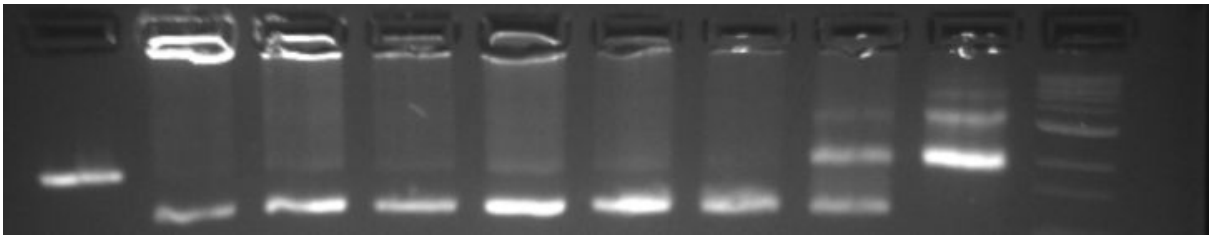
(Design process)
In this project, two DNA origami nanostructures, a gear and track, were created. The lattice cross section was formed using 14 double helix strands for both structures,

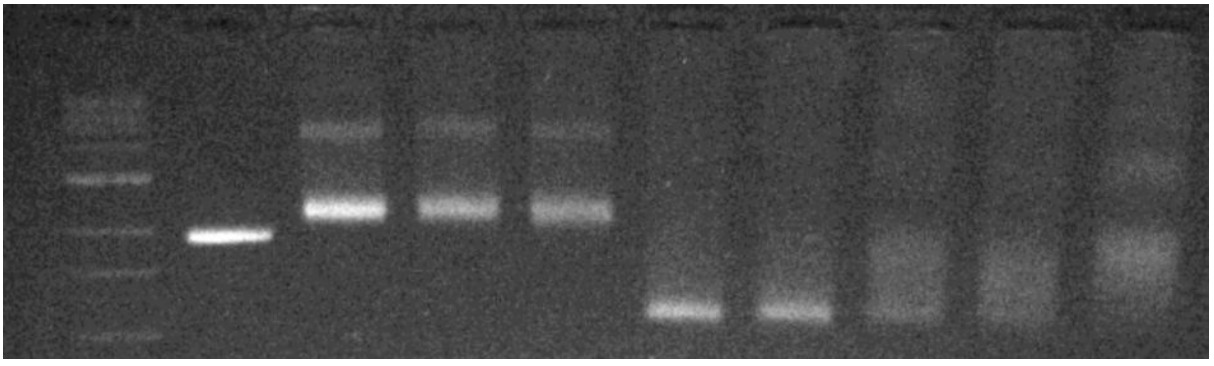
Gear and track without overhangs

(Magnesium screen, 2.5-d slow fold, w/o overhang)
For both gear and track, the final concentration of magnesium ion was defined in gradient of 12 mM to 26 mM with 2 mM increment. A 2.5-day slow folding process was performed to determine the acceptable range of concentration of magnesium ion in the sample. An acceptable magnesium concentration needs to be selected for future folding.
(Track w/o overhang)Set magnesium screen from 12-26mM, 2.5day fold then run gel.



18 mM MgCl2 is chosen.

