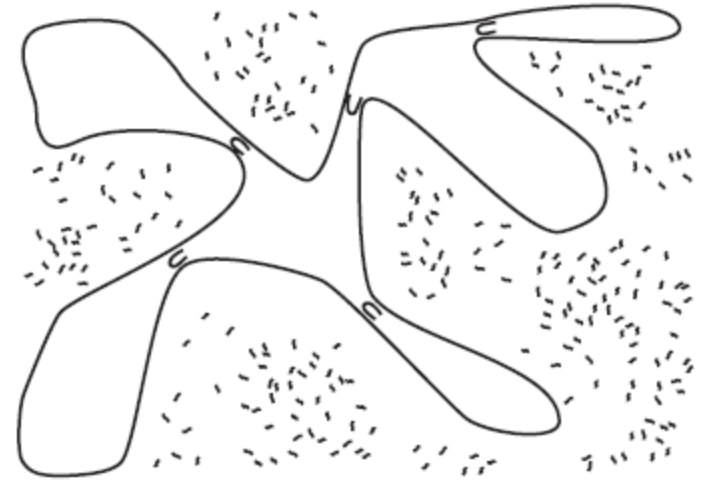
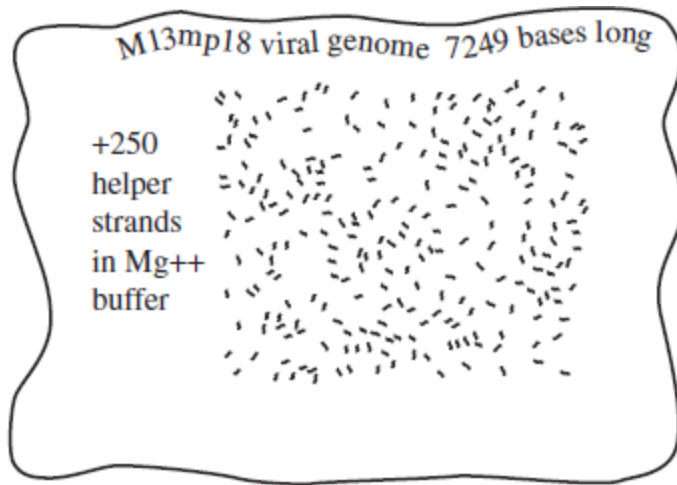
Shawn Douglas



Hendrik Dietz



Origami Refresher

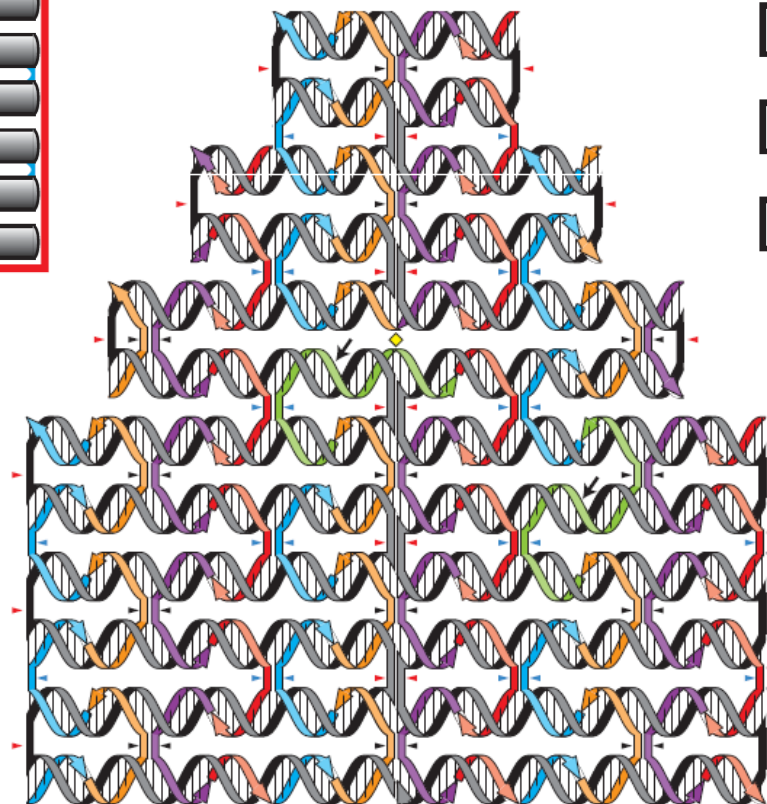
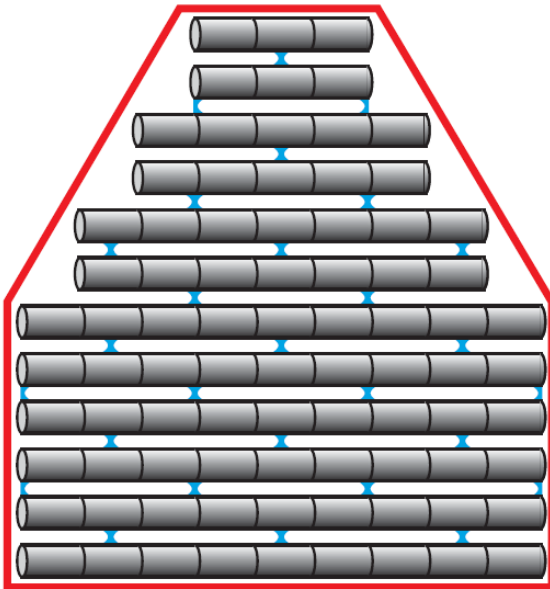


100 nm



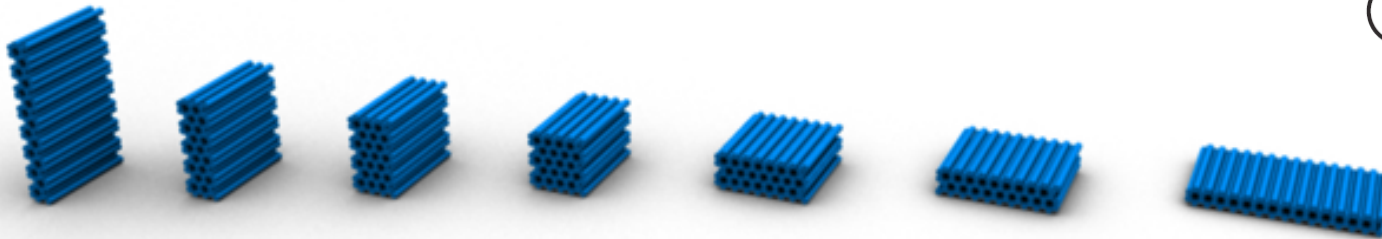
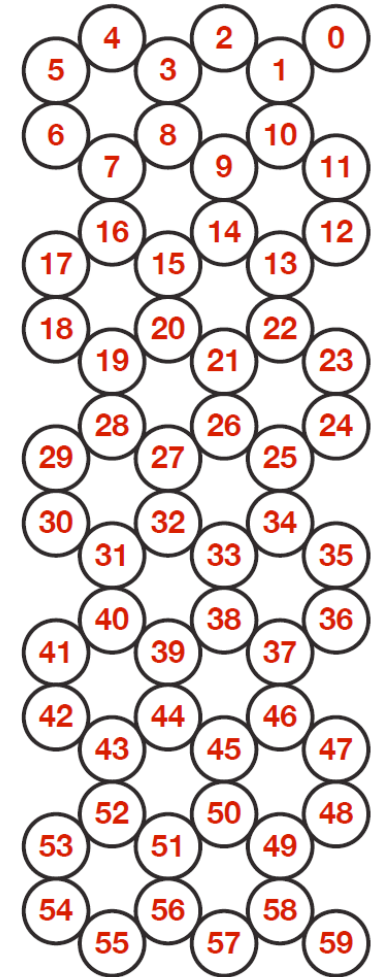
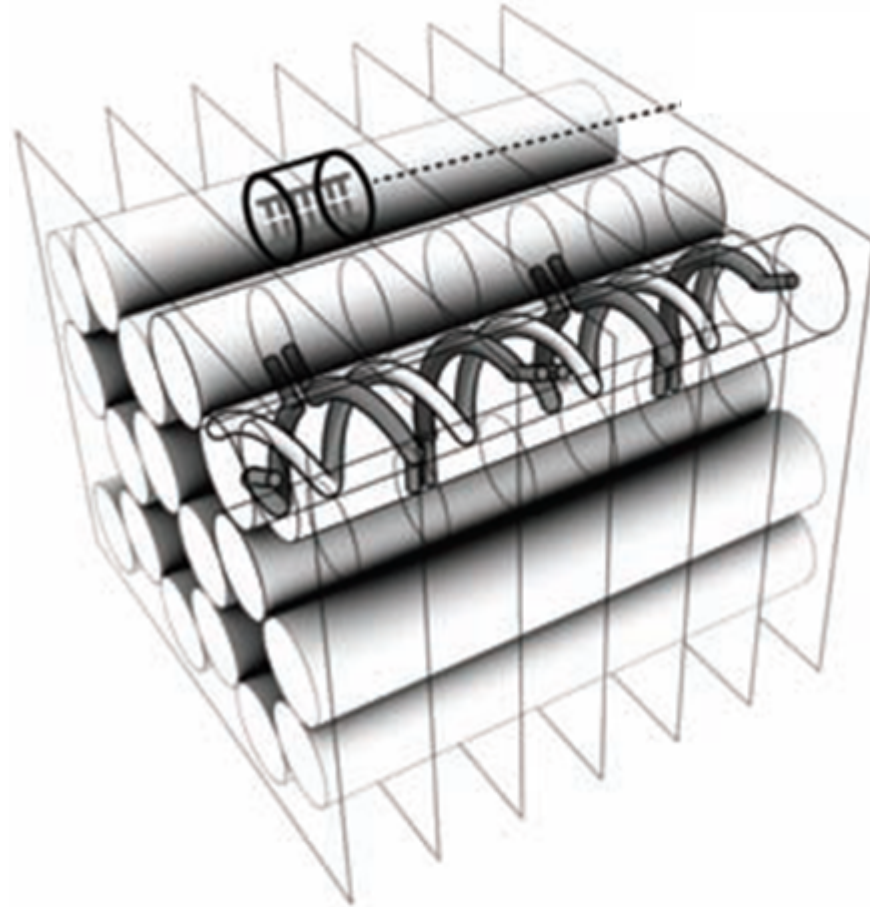
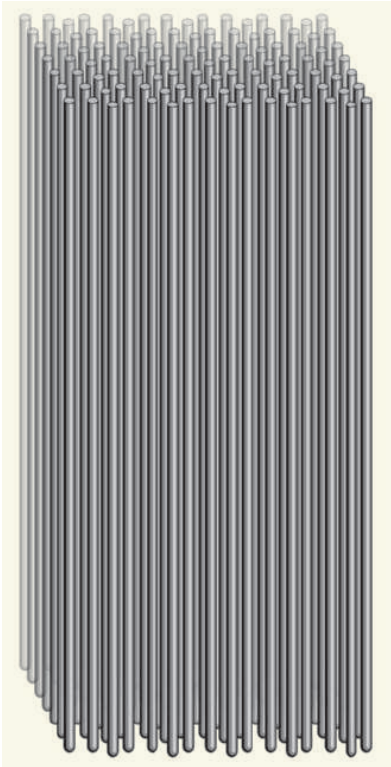
Design of 2D Origami

