Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Structure, stability and elasticity of DNA nanotube

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Supplementary Information

1. Construction of 6-helix and 8-helix DNA Nanotubes.

To construct the 6-helix and 8 helix DNA nanotubes, we kept the double helix at the vertices of hexagon and octagon respectively. To arrange the helices into closed bundle, we fused the double helical arms of DNA with crossovers according to the closing geometrical angle of hexagon and octagon.

For example for a DNA with 10 base-pairs per turn, we can design the crossovers at 7 or 14 base- pairs spacing which will give us a closed angle of 120 ˚. In the case of 8 helix DNA nanotubes, we cannot get a perfectly closed regular octagon with geometrical angle 135˚ with DNA crossovers since it does not give any integer value of number of base pairs. The crossover for the corresponding geometry can happen either after a specified number of base pairs, or multiples of those to get a closed tube like structure. These spacing are as follows,

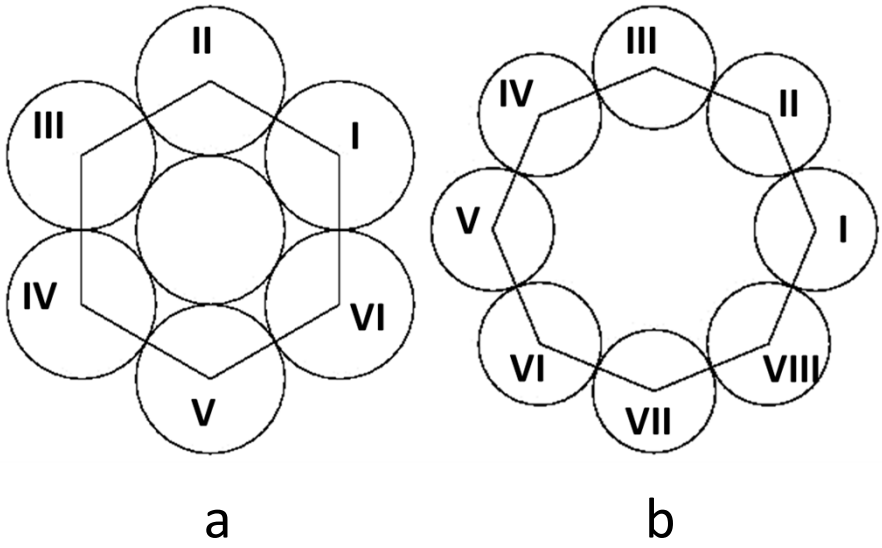

Fig. S1: Cross sectional view of **(a)** 6-helix and **(b)** 8-helix DNA Nanotubes. The numbers mentioned inside the circle are the helix identities.

To construct these nanostructures, we have designed a code using NAB module in AmberTools. This program takes the details about the structure from a sequence file. The sequence file is of a

specific file format, which the program can read and use to create the structure. A portion of a sequence file is displayed below

```
cgactt  gatggagcaga|1|gctactt|2|ctacatc| |gcattca|3|gtgctca|4|ggtacta|
------||ctacctcgtct   cgatgaa   gatgtag   cgtaagt   cacgagt   ccatgat

cggtac  gtgacgatagg   acacatc   agatgtc   ttaggag   aggtcac   agtaacc
------||cactgctatcc|1|tgtgtag   tctacag|5|aatcctc|3|tccagtg   tcattgg
```

The first line represents the sense strand and the second line represents the antisense strand and so on. Together they form one double helix. An empty line is used as separators between individual double helices. Vertical bars are used to indicate breaks or nicks in the structure. A dash (-) is used when some regions are needed to be left single stranded. The number in between vertical bars serves as labels to make crossovers. A crossover is constructed between similarly labeled breaks. In the above example, crossovers are made between the 1 and 3 labeled breaks. The program generates the correct topology based on this input sequence file. To get the right orientations, specific NAB code has to be written in an orient function according to the requirements of the molecule. A generic orient function also exists which assumes a tubular structure for the entire molecule. It assumes that the molecule is made of parallel DNA double helices which have crossovers between them. The program takes as input a file containing the sequences and markers for the crossover point's locations. The program first reads the individual double helices and constructs broken helix structure for each of them. Next the individual broken helices are oriented about each other by the generic orient function. This is done in such a way as to minimize the root mean square distances between the atoms that need to be bonded. During the orientation process the double helices are only given three degrees of freedom to move, namely:

1. Rotation about their own helix axis.
2. Revolution about the helix axis of the molecule to which it is being bonded to.
3. Z-axis or vertical translation of the double helix such that all helix axes are always parallel.

The three parameters for the above three degrees of freedom are chosen by iterating over all possible values with appropriate step sizes and the values for which the RMSD is minimum, are chosen. Also the values are chosen such that there will not be any overlap with other helices and

that they maintain a certain distance from each other. Next the individual bonds are made across the broken helices and the strands are merged so that the strand identity is preserved, as in the original molecule, so that the residues on each strand are correctly represented and numbered in the output. Finally, we get the PDB file of the structure for the AMBER MD simulation.

2. The connectivity of triangular DNA nanotubes: TBZ molecule

We have used the corner molecule to connect the triangular rungs of the triangular DNA nanotube. The molecule has been introduced to join the triangular rungs to the outer DNA forming the sculpture of triangular DNA nanotubes. The same molecule is used by Sleiman et. al[1], to construct the tri-tube geometry. This molecule has been designed with the xLeap module of AmberTools. GAFF[2] has been used to describe the interaction parameter for this molecule. Figure S2 shows the structure of TBZ molecule with GAFF atom type. Here is the picture of the corner molecule named as TBZ, (Fig. S2).

