Mechanical Design of DNA Origami in the Classroom

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Supporting Information

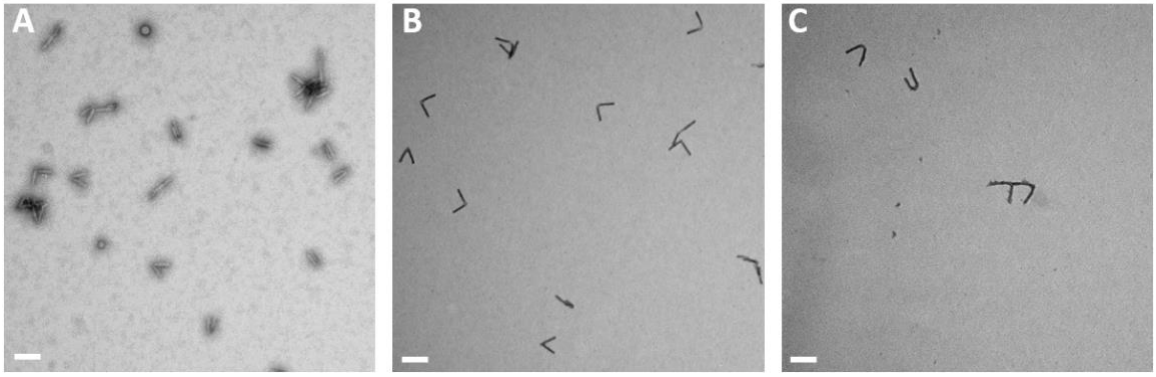


Figure S1 Typical TEM images for laboratory folded (i.e. 2.5 day fold purified in agarose gel electrophoresis) CHJ structures. (A) HF TEM image (B) MF TEM image (C) LF TEM image. Scale bars 100 nm.

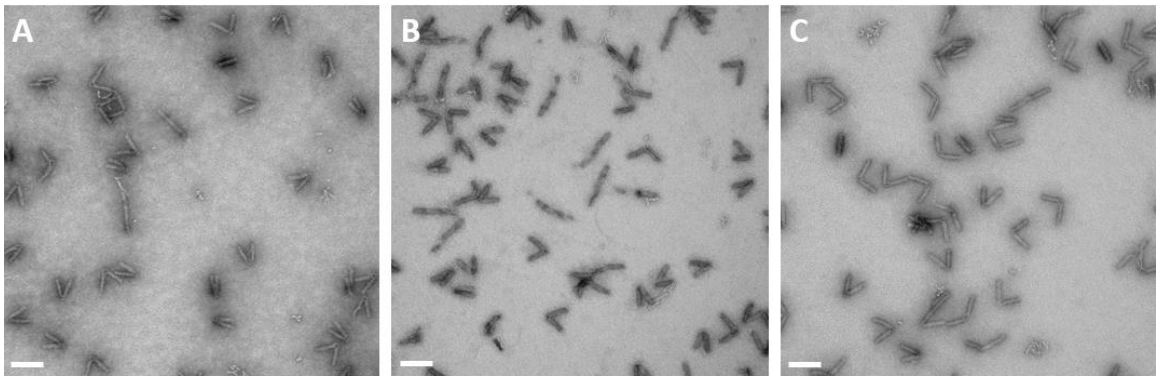


Figure S2 Typical TEM images (for fig2 bot panelB). (A) HF folded at 50ºC for 10 minutes (B) MF folded at 50ºC for 10 minutes (C) LF folded at 50ºC for 10 minutes. Scale bars 100 nm.