- Scaffold crossover
- Staple crossover



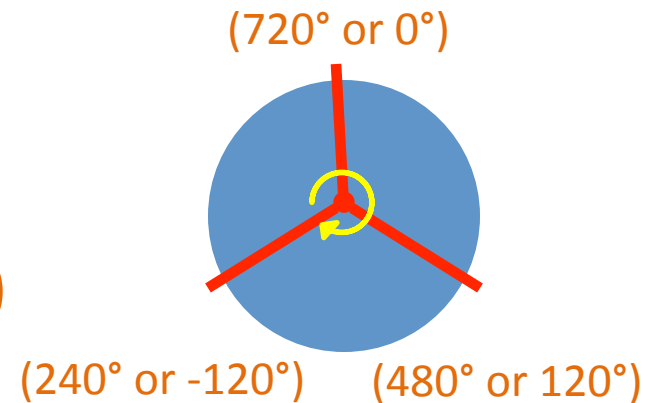


Honeycomb Lattice



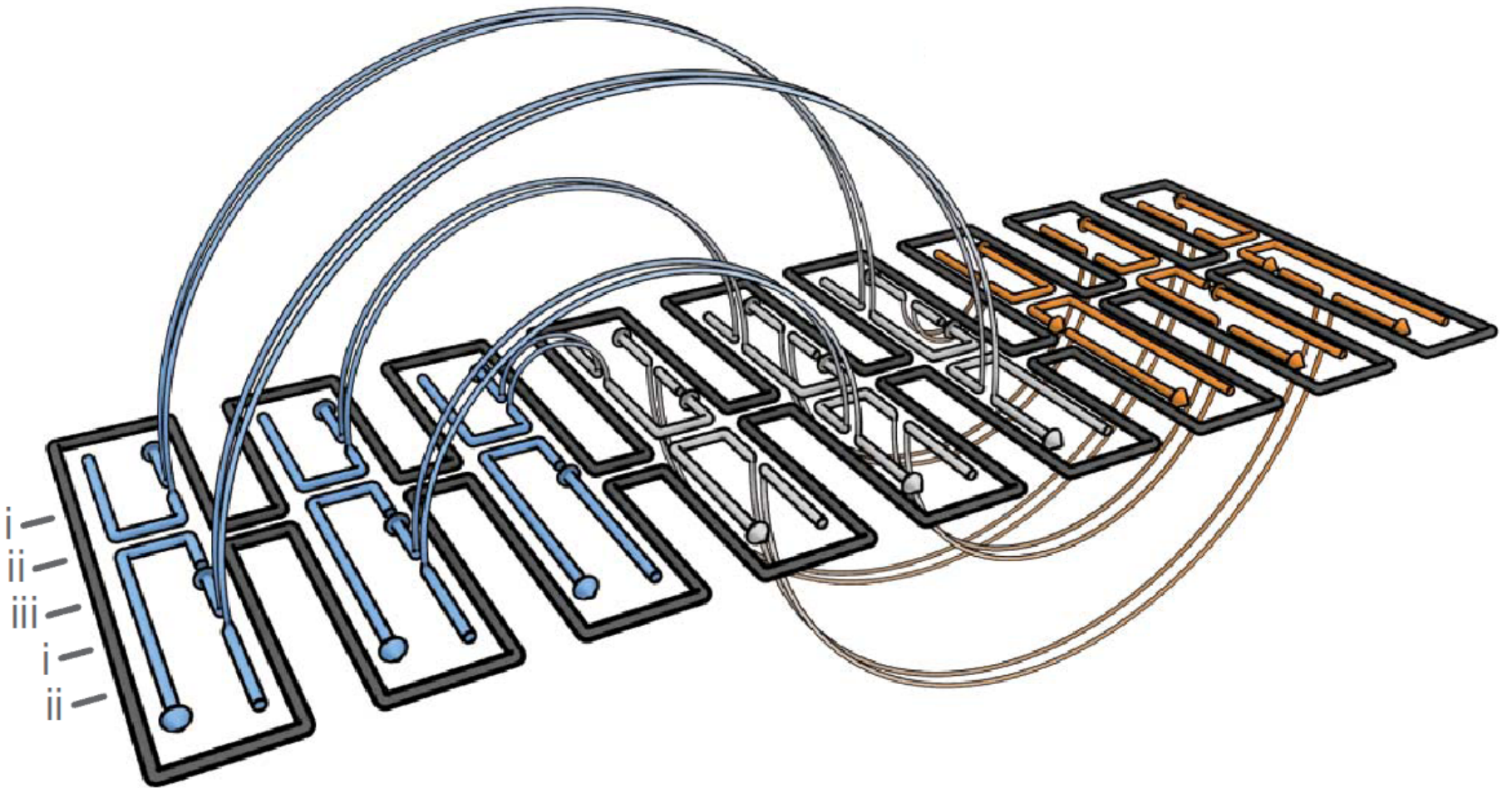
Design Rules for the Honeycomb Lattice

- Potential crossovers every 7 bases
 - 7 bases = $\frac{2}{3}$ rd turns (240° or -120°)
 - 14 bases = $1 \frac{1}{3}$ 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)



