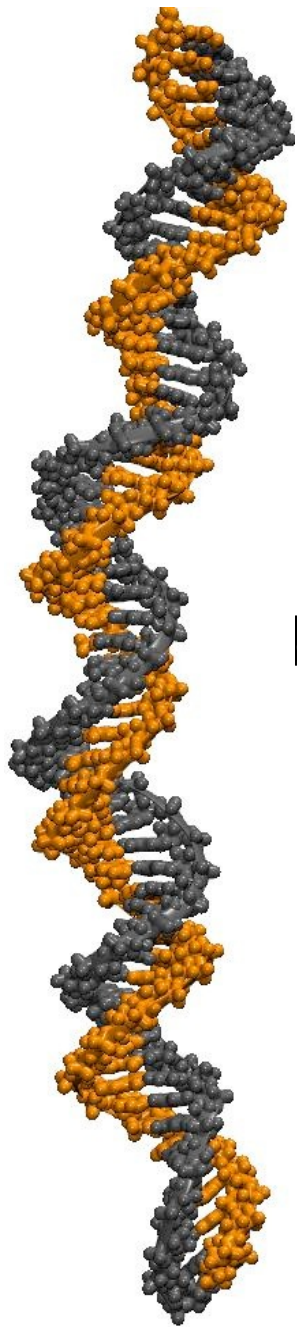


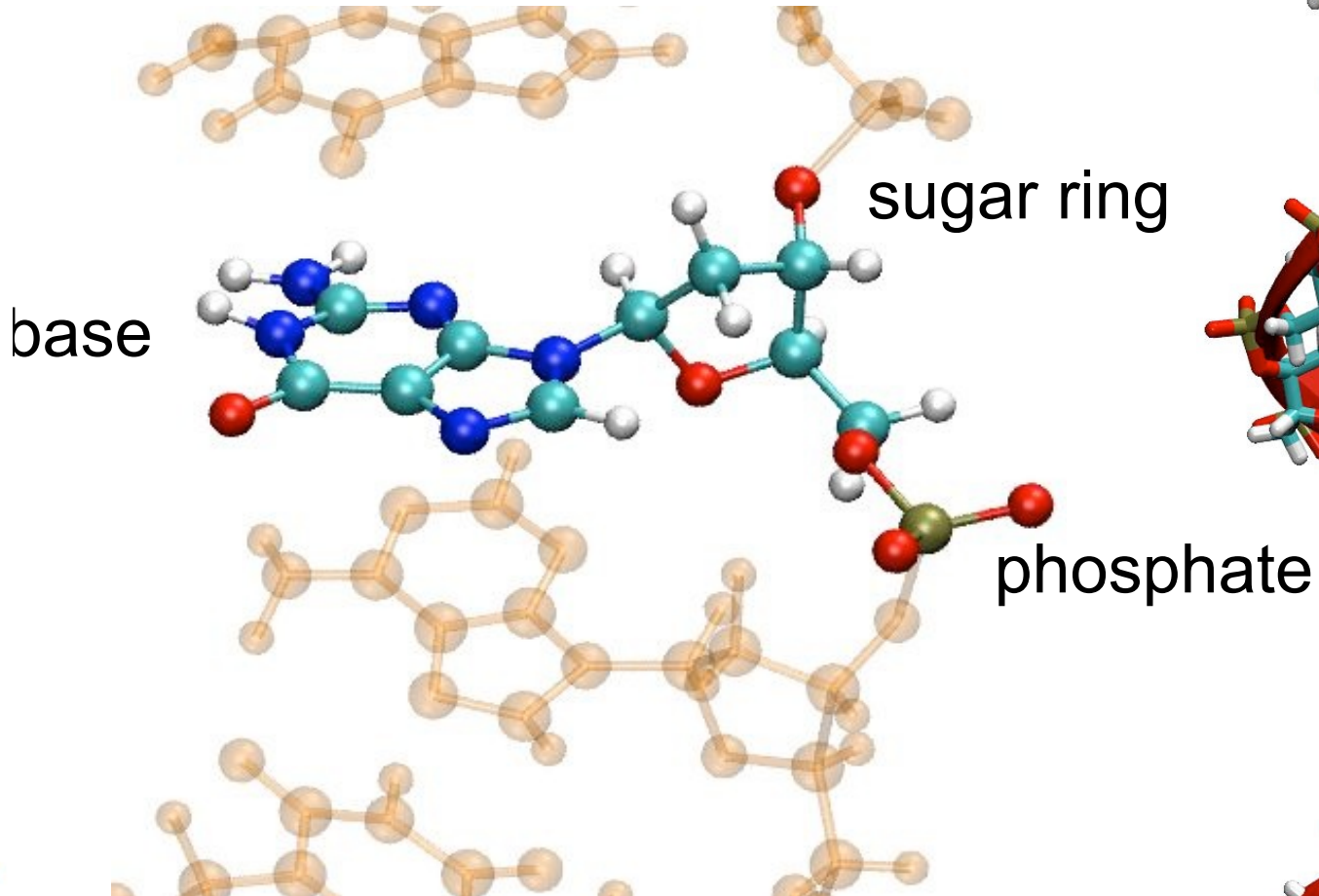
DNA nanotechnology

Aleksei Aksimentiev
Department of Physics, UIUC