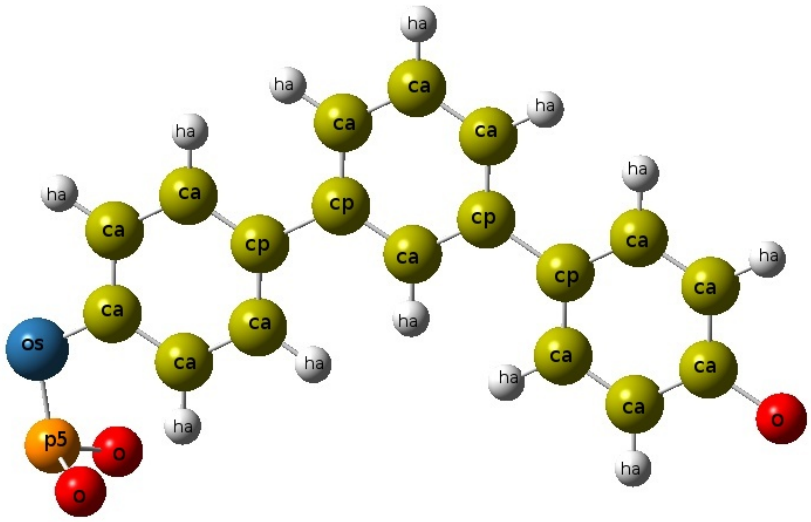

Fig S2: The connecting molecule TBZ for the triangular nanotube.

3. Snapshots of simulation.

The simulation without single stranded overhangs has also been done to compare the effect of overhangs. We have performed two sets of such systems, AT rich and GC rich nanotubes. The triangular nanotubes are also stable during the simulation. Here are some snapshots of the 6-helix nanotube as well as the triangular nanotubes during MD simulation.

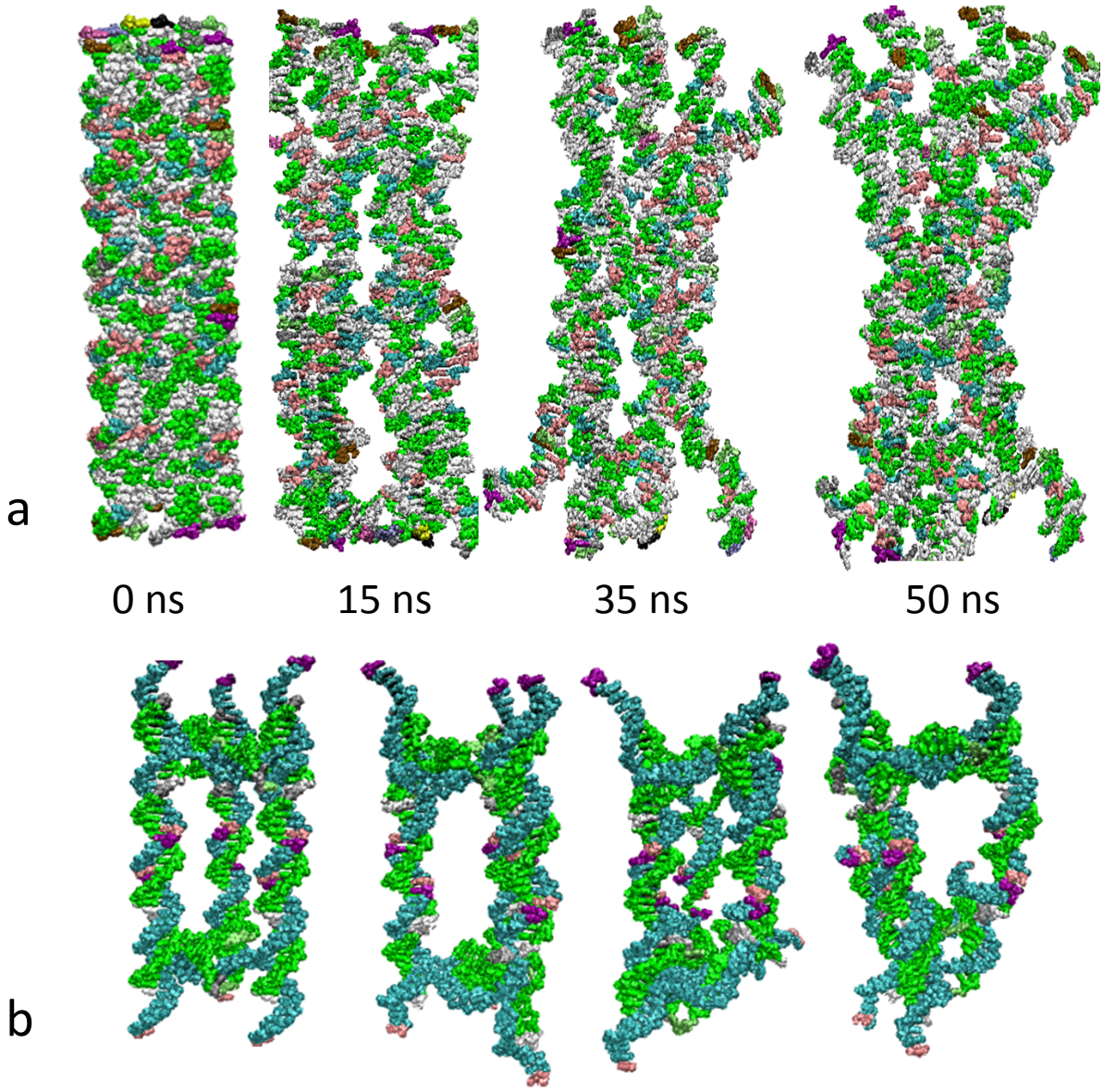

Fig. S3: Snapshots of simulation trajectories at various time steps,

(a) 6-helix DNA nanotube structure without Single stranded overhangs, **(b)**Triangular AT DNA nanotube during simulation.