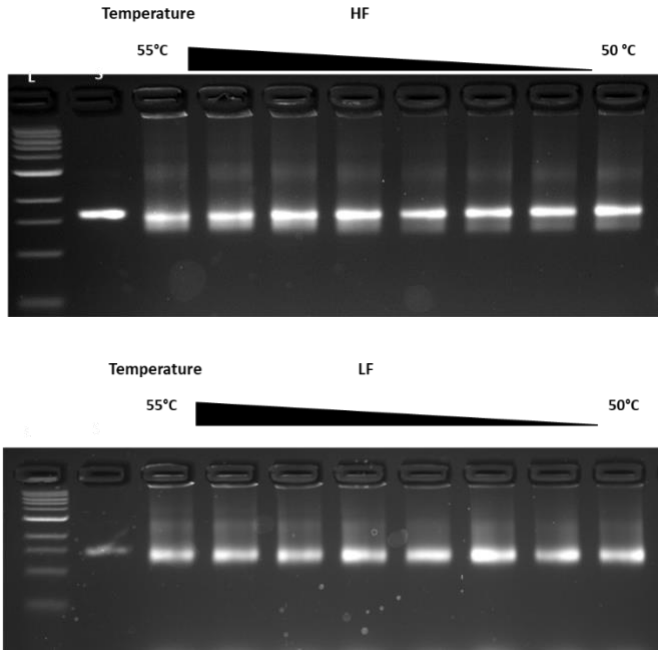


Figure S3 Temperature titration of CHJ.HF (top) and CHJ.LF (bottom) from 55ºC to 50ºC.

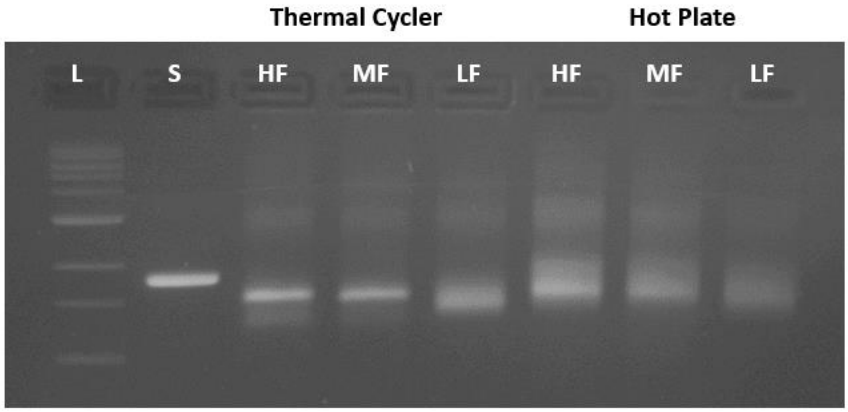


Figure S4 Agarose gel electrophoresis using the laboratory protocol showing CHJ structures folding for 10 minutes in the thermalcycler and CHJ structures folded for 10 mintues on the hot plate.

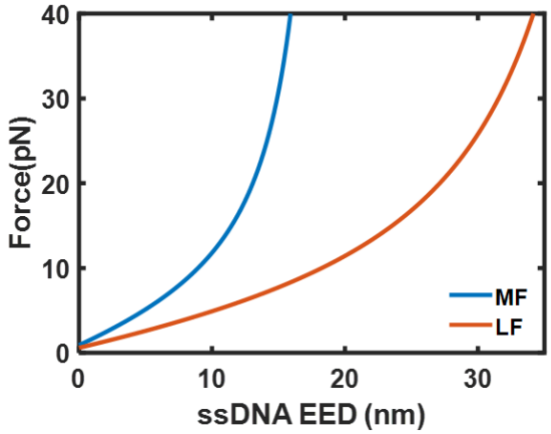


Figure S5 WLC curve illustrating force as a function of end-to-end distance (EED) of the ssDNA for MF and LF designs. The WLC does not hold for HF, since 3 ssDNA linker strands are 0 bases long, and each of them will lead to unrealistically large force (i.e. like other deformation mechansims occur in other parts of the structure since those cannot deform).

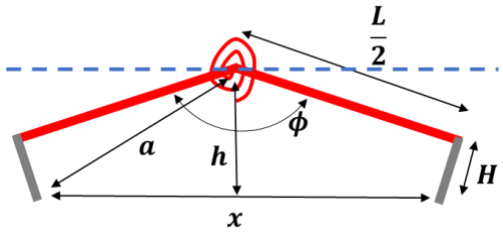


Figure S6 Parameters used for PRBM. Note that ϕ and h are the function of x that can be determined by geometric relationships. Define a as the length between tip (where the ssDNA springs connect to the outer bundles) and vertex, the relations between ϕ, h, and x are as follows: $a = \sqrt{H^2 + \frac{L^2}{4}}$, $\phi(x) = \cos^{-1}(\frac{2a^2 - x^2}{2a^2}) + 2\tan^{-1}(\frac{2H}{L})$, and $\mathrm{h}(x) = \frac{L}{2}\cos(\frac{\phi(x)}{2}) + H\sin(\frac{\phi(x)}{2})$