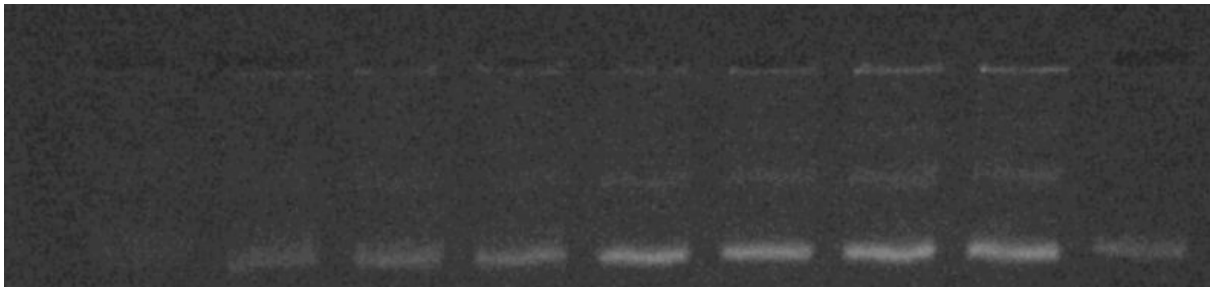
(Rapid fold, 4-hr folding, w/o overhang)
Once an acceptable magnesium concentration was selected, a 4-hour rapid fold needs to be performed to determine the folding temperature of the structure. For both gear and track, a temperature range of 40 C to 60 C was initially selected to be confined.
(Track w/o overhangs 60-40 degree)



60-40 degree rapid fold. Lane 4 and lane 5 are chosen.(Find temperature in the folding machine, maybe 52-56 degree then 53 degree is chosen)

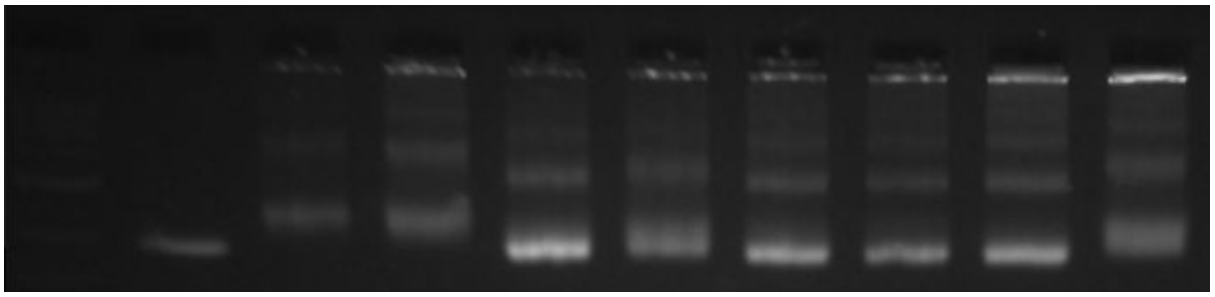
(TEM image for 18mM, 53 degree folded track)

(Gear w/o overhang)
Set magnesium screen from 12-26mM, 2.5day fold then run gel.