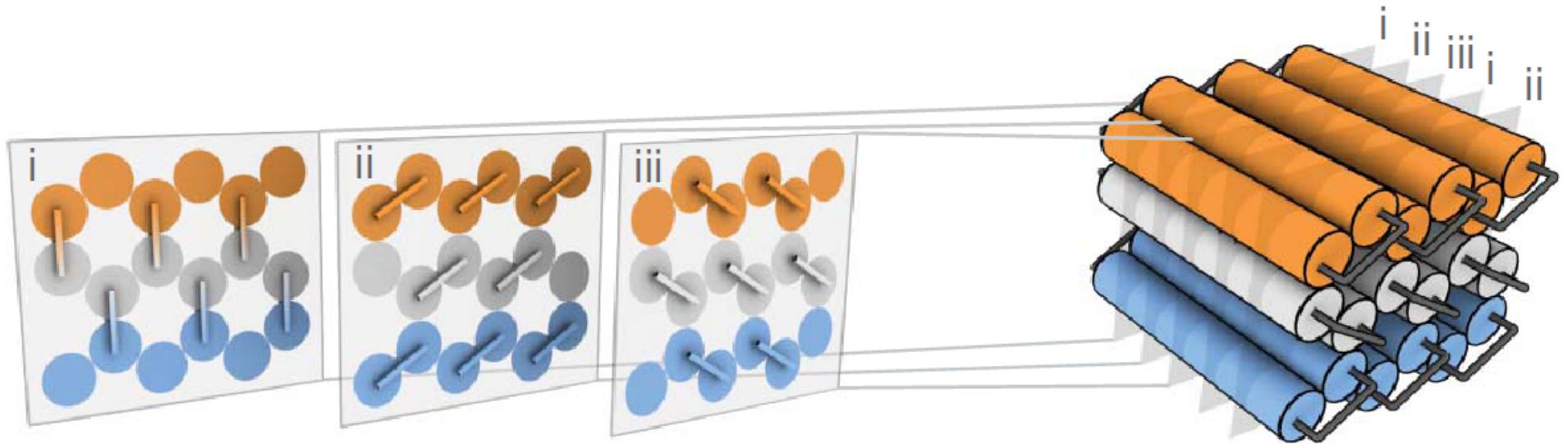


Crossover Rules

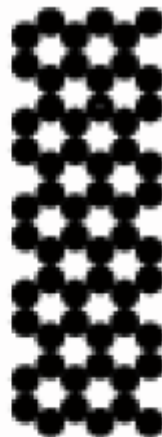
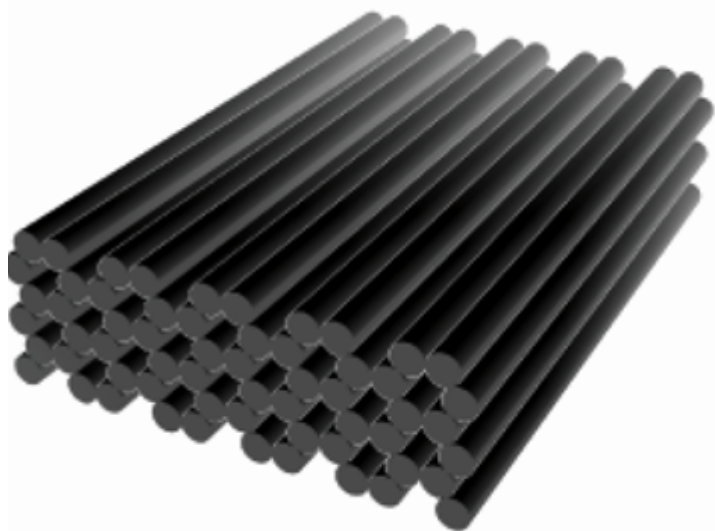




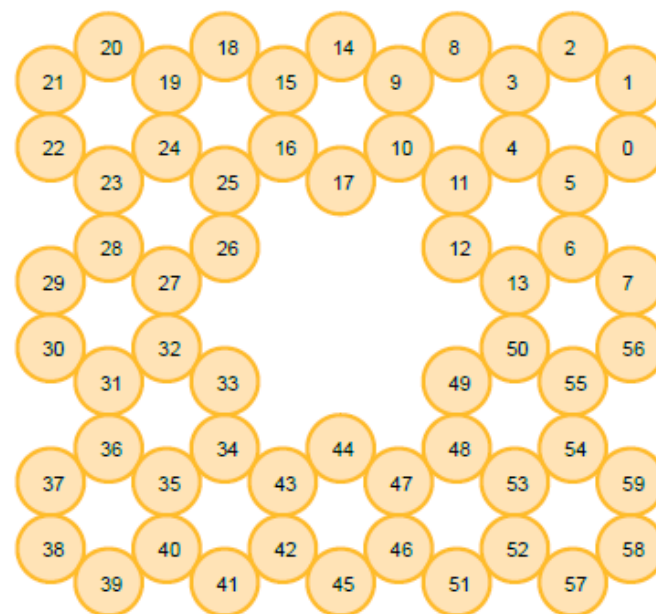
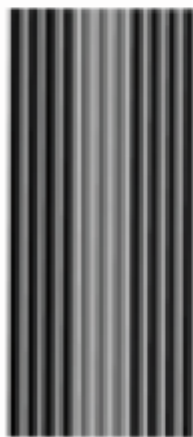
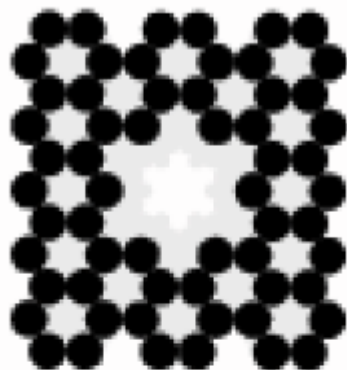
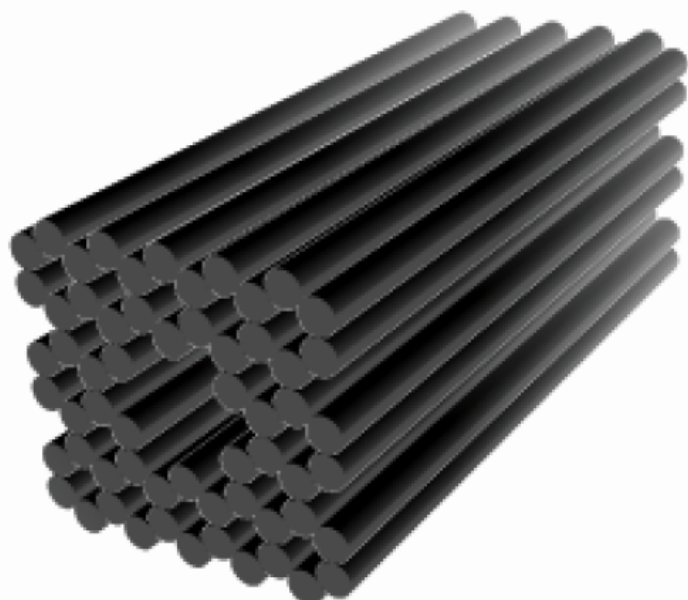
Crossover Rules