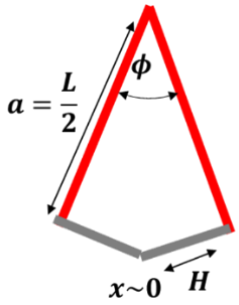


Figure S7 Special case for HF. The ssDNA extension is modeled as having zero length, thereby simply imposing a geometric constraint holding the two arms together at that location, and the large bending beam is modeled as two solid bars. Due to the symmetry of the structure, the angle in equilibrium can be determined as: $\phi = 2\tan^{-1}\left(\frac{2H}{L}\right) \approx 60°$.

Symbol	Parameter	Values
D	Diameter of dsDNA strands	2.5 nm
L_b	Length of one base pair	0.337 nm (dsDNA)
	Length of one base	0.65 nm (ssDNA)
L	Nominal length of the beam	28.31 nm
L_p	Persistence Length	50 nm (dsDNA)
		2 nm (ssDNA)
H	Offset distance of ssDNA spring to the joint	8.125 nm
c	PRBM correction factor	1.5

Table S1: Basic parameters used in the analytical model

Conductor	Resistance (MΩ)
Double Distilled Water	~4
137.5mM MgCl2 Solution	~0.05
13.75mM MgCl2 Solution	~0.1
1xTBE Buffer	~0.9
0.25xTBE Buffer	~1.3
0.5xTBE+6mM MgCl2 Buffer	~0.6
0.17xTBE+2mM MgCl2 Buffer	~1

Table S2: The resistance for several ionic liquids. The resistance was measured by multimeter and the probes were placed at gel position in the electrophoresis rig. Note that the resistance is dependent on the probe position and the liquid spatial distribution. The MiniOne gel electrophoresis system only works around 1xTBE condition, and the dilution of running buffer with magnesium is to achieve a consistent liquid resistance.

Design Software	Websites
Cadnano	https://cadnano.org/
MagicDNA	https://github.com/cmhuang2011/MagicDNA
vHelix	http://www.vhelix.org/
DEADALUS	http://daedalus-dna-origami.org/about/
Simulation/Visualization Tools	**Website**
oxDNA	https://oxdna.org/
Adenita	https://edellano.github.io/Adenita-SAMSON-Edition/
oxview	https://sulcgroup.github.io/oxdna-viewer/#

Table S3: Current DNA nanostructure design softwares and simulation/visualization tools. Websites where software tools can be accessed are shown to the right of the softwares/tools.

Students will:
Gain exposure to the emerging field of DNA nanotechnology
Learn basic concepts of DNA self-assembly
Learn basic biology wet-lab skills through pipetting and gel electrophoresis
Gain knowledge of deformation in DNA nanostructures through two mechanical models: Pseudo Rigid Body Model and Small Deflection Model
Gain exposure to image analysis software

Table S4: Table of learning objectives for educators to implement into the classroom protocol. This can be modified or added to as desired.

A Tutorial for Experimentally Determining the Hinge Angle Distribution from Raw TEM Images