4. RMSD with aqvist parameter.

The ion-water and ion-DNA interactions play an important role in the stability of DNA structure. So while studying the thermodynamic stability of DNA nanotubes with respect to various sequences; we have also simulated 6-helix and 8-helix structures with aqvist ion parameter for Na+ ion[3] and compare the results with those obtained using Joung and Cheatham ion parameters.[4] Structures simulated with aqvist ion parameter show higher RMSD compared with structures simulated using Joung and Cheatham parameters. Subsequently all the nanotubes structures reported in this paper have been simulated using Joung and Cheatham parameters. This implies the better suitability of Joung and Cheatham parameter for water-alkali ion-nucleic acid interactions.

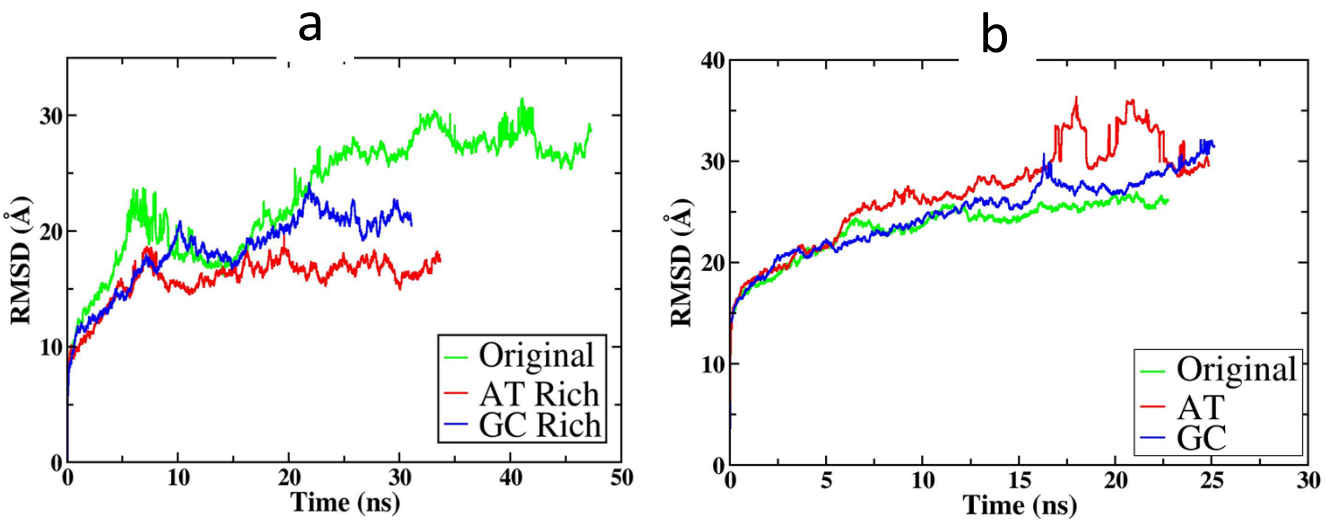

Fig. S4: RMSD for (a) 6-helix structures and (b) 8-helix structures with simulated using Aqvist ion parameter. The RMSD is calculated with respect to the initial minimized structure.

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5. The 8 helix open structure.

Following the similar protocol used to build 6-helix DNA nanotubes, we put ds-DNA at the vertices of octagon in order to get the 8-helix DNA nanotube structure. But this protocol leads to a quit open structure, because of the geometry of B-DNA. Fig**. S5** shows the open helices of 8-helix structure. In order to get the closed tubular structure, we forcefully fused the helical domain of adjacent ds-DNA, which ultimately led to a highly strained structure. These structures try to minimize this dihedral strain during molecular dynamics simulation resulting in highly distorted tubular structures which can be easily seen in the instantaneous snapshots shown in **fig 3b**.

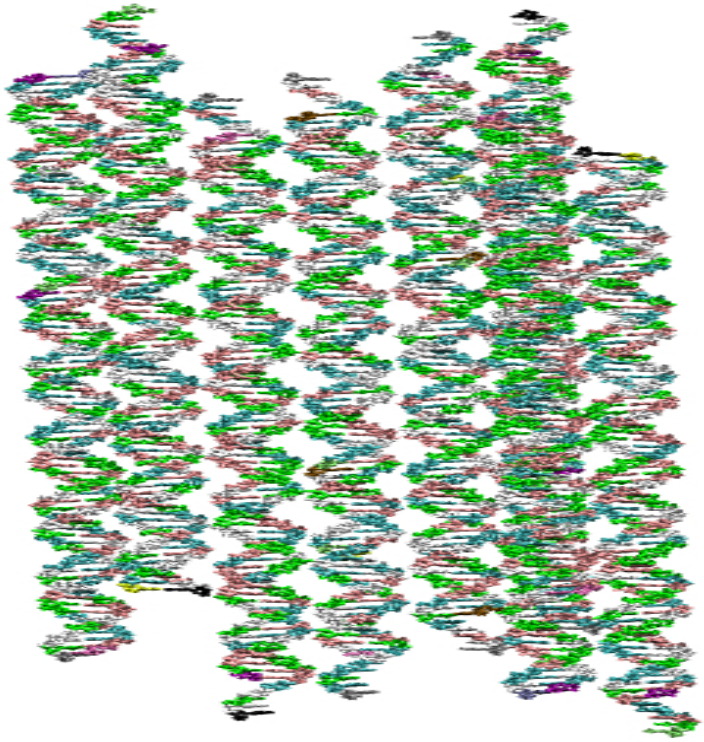

Fig. S5: The open structure of 8-helix bundle. Using the NAB code we make transformation and design crossovers to get a closed geometry.

