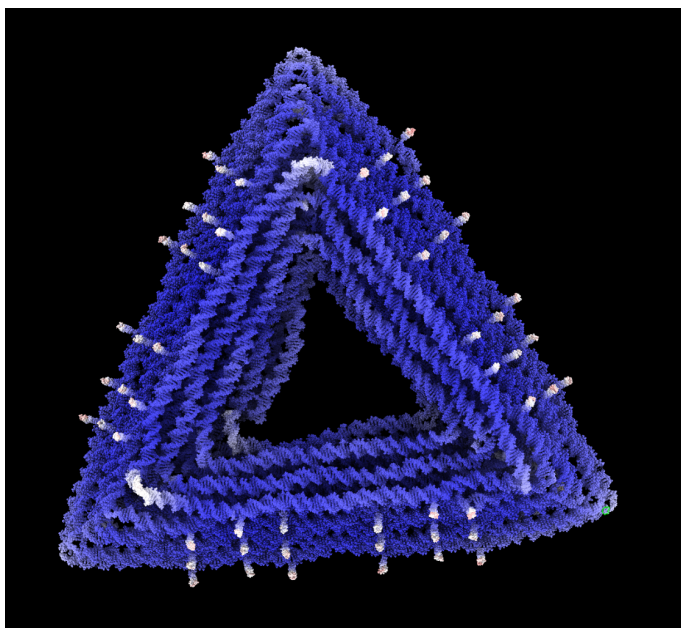


Wet Lab Section: Building DNA Origami Tubule

Introduction: We use DNA origami to build different types of structures, such as sheets, capsids, tubules, etc. In this section, we will walk through the experimental workflow of building long tubules and observe them under an epifluorescence microscope.

A single monomer (See the triangle from OxDNA simulation below) is the fundamental building block of our DNA origami assemblies. The monomer is folded with a long scaffold (a 8064 base pair single strand DNA) and two-hundred staples (20-60 base pair single strand DNA).



On each surface of the monomer, there are 18 single-strand DNA pulled out from the surface. They serve as interaction sites for the monomers. We can program the interaction based on the structure we want to build. Each side may have a different bevel angle depending on the target assembly.

Today, we use an equilateral triangle with 34.8, -17.8, and 34.8 degrees bevel angles and activate 6 out of 18 interaction sites to build long tubules. Each side is self-complementary with itself.

In the wet lab section, we hope to get through the following (with a bit of cooking show magic!):

1. fold the monomers
2. gel extract monomers
3. make assembly solutions, and
4. observe the tubes under a fluorescence microscope.

Task 1: Fold the DNA origami triangles

Fold the monomers from single-stranded DNA and load the folding solution into the thermocycler.

Step 1: Calculate the recipe for the folding solution

We will make a folding solution with a volume ratio: $V_{\text{scaffold}}:V_{\text{staple}}:V_{10X \text{ Salt}}=5:4:1$

Materials	Volume
100nM Scaffold	
Staple Working Stock (500nM for each staple)	
175mM MgCl_2 (buffer)	
Total Volume	200ul

Step 2: Grab a 200 μL strip tube, pipette the amount of solution calculated from step 1, then vortex and centrifuge the solution to make sure that it is well-mixed

Step 3: Load the solution into the thermocycler (max 50 μL per tube) and run the folding protocol.

Annealing Protocol: heat to 65C for 15mins; 58C-51C, -1C/hour; stay in 51C

Task 2: Gel electrophoresis

After annealing the folding solution, we want to extract only the well-formed monomers in the solution. Gel electrophoresis allows us to separate different structures based on the electrophoretic mobility.

Step -2: Prepare gel and gel buffer. The following is the recipe for an agarose gel and gel buffer.

IRG1 members prepared the gel, gel buffer, and incubated folding solution ahead of time

Agarose Gel Recipe

Materials	Volume
Agarose	2.4 g
10xTBE	8 mL
1.375M MgCl ₂ buffer	640 μL
SYBR safe (DNA binding dye)	8 μL
water	152 mL
Total Volume	160 mL

Gel Buffer Recipe

Materials	Volume
10xTBE	30 mL
1.375M MgCl ₂ buffer	2.4 mL
water	570 mL
Total Volume	600 mL

Step -1: pour buffer, take the comb out carefully.

Step 1: Pipette the loading dye* into a folding solution and pipette-mix them. $V_{\text{folding}}: V_{\text{dye}}=5:1$. Prepare a Parafilm on the bench, pipette 10 μL DNA solution on top of film, then pipette mix 2μL of loading dye with DNA.

*loading dye: DNA has a lower density than water and is transparent. Mixing loading dye increases the density and makes it visible in the gel.

Step 2: Load the folding solution into an agarose gel. For each well pipette 5 μ L solution. If pipetted carefully, the solution should sediment to the bottom of the well.

Step 3: Connect the power supply to the gel box. Run the gel at 110V for 2 hours. To check the connection of power, we are expected to see bubbles coming from electrodes.

Step 4: After two hours, scan the gel with the Typhoon gel scanner or check through the blue transmission light. A monomer band could be found. (Only Show gel picture in the section)

Task 3: Assemble single monomer into long tubes

Prepare 10 nM assembly solution. IRG1 members prepared monomers and stored them in 5 mM MgCl₂ buffer.

Step 1: Calculate the recipe for 50 μ L assembly solution with 10nM DNA concentration, 10 mM MgCl₂ concentration.

Materials	Volume
DNA monomer in 5mM MgCl ₂	
FoB___	
FoB___	
FoB___	
Total Volume	50 μ L

Step 2: Grab a 200 μ L tube and pipette the solutions we calculated in step 1

Step 3: Vortex and centrifuge the sample

Step 4: Load into thermocycler at 36C

Task 4: Check the long tube with epifluorescence microscope

An epifluorescence microscope is a type of light microscope used for observing fluorescently labeled specimens. Fluorescence microscopy detects fluorophores, which are molecules that can absorb light at one wavelength and emit light at a longer wavelength.

We will stain the DNA tube assembly solution with Yoyo-1 fluorescent dye and image the sample under an epifluorescence microscope.

Step 1: Dilute 10 nM tubes into 5nM with FoB20

Step 2: Pipette Mix 2 μ L of 5 nM tubes with 2 μ L 1.25 μ M Yoyo-1. Wait for 10 mins

Step 3: Blow compressed nitrogen gas on both the glass slide and coverslip to remove dust on the surface

Step 4: Pipette 1.8 μ L of the solution onto the slide and cover with a coverslip

Step 5: Imaging on the microscope