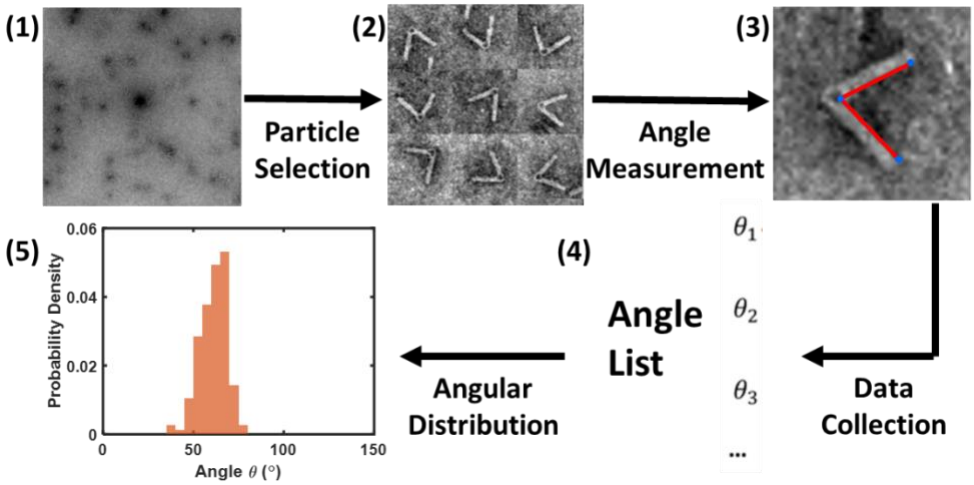


Figure S8 Workflow for experimentally determining the hinge angle distribution

1. Download the newest version of ImageJ: https://imagej.net/software/fiji/downloads
2. Use ImageJ to open raw TEM image 'tif' file
3. Specify the particle box size for both width and height that slightly larger than hinge (Edit---Selection---Specify)
4. In ROI manager (Analyze—Tools-ROI manager)
    a. Move the box to fit one of all hinges
    b. Capture its coordinates (shortcut 'T' on keyboard):
    c. Repeat a,b until all hinges are covered
    d. enable 'show all' to see all boxes
5. Crop Particles (In ROI manager-Muticrop)
6. Combine particles into gallery (TEM---pub montage)
7. Use angle tool to specify three 3 critical points (2 arms tip and 1 vertex) and use Ctrl+M to record the angle
8. Save angle data into 'csv' file
9. Use MATLAB, Excel, or other statistic tools to generate the angular distribution (Example code for MATLAB attached)

```
data= ;         %import your angle measurement in an array

%generating the histogram bin information
[CdfF,CdfX] = ecdf(data,'Function','cdf');
BinInfo.rule = 5;
BinInfo.width = 3;
BinInfo.placementRule = 1;
[~,BinEdge] = internal.stats.histbins(data,[],[],BinInfo,CdfF,CdfX);
[BinHeight,BinCenter] = ecdfhist(CdfF,CdfX,'edges',BinEdge);

% plot the histogram
hLine=bar(BinCenter,BinHeight,'hist');
set(hLine,'FaceColor',[0.8500 0.3250 0.0980],'EdgeColor','none',...
'LineStyle','-', 'LineWidth',2,'FaceAlpha',0.7);  %orange
xlabel('Angle (deg)')
ylabel('Probability density')
set(gca,'FontSize',18,'FontWeight','bold')
ax = gca;
ax.LineWidth = 3;
set(gcf,'Color',[1 1 1])
```

Table S5: Example MATLAB code for generating the angular distribution

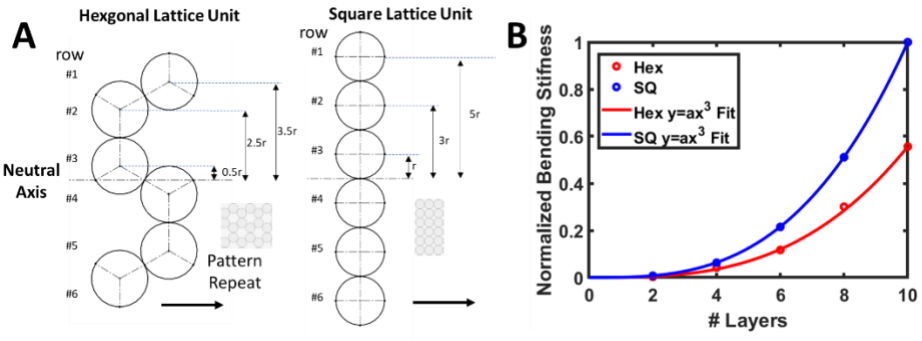


Figure S9 A general comparison of bending stiffness with different number of layers and lattice type, hexagonal lattice and square lattice. (A) Schematics for showing displacement of DNA bundle to neutral axis. Note uneven pattern in hexagonal lattice. (B) The bending stiffness is increased by add more layers. With the same number of total bundles, square lattice has higher bending stiffness. They both satisfy the cubic increasing pattern.