Design Examples: Monolith



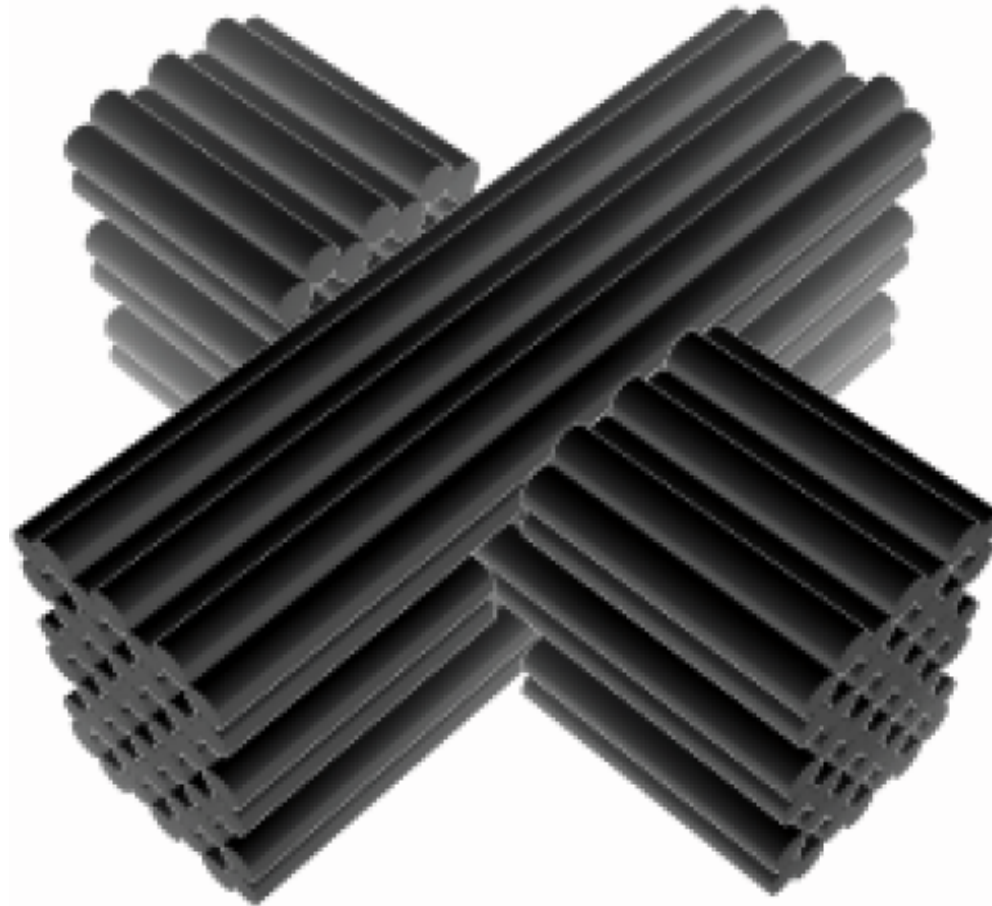
Design Examples: Square Nut



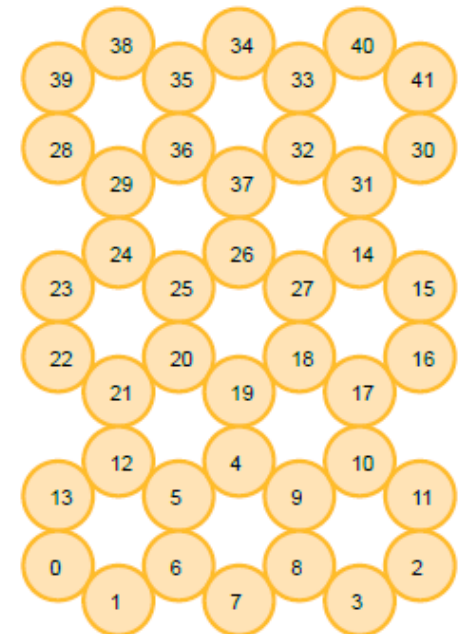
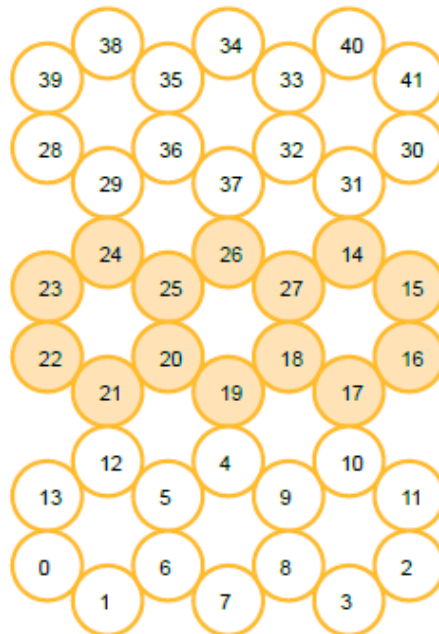
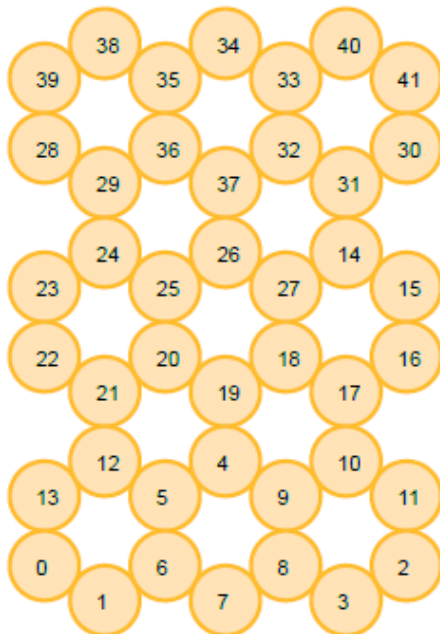
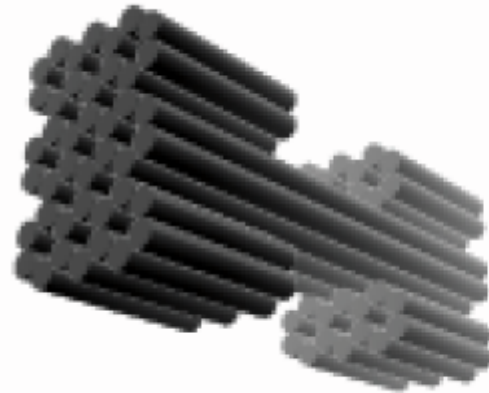


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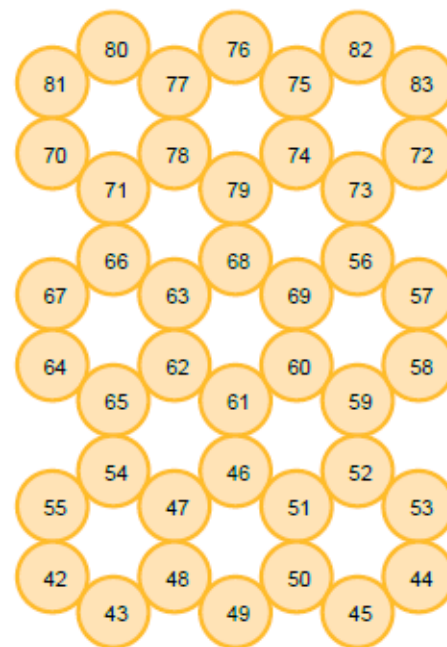
Design Examples: Slotted Cross



Design Examples:

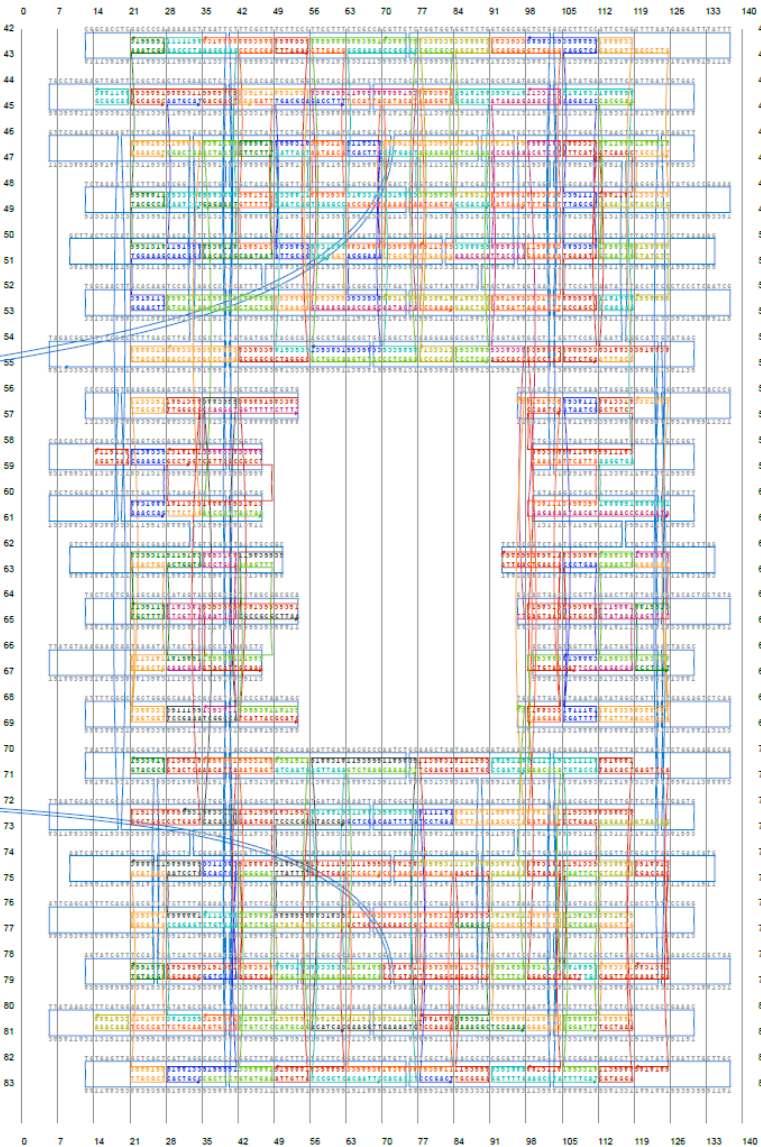
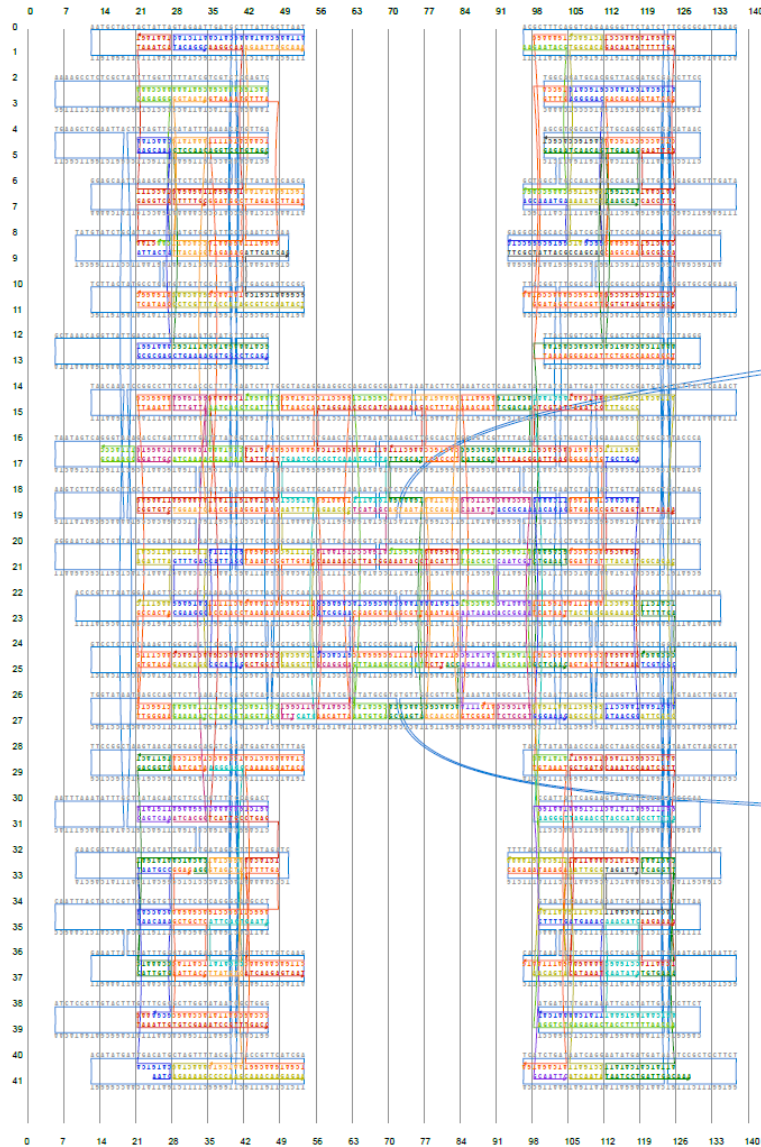