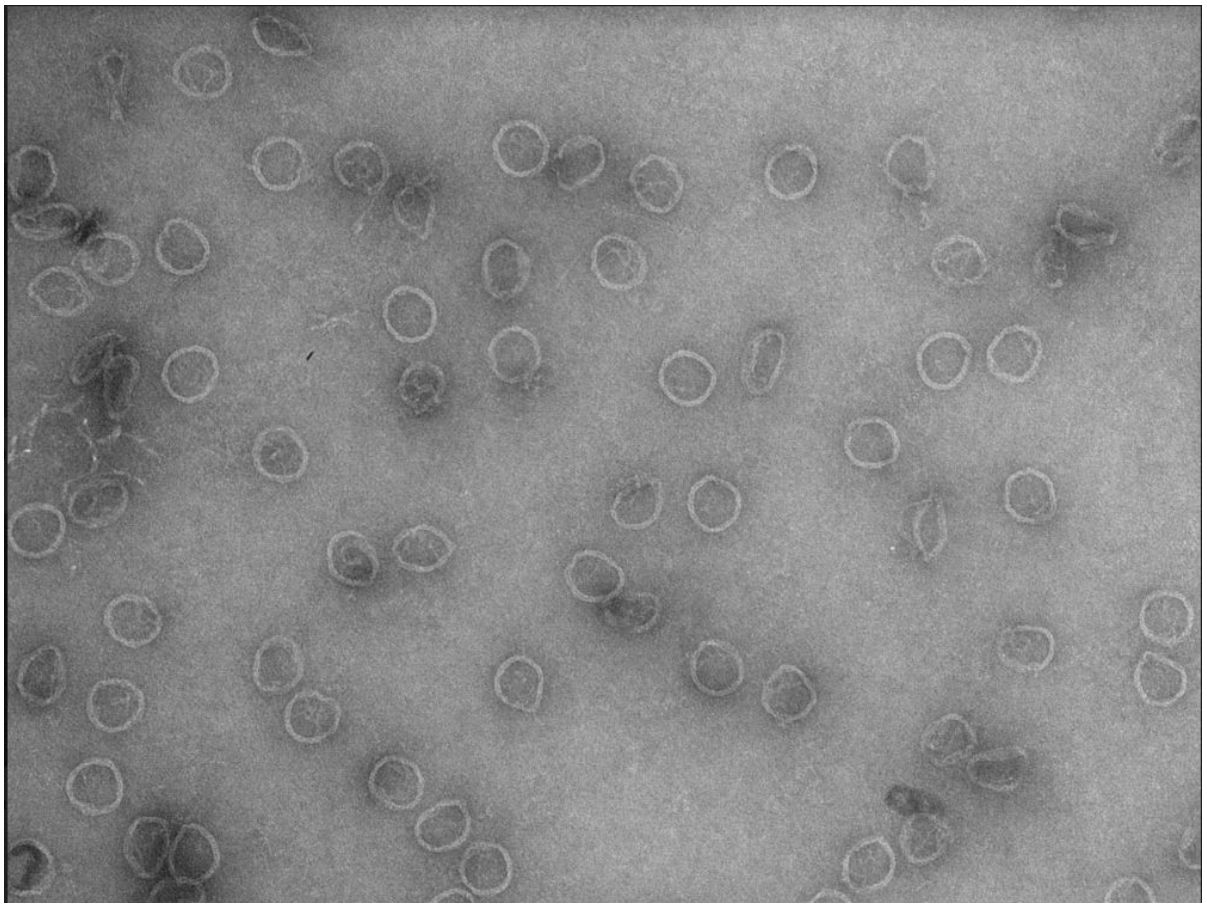


24mM is chosen

56-50 degree rapid fold



Lane 3 is chosen (53 C is chosen).



(TEM image for 24mM MgCl2, 53 C folded gear)

Gear and Track with overhangs

Add gear overhangs to prestock

The prestock sheet was revised to replace “overhang sites” with new arrived overhangs. New prestock and new working stock were prepared for the final structure.

(Magnesium screen, 2.5 d, with overhang)

After the addition of the overhang, the structures for gear and track were changed, new concentration and new folding temperature needs to be redefined for both gear and track.

(Rapid fold, 4 hr, with overhang)

Folding temperature needs to be determined under the new magnesium concentration.

(PEG Purification)

PEG purification is able to provide a significantly higher concentration than gel purification.

(Actuation, blockers and removals)

To enable the gears to roll on the tracks, strand displacement was performed step-by-step.

Step 1. Track and gear were diluted to 1:2 in concentration. In this project, 10 nM track and 20 nM gear were used.

Step 2. Blockers C, D, E, and F were added to the track, with concentration of the mixture of blockers to be 10x of the concentration of the track. Once the blockers were added, the tube was labeled as “reaction tube” or “RT”, and was put into the thermomixer at 25 C, 250 rpm for 30 minutes.

Step 3. Overhangs C, D, E, and F on the track were blocked, while overhangs A and B were remained open. Gear was added to the reaction tube, and the reaction tube was put into the thermomixer at 25 C, 250 rpm for 24 hours.

Step 4. After 24 hours, gears and tracks have overhangs A and B binded. To perform strand displacement, one previous overhang on the track was blocked and the next overhang blocker was removed to allow the gears to move one step on the track. (i.e. Add blocker A to disconnect the binded overhang A, and add removal C to remove blocker C on the tracks, so that gears and tracks will have overhangs B and C binded)