6. Zigzag radius profile of 8-helix DNA nanotubes.

During several nanosecond long MD simulations, the 8-helix structures try to minimize the dihedral strain which results in highly deformed nanotube structure. This deformation gives rise to the erratic radius profile of all three 8-helix structures along the tube length. So overall, 8-helix structures are less stable due to its inherent closing angle which is not appropriate for crossover switching among helices.

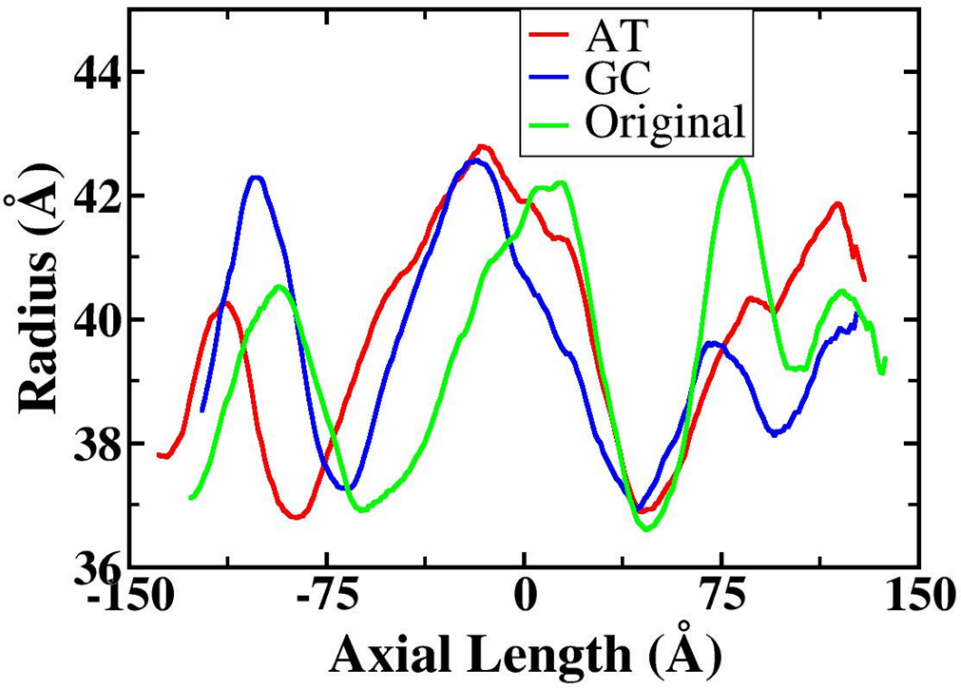

Fig. S6: The radius profile of 8-Helix DNA nanotubes with respect to the axial length.

7. Elastic properties of dS-DNA in constant velocity pulling simulation.

To explore the elastic response of DNA nanotubes, we pulled them in steered molecular dynamics (SMD) simulations in constant velocity ensemble. Note that the pulling rates in simulation are order of magnitude higher compared to the rates used in the experiments. Figure S7 shows the strain vs applied force (constant velocity ensemble) for 38-mer dS-DNA. From the linear region of this plot, we extract the value for stretch modulus for this structure.