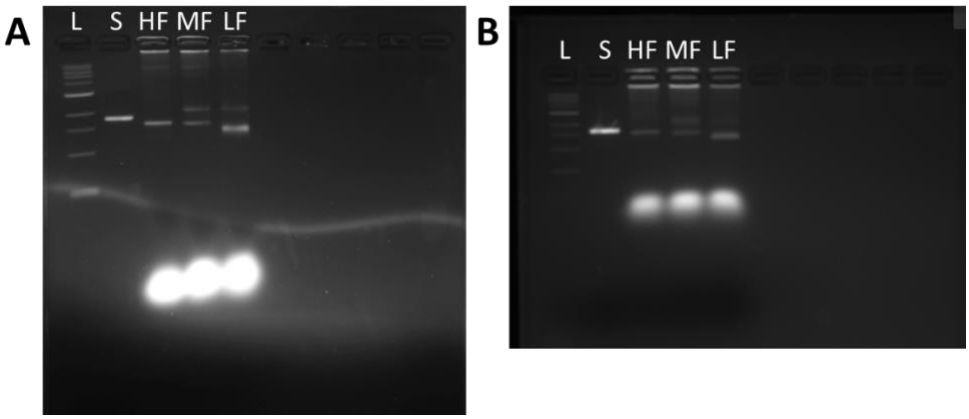


Figure S10 (A) Original full gel image from figure 1D with the gel run for 180 min. (B) Another representative gel image for the same set of structures with 2.5-day folding and run for ~90 min.

Classroom Protocol

Here we lay out a proposed procedure for a ~2 hr experiment module for classroom implementation of the Mechanical Design of DNA origami for the folding and gel electrophoresis analysis of three configurations of a DNA origami compliant hinge joint (CHJ) as shown in Figure S11B (from Figure 1 in main text). The protocol below is based off a similar protocol presented in a prior DNA origami education module.[1] The procedure consists of three main steps: 1) Preparing the Gel for Electrophoresis, 2) Running the Folding Reaction, and 3) Running the Gel. Each step entails preparation time (which will vary based on the students' prior experience with lab work (pipetting, measuring reagents, etc.)), and each step has a rate limiting step described below. Below we provide an overview of each step, and detailed protocols can be found in the Methods section of the main text.

Step 1 - Preparing the Gel for Electrophoresis (~35 min):

Students will prepare the gel running buffer and cast the gel. This involves both standard laboratory measurements as well as pipetting. Preparing the gel takes approximately fifteen minutes. The rate limiting step here is waiting for the gel to solidify, which takes ~30 min at room temperature. Students can prepare the folding reaction or bring water baths up to target temperatures while waiting for the gel to solidify.

Step 2 - Running the Folding Reaction (~30 min):

In this step, students will prepare the two water baths bringing them to the desired target temperature range, mix the folding reaction, and perform the folding thermal cycle. Students will need to calculate appropriate dilutions to make 200 mM $MgCl_2$, and then mix the five ingredients at proper volumes and concentrations (scaffold, staple strands, folding buffer, salt buffer, and water). This process takes ~15-20 min. The rate limiting step is folding the structure for 20 minutes (5-min melt, 10-min fold, and 5-min cooling).

Step 3 - Running the Gel (~50 min):

Here, students will perform gel electrophoresis by setting up the gel equipment, mixing the folded structure solution with gel loading dye, loading the samples into wells, and running the gels. Preparation takes ~10 min, and running the gel takes 30-40 min to visualize the gel shift results. Students will compare their results to expected results shown in Figure S11C (Figure 4A from main text), depending on the electrophoresis setup being used.