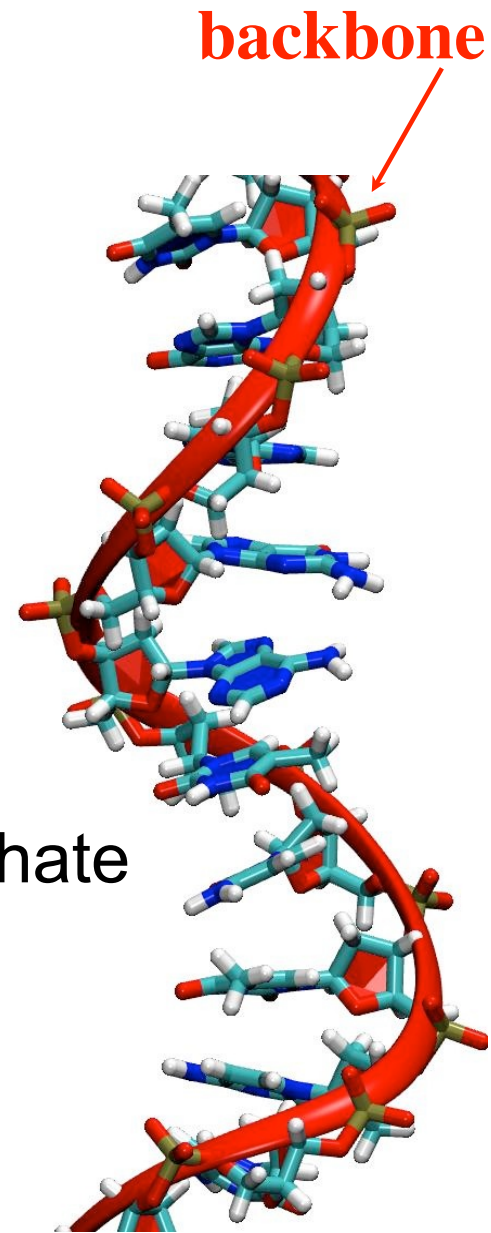
DNA up-close



Double stranded DNA

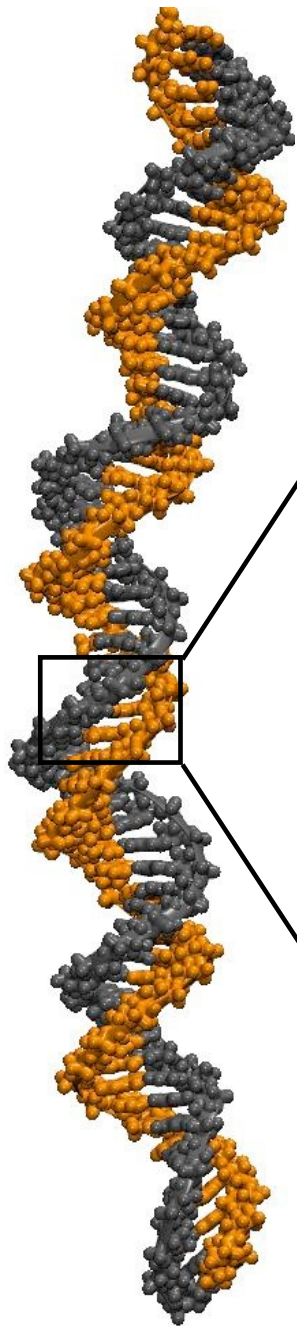


5'-AAGCTGGTTCAG-3'