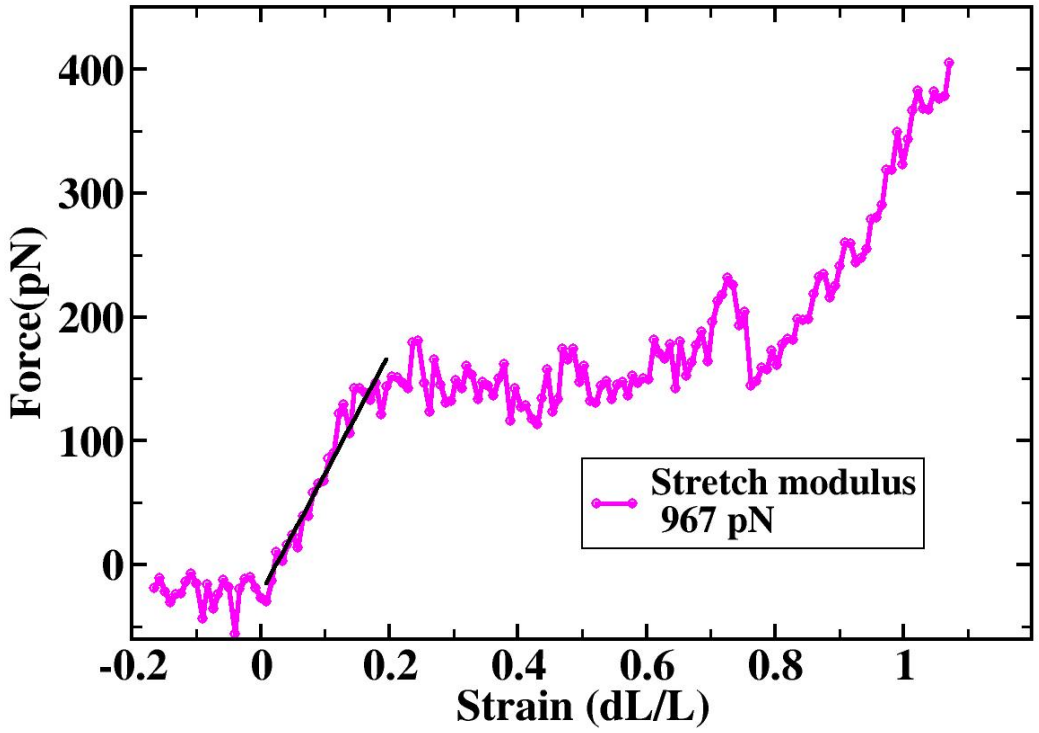

Fig. S7: The stress vs strain curve for 38-mer dS-DNA. The stretch modulus has been calculated from the linear region of the plot.

8. Snapshots of structures during constant velocity pulling.

Below we give instantaneous snapshots of the various nanotube structures at various strains during steered MD simulation. The tubes have been pulled from both the ends.

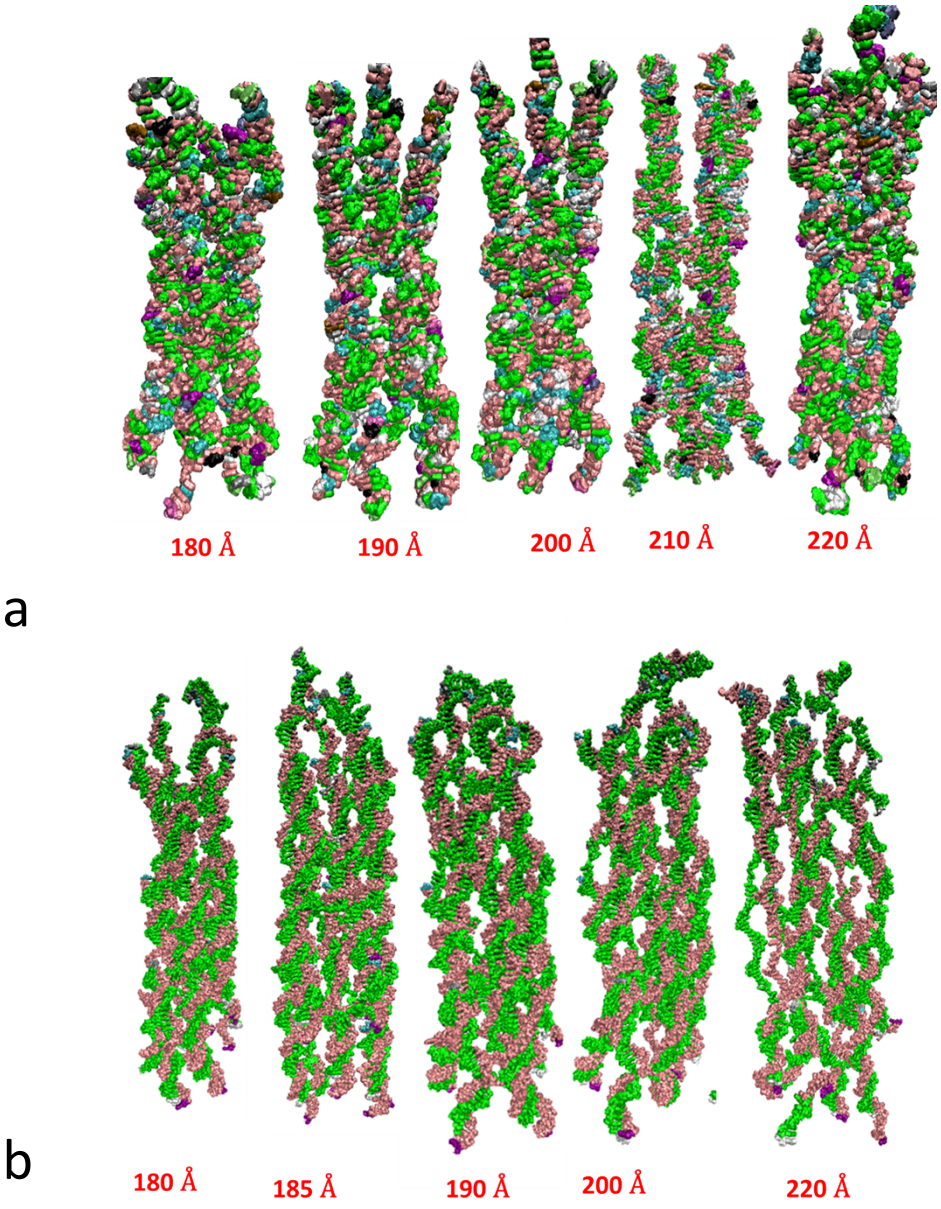

Fig. S8 : Snapshots of (**a**) 6-helix, **(b)** 8-helix DNA nanotube during SMD simulation.

9. Free energy calculation using WHAM analysis

From the SMD simulation we have calculated the free energy of the nanotube structure as a function of nanotube length using WHAM technique The free energy as a function of the tube length for various nanotube geometry have been shown in figure S9. This gives us an estimate of the equilibrium length of these tubes.