Design Examples: Slotted Cross





Design Examples: Slotted Cross



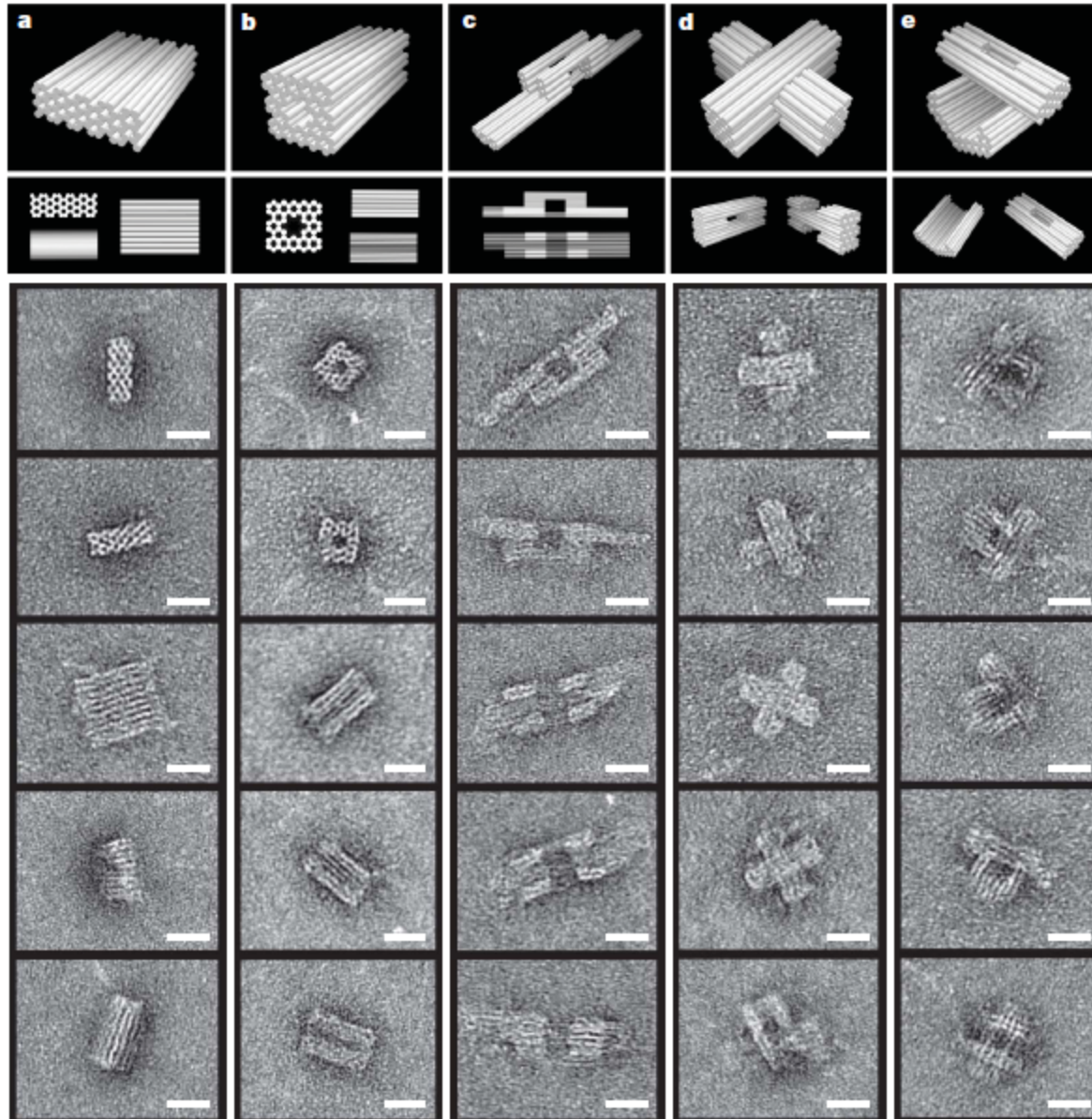
- Seven different scaffolds prepared in the lab
 - p7308, p7560, p7704, p8064, p8100, p8364, pEGFP
- Reverse phase cartridge purified staples
 - DMT protecting group retained at the 5'-end upon the completion of the last cycle of synthesis
 - Synthesized oligos are transferred to a resin that can bind 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

- 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

- 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



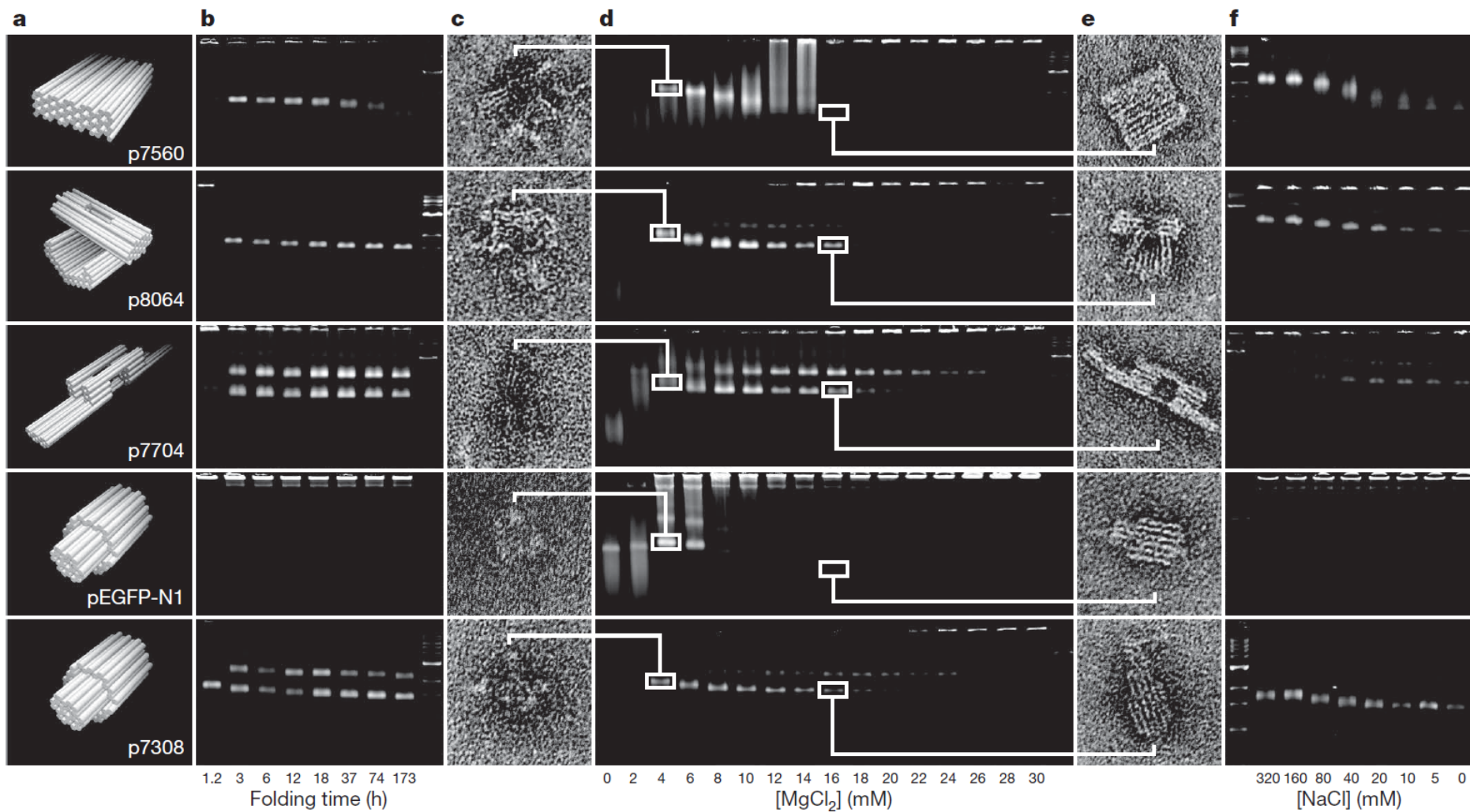
Results



Factors Affecting Yield

- Duration of thermal ramp
- Divalent cation concentration
- Monovalent cation concentration