The total protocol can be completed in a single, two-hour lab session. If broken up into two one-hour sessions, the initial session would consist of Step 1 and the preparation for Steps 2 (i.e., mixing folding reactions). Session two would then include running the folding reaction from Step 2 as well as running the gel in Step 3. Part of the preparation of Step 3 (i.e., preparing loading dye) can be done during the folding reaction, as long as students can also carefully monitor the water bath temperatures

simultaneously. The protocol can also be completed in a single one-hour session if the instructor prepares the gel, water baths, and folding reaction mixtures ahead of time. Students would then perform the folding reaction, mix with loading dye, load and then run the gel for ~30 min. This shorter method would be ideal for younger students or students with no prior lab experience.

Table S6 shows the equipment and reagents needed to complete the entire procedure. Many items on the equipment/supplies list (E1-E12) are commonly found in classroom science laboratories or can be purchased at a low cost. E13-E15 are not as common, however, inexpensive classroom versions exist such as the MiniOne Gel Electrophoresis Kit used in this research (< $300).

The reagents R1 and R2 are readily found in science laboratories. R3-R7 can also be purchased at low cost individually or in kits (in these experiments, reagents R3-R7 were purchased from MiniOne). The reagents R8-R10 can be provided in small quantities for those interested in performing the procedure.

Equipment		Reagents	
E1	Scale	**R1**	Distilled Water
E2	Chemical Spoon	**R2**	MgCl2
E3	Beakers	**R3**	GelGreen DNA stain
E4	Microwave	**R4**	Agarose
E5	Timer	**R5**	0.5x TBE buffer
E6	Floating tube rack	**R6**	1 kb DNA ladder
E7	Graduated cylinder	**R7**	Loading Dye
E8	Hotplate	**R8**	Folding Reaction Buffer
E9	Thermometer	**R9**	M13mp18 DNA scaffold
E10	Gloves	**R10**	Compliant Hinge Joint oligos
E11	Calculator		
E12	Eppendorf tubes		
E13	Pipette and Tips		
E14	Gel Casting Equipment		
E15	Electrophoresis System		

Table S 6 List of Equipment and Reagents for classroom protocol

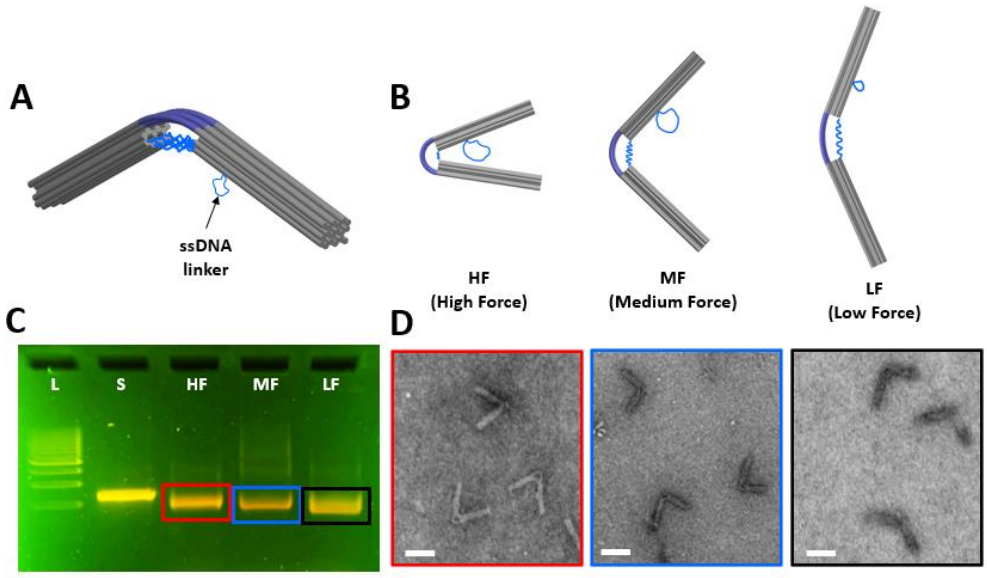


Figure S 11 Compliant Hinge Joint (CHJ) overview (A) CHJ schematic (B) CHJ schematics with varying ssDNA linker lengths (C) Agarose gel electrophoresis image with anticipated results of each configuration. Left to Right: 1kb ladder, 7560 scaffold, high force configuration, medium force configuration, and low force configuration

A sample lab assignment focusing on mechanics. (Questions with Solutions)

Question 1:

From the configuration of HF, MF, LF structure, ignoring their names, can you qualitatively order them in terms of the level of force on the hinge vertex?