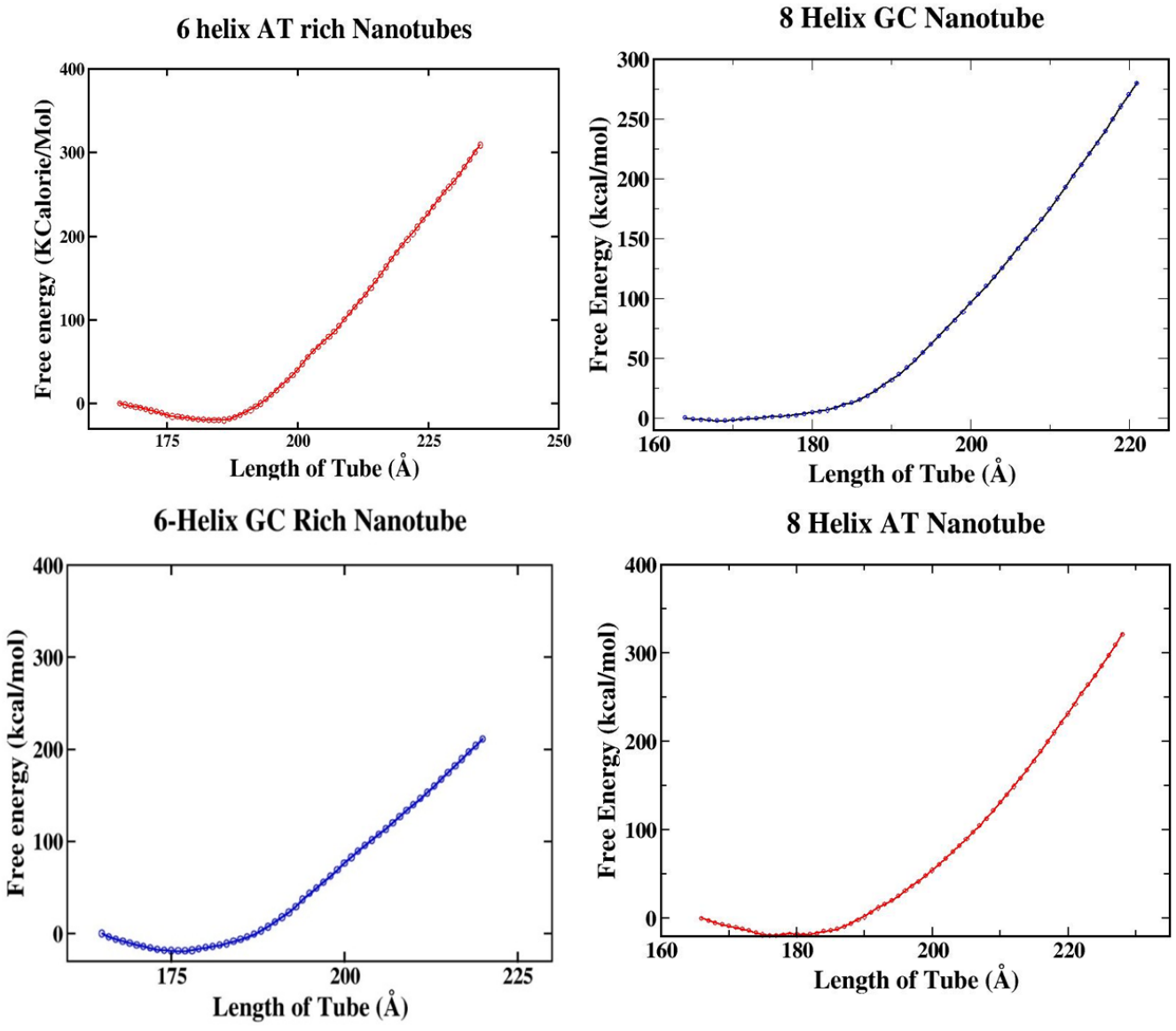

Fig. S9: Free energy for various DNA nanostructures as a function of the nanotube length obtained from the steered Molecular dynamics simulation. The minima of free energy plot correspond to the equilibrium length of these DNA nanotubes.

10. 6-helix DNA nanotubes made of pure AT and pure GC base composition.

To understand the effect of sequence on the stability of the 6-helix topology, we have done simulation of structures with only AT and only GC base sequences. Figure S10 (a) and (b) shows the RMSD and the radius profile for these structures. Structures with only AT base pairs are more stable. We have pulled these structures in constant velocity ensemble using steered molecular dynamics to calculate the stretch modulus of these structures. We see that the DNA nanotube composed of AT sequence is more stable which is clear from both RMSD and radius analysis. The stretch moduli of AT nanotube and GC nanotube are 4397 (± 216) pN and 4507 (± 213) pN respectively.