Gel Data

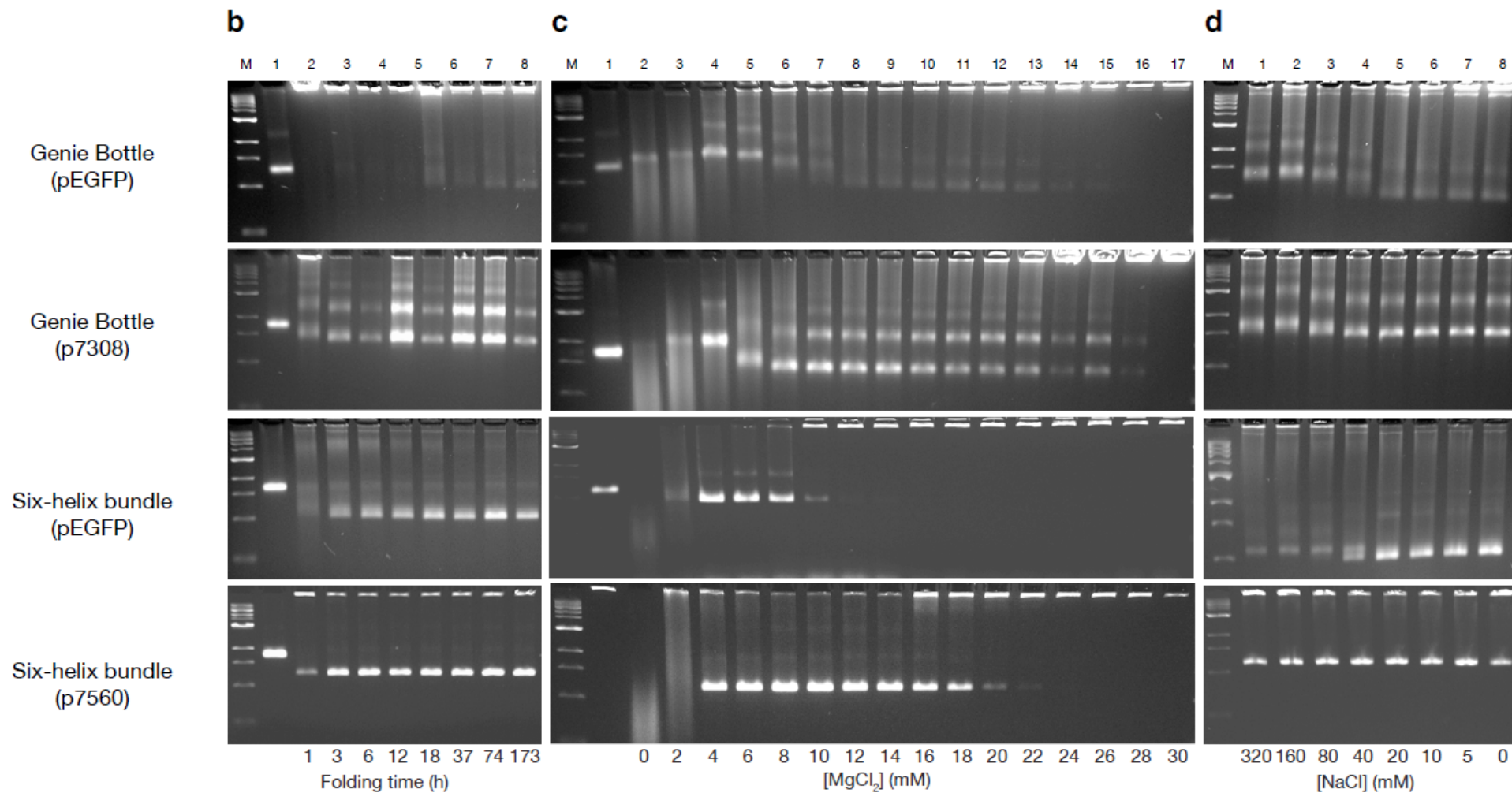




Trends

Factor	Low	High
Thermal Ramp	Slow migration Poorly formed Structures	Fast migration Well formed Structures
Divalent Cations	Slow migration Poorly formed Non Aggregate Structures	Fast migration Well formed Aggregate Structures
Monovalent Cations	Slow migration Well formed Aggregate Structures	Fast migration Poorly formed Non Aggregate Structures

More Gel Data



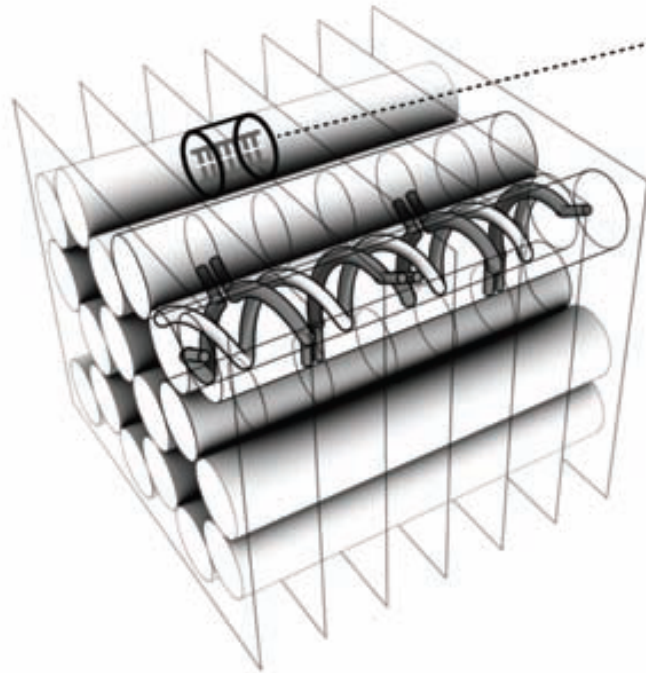
Typical Conditions

- Duration of thermal ramp: 173 hrs
- Divalent cation concentration: 16 mM Mg
- Monovalent cation concentration: 5 mM Na



Twisting and Bending

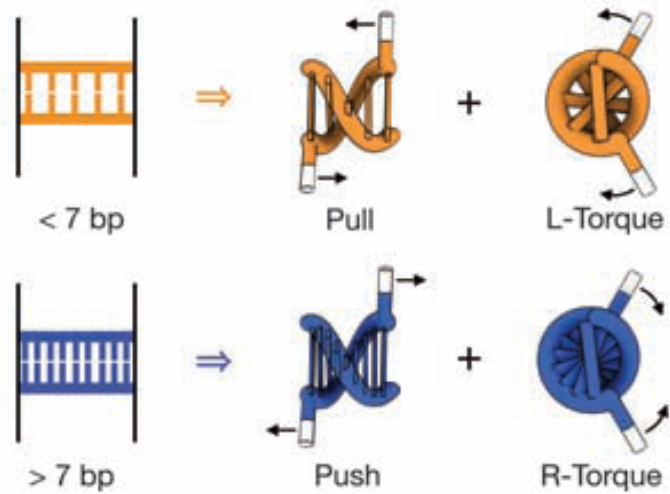
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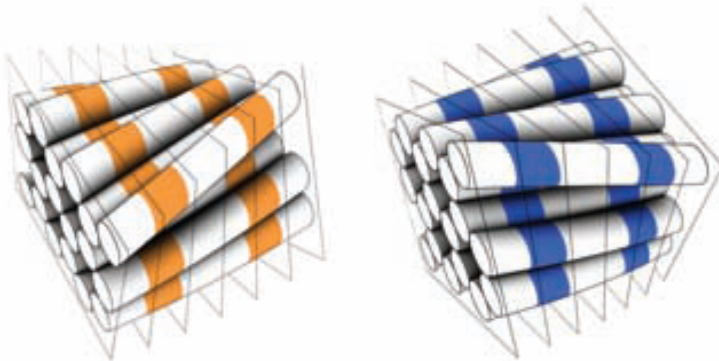
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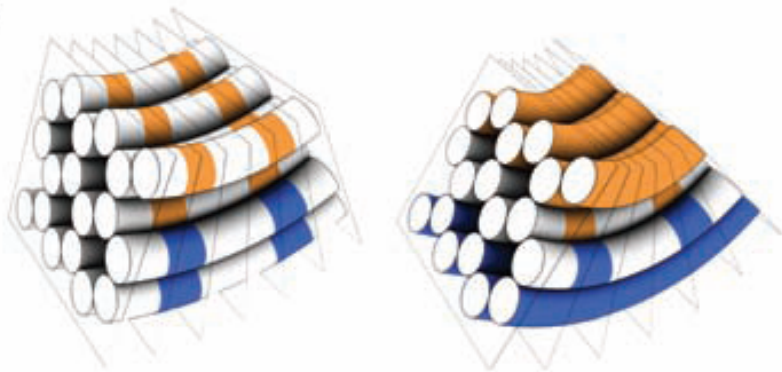
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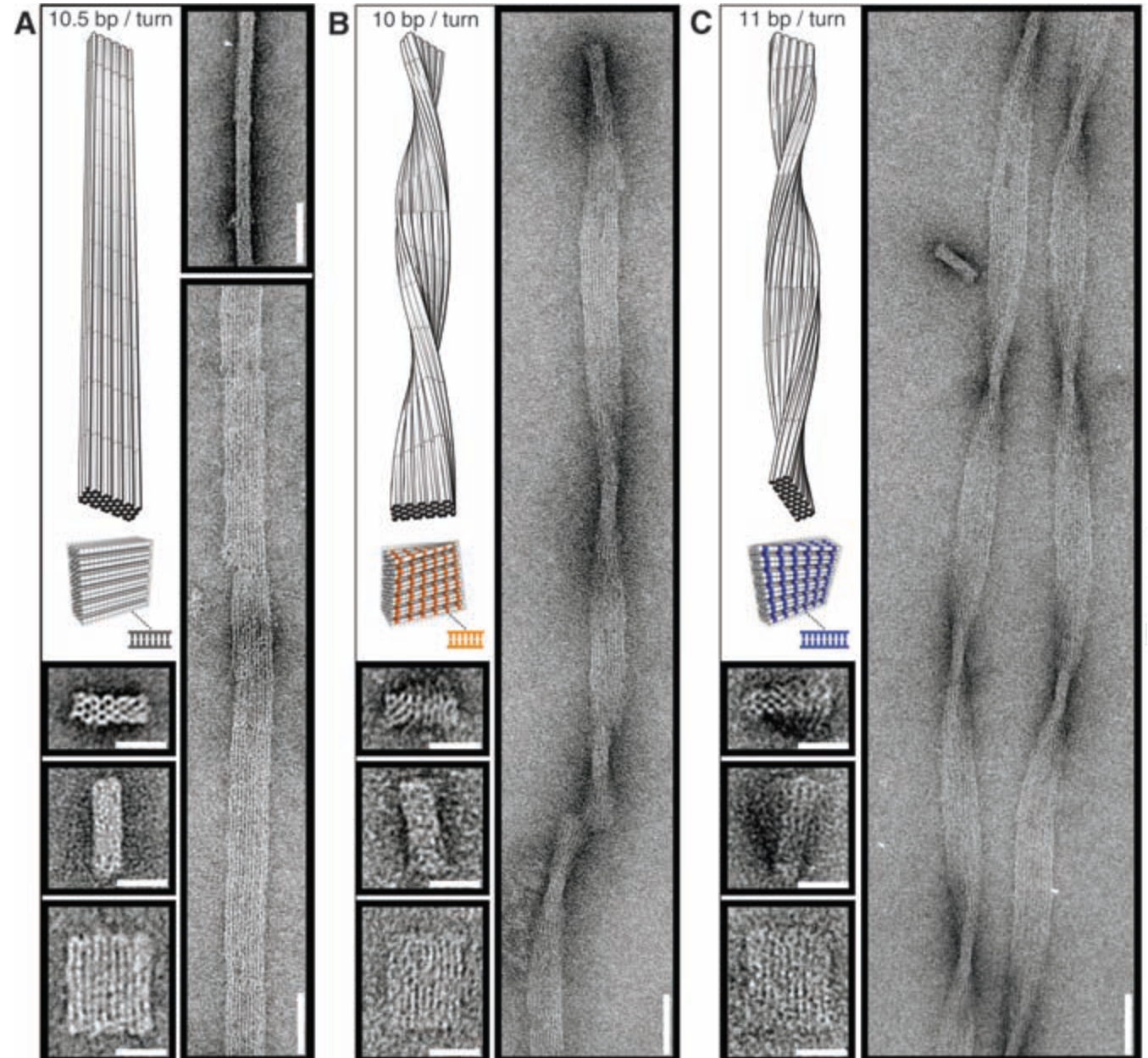
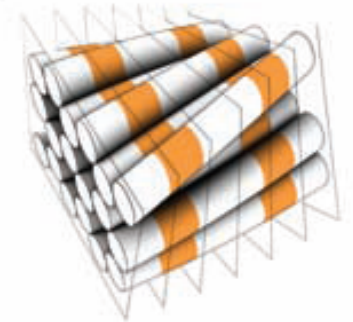