Solution:

For determining force order, we can first consider two extreme cases where we have no force and very large force. For example, in a zero force case, all DNA bundles are supposed to be straight with no bending; therefore, the CHJ should exhibit a 180 deg angle (i.e. straight configuration). On the other hand, assuming a homogeneous material, a very large force would cause large bending where the two arms would touch each other. Overall, for intermediate cases, we can conclude that larger hinge angles (i.e. closer to straight configuration) have lower bending deformations and lower applied forces.

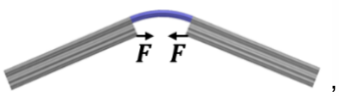
Question 2:

If we consider the ssDNA springs and the bent beam as two separate bodies, draw a free-body diagram of both CHJ components when the forces are in equilibrium.

Solution:

The gravitational force for each hinge is ~7560x660/Na/1000x9.8=8.1x10^(-20)N which is far below the pN level, and therefore we do not consider it in the FBD.

For the beam part:



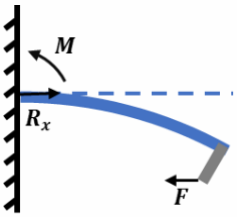
,

For the ssDNA part:

Question 3:

Due to the symmetry of the structure, we can consider the CHJ as mechanically equivalent to a cantilevered beam that is half of the geometry (left half or right half). Draw a free-body diagram of this cantilevered beam. Relate the force applied by the springs to the reaction loads at the cantilevered end assuming we know the vertical offset to where the force is applied, y.

Solution:



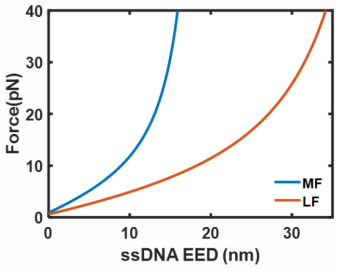
Rx = F

M = F*y y is vertical offset from tip to horizontal axis

Question 4:

In this CHJ, if we consider ssDNA as a Worm-Like-Chain (the equation is given), plot the force vs extension curve for MF and LF. What is the difference compared with a spring that satisfies Hooke's law?

Solution:



For a spring that satisfies Hooke's law $F=k\,\Delta x$, the force vs. extension curve is a line with a slope of stiffness k.

Question 5:

Estimate the bending angle of the CHJ when the springs apply a total force of 10 pN using the SDM.

Given that: Beam Length: L=28.31 nm, Beam bending stiffness: EI=2.67x10^3 pNnm^2, Beam offset (vertical offset to location of force application): H=8.125 nm

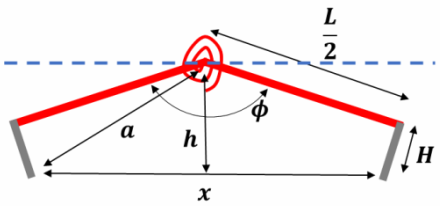
Solution:

$$\theta = \frac{L/2}{EI} FH = 0.47 = 26.7°$$

$$\phi = 180° - 2\theta = 126°$$

Question 6 (extension):

Can you estimate the force required to bend the CHJ into the experimentally measured bending angle for the MF and LF versions of the design (average bending angle for MF is 95^o, and average angle for LF is 126^o)?



Solution:

Case MF:

$$\theta = \frac{180° - \phi}{2} = 42.5° = 0.74$$

Case LF:

$$\theta = \frac{180° - \phi}{2} = 27° = 0.47$$

In SDM:

$F = \frac{\theta EI}{HL/2}$ = 17.2 pN (MF) or 10 pN (LF)

In PRBM:

$K = c\frac{EI}{L_{half}}$ =283 pNnm

$\mathrm{h} = \frac{L}{2}\cos(\frac{\phi}{2}) + H\sin(\frac{\phi}{2})$=16.13 nm (MF) 13.66 nm (LF)

$F = \frac{K\theta}{h}$ =13 pN (MF) or 9.7 pN (LF)