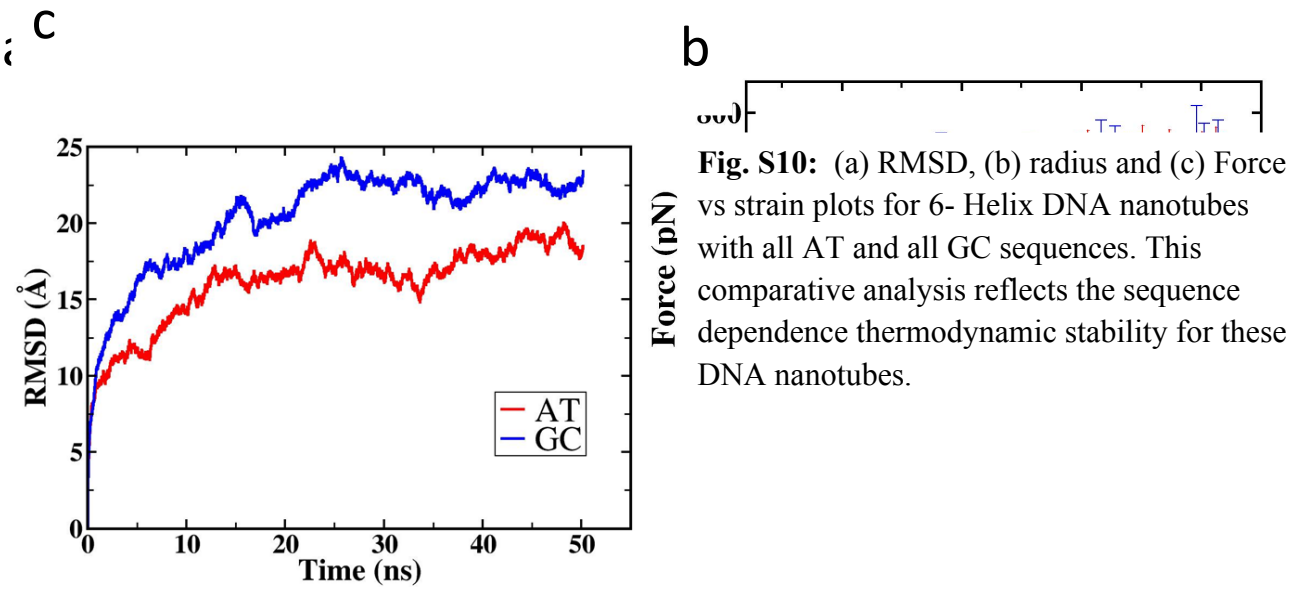

Fig. S10: (a) RMSD, (b) radius and (c) Force vs strain plots for 6- Helix DNA nanotubes with all AT and all GC sequences. This comparative analysis reflects the sequence dependence thermodynamic stability for these DNA nanotubes.

11. Effect of pulling velocities on DNA Nanotubes.

6-helix AT rich structure has been pulled with three different velocities to explore the role of pulling velocities to the elastic response under constant velocity SMD simulation. We see that the slope of the linear region of force vs strain plot is almost similar with respect to all three pulling velocities. As expected, the pulling force required is less with low pulling velocity compared to high pulling velocity for the same strain in the structure. The plateau region begins at lower forces for slow pulling velocities. While pulling with 0.05 m/s, the simulation is more realistic but it is computationally expensive.

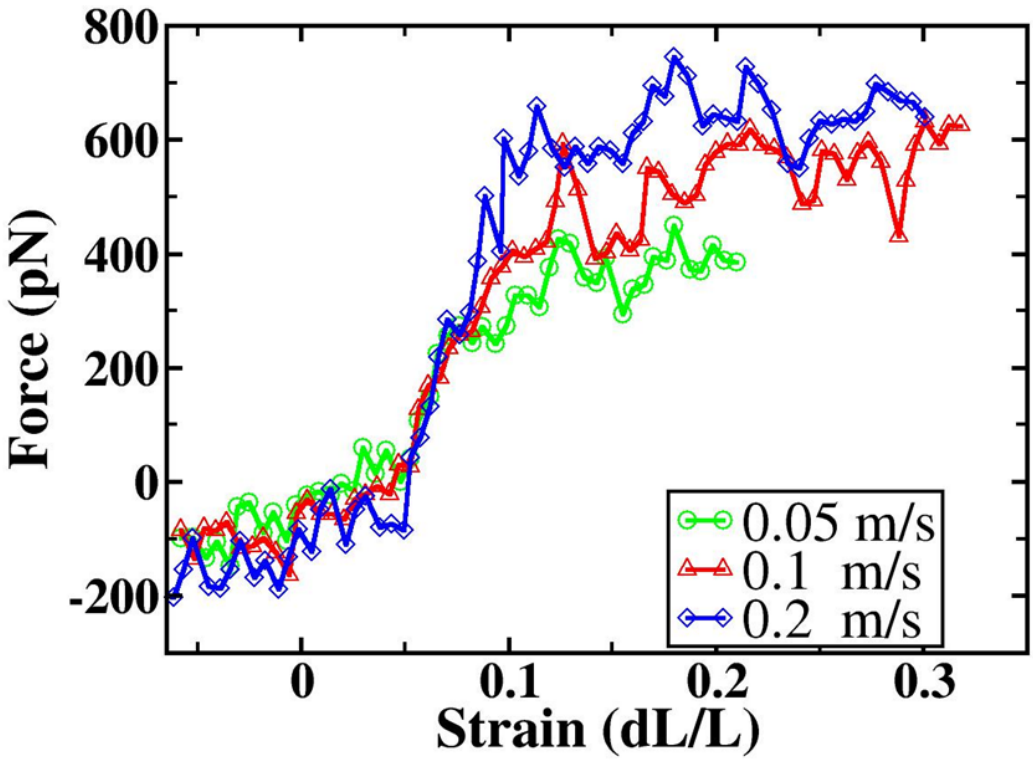

Fig. S11: Force vs Strain plot for 6-helix AT rich structure at different pulling velocities. The plateau region approaches to the smaller force values as we decrease the pulling velocity.