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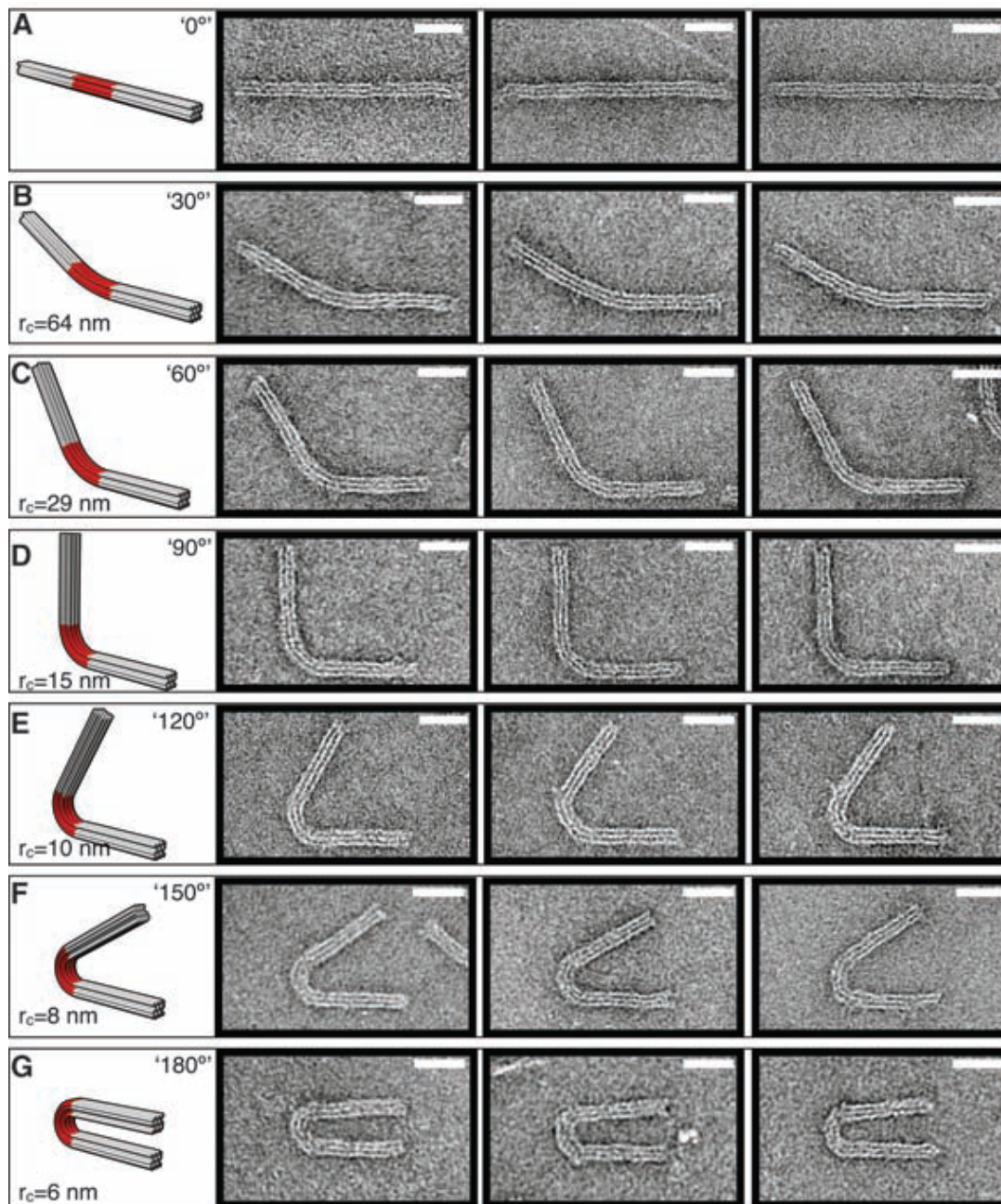