12. Snapshots of the cross sectional view from the top of the nanotubes

This figure shows the variation of the cross section of DNA nanotubes tubes with respect to the simulation time.

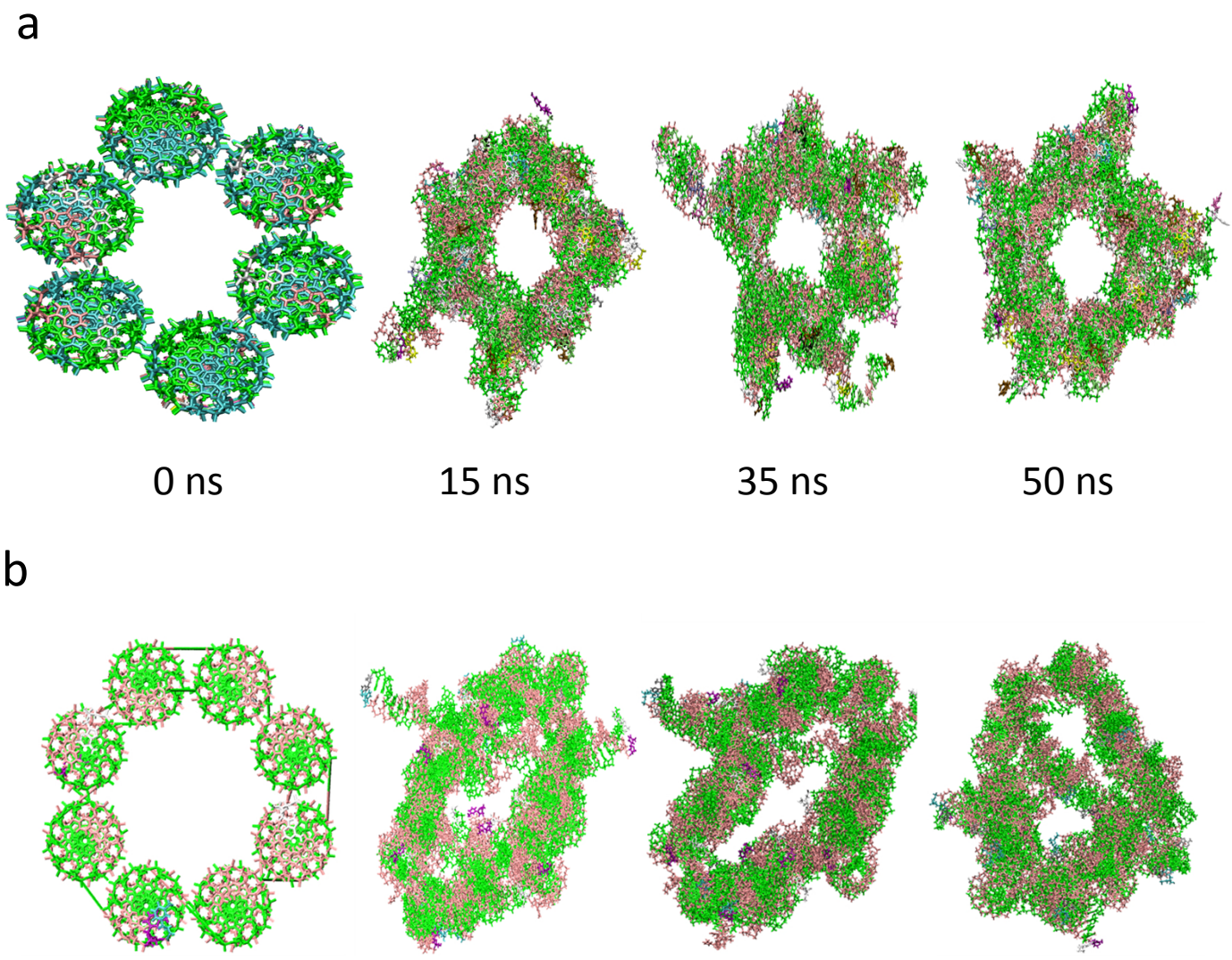

Fig. S12. The cross sectional view of DNA nanotubes at various times steps during the simulation. (a) 6 helix DNA nanotubes. (b) 8 helix DNA nanotubes.

Supplementary Video V1:

Trajectory of 6-helix nanotube in all atom MD simulation for 50 ns time scale.

Supplementary Video V2:

Evolution of RMSD profile 6-helix AT rich nanotube with respect to simulation time.

Supplementary Video V3:

The pulling of 6- helix AT Rich structure. The red atoms are the O3 and O5 atoms of the sugar phosphate back bone where the force has been applied.

References:

1 Lo, P. K. *et al.* Loading and selective release of cargo in DNA nanotubes with longitudinal variation. *Nature Chemistry* **2**, 319-328, doi:10.1038/nchem.575 (2010).

2 Wang, J., Wolf, R., Caldwell, J., Kollman, P. & Case, D. Development and testing of a general amber force field. *Journal of computational chemistry* **25**, 1157-1174, doi:10.1002/jcc.20035 (2004).

3 Aqvist, J. ION WATER INTERACTION POTENTIALS DERIVED FROM FREE-ENERGY PERTURBATION SIMULATIONS. *J. Phys. Chem.* **94**, 8021-8024 (1990).

4 Joung, I. S. & Cheatham, T. E. COMP 270-Determination of alkali and halide monovalent ion parameters for use in explicitly solvated biomolecular simulations. *Abstracts of Papers of the American Chemical Society* **236** (2008).