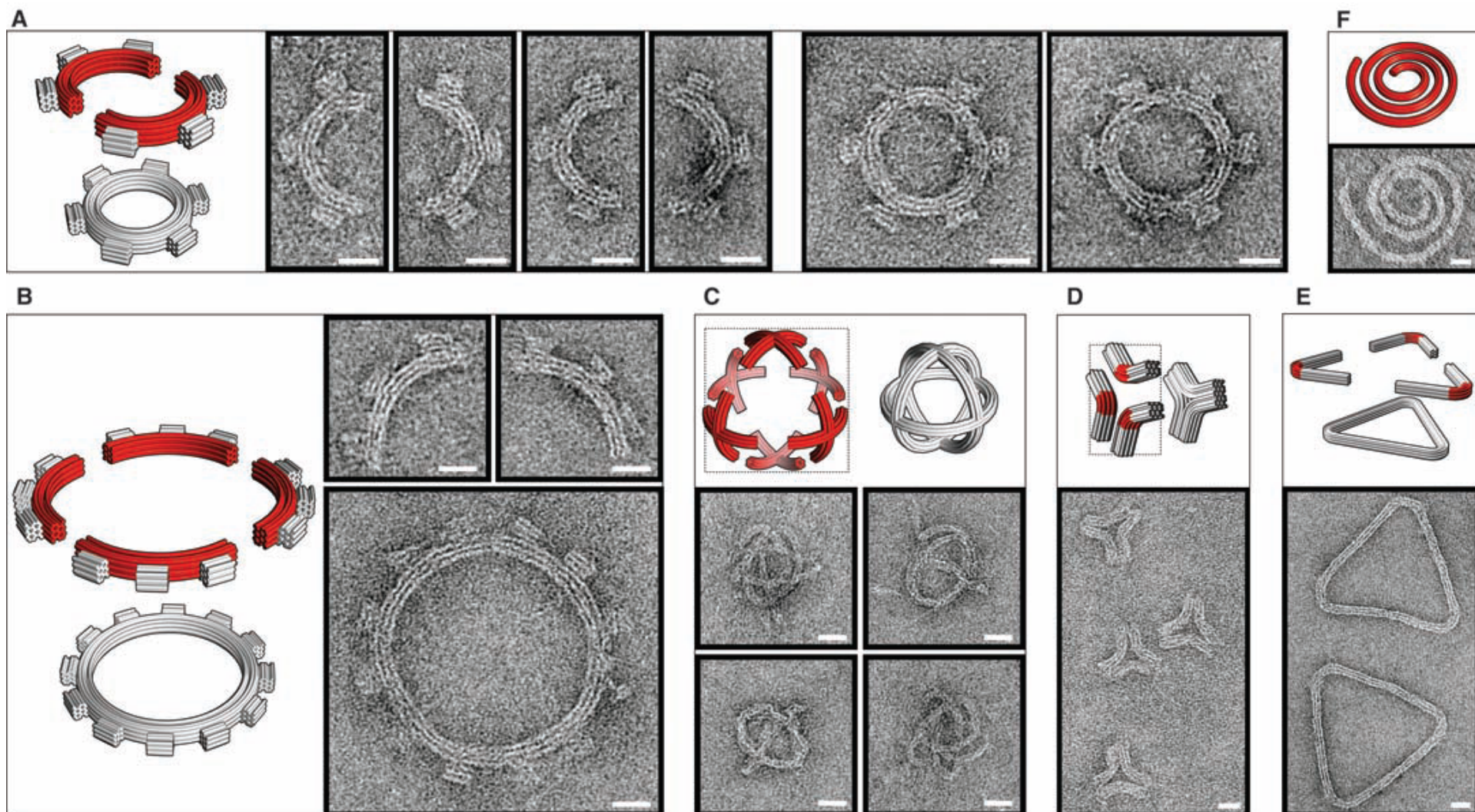
Twisting, No Bending



Bending, No Twisting

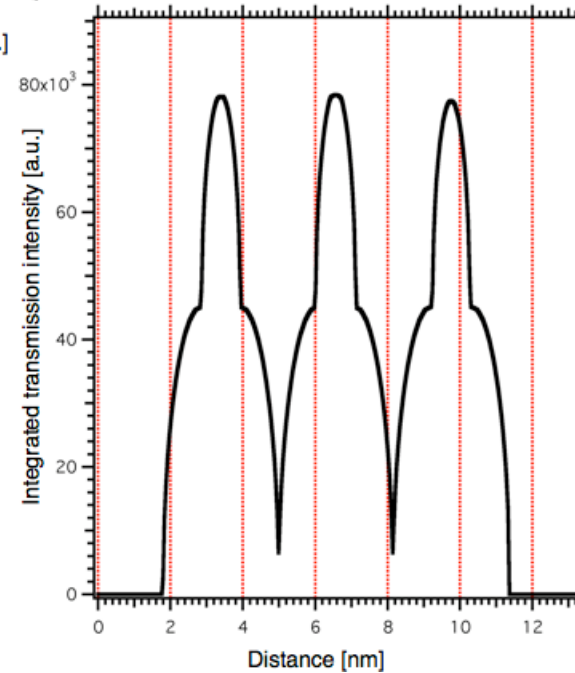
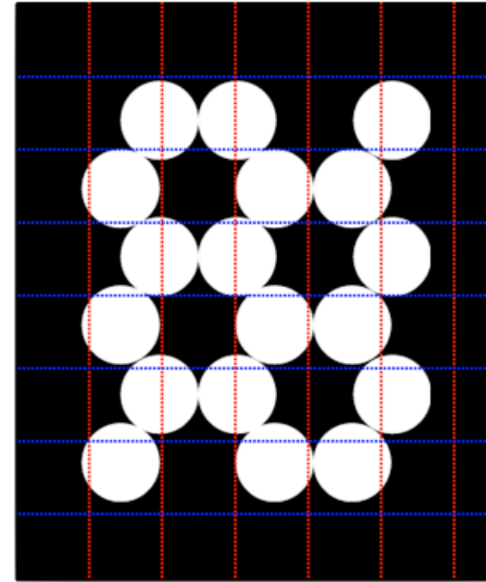
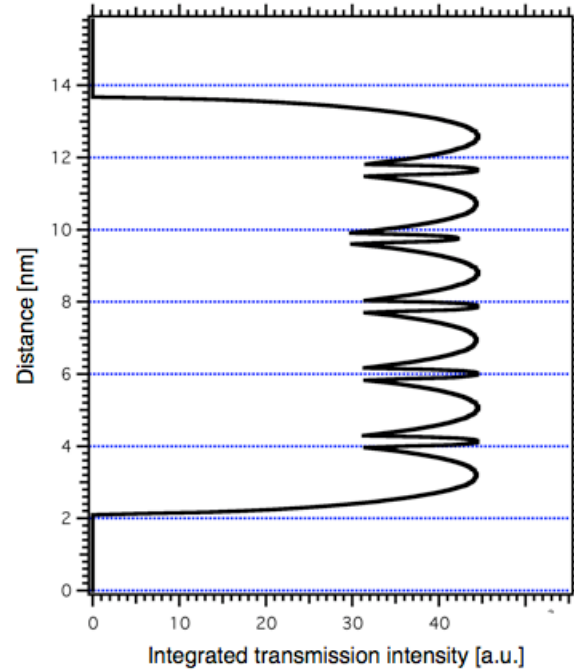


More Exotic Stuff





Origin of Stripes

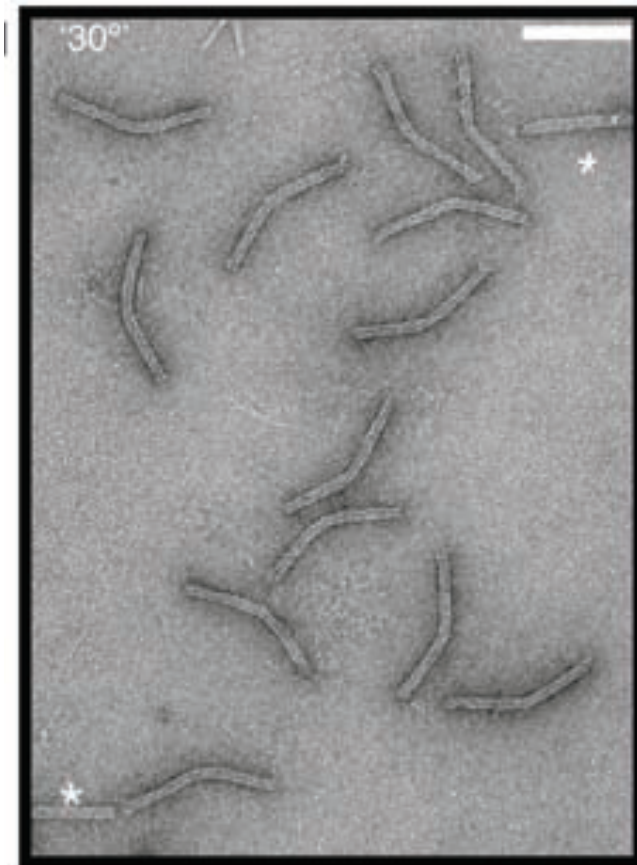
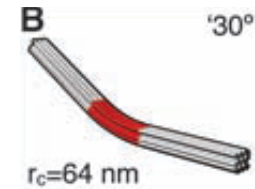
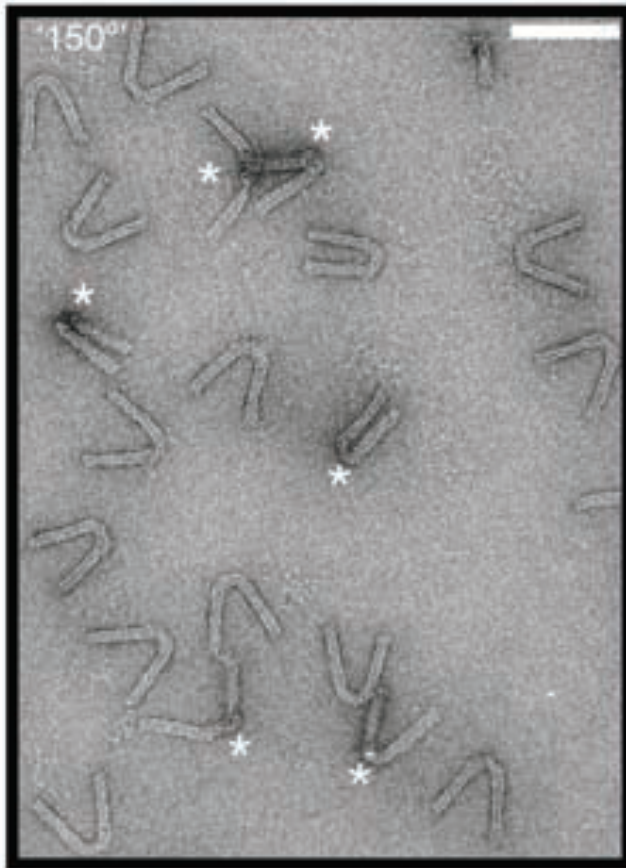


Claim: Clear stripes indicate well formed structures.



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Yield Analysis



* are not included in the yield calculation
since the stripes are not clear

- Yield \sim 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