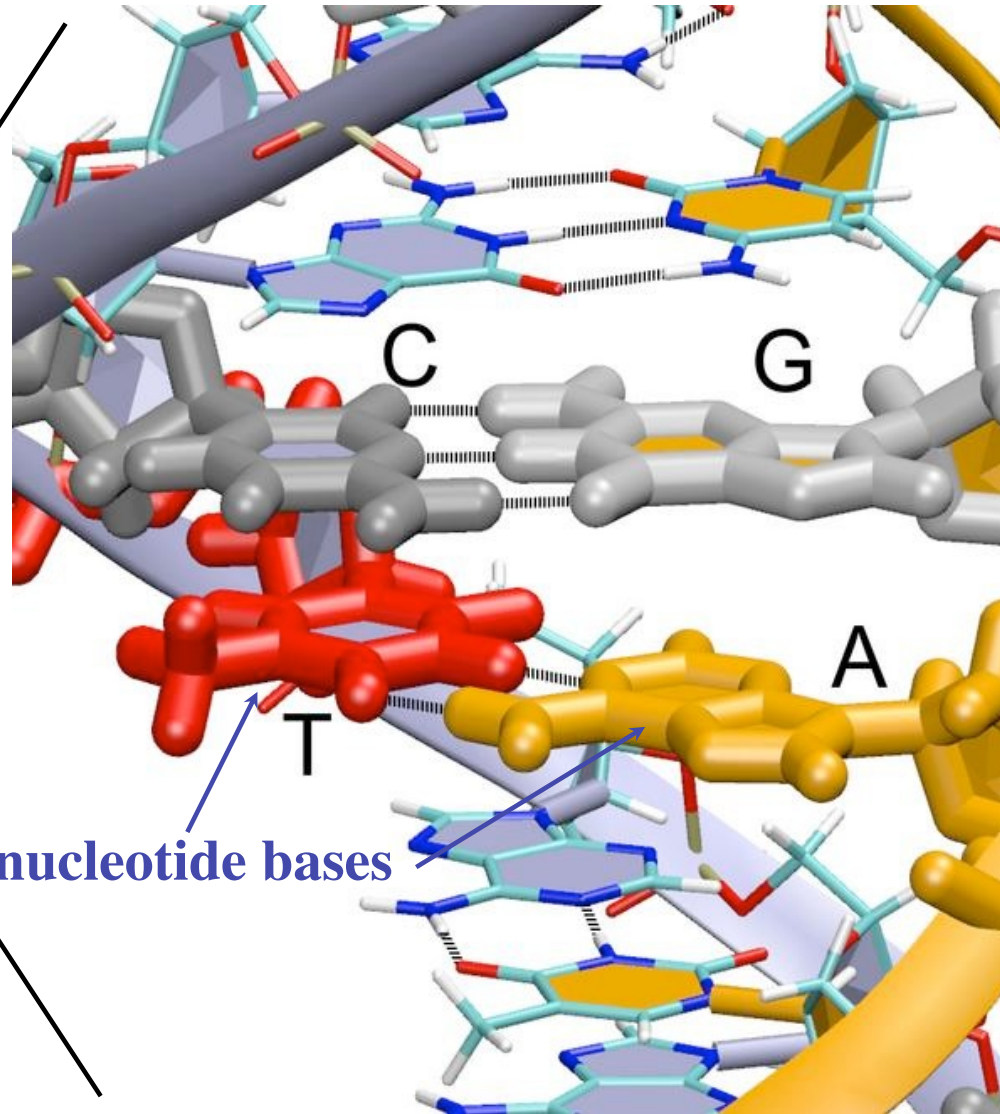


Single stranded DNA

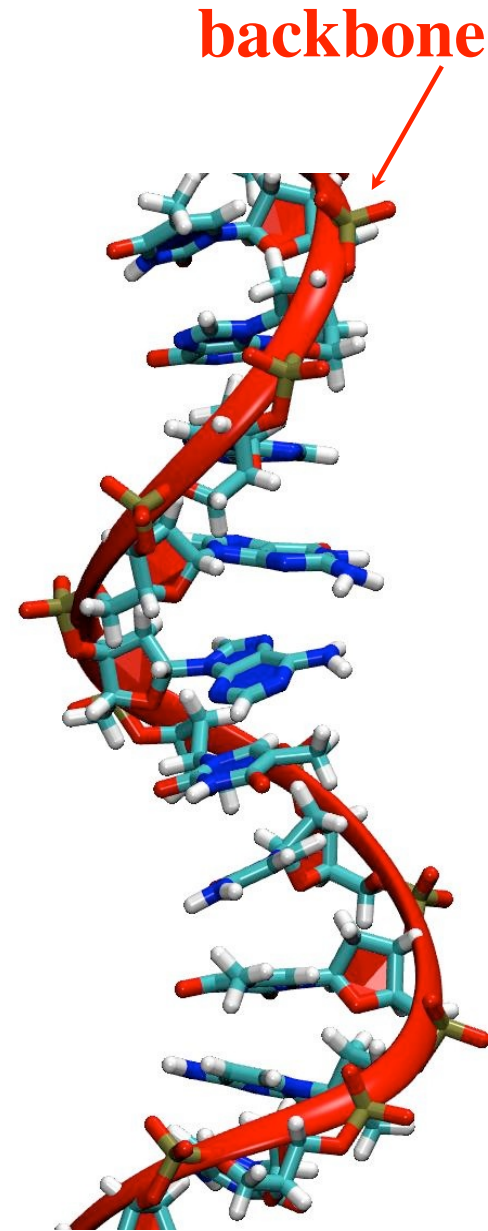
DNA up-close



Double stranded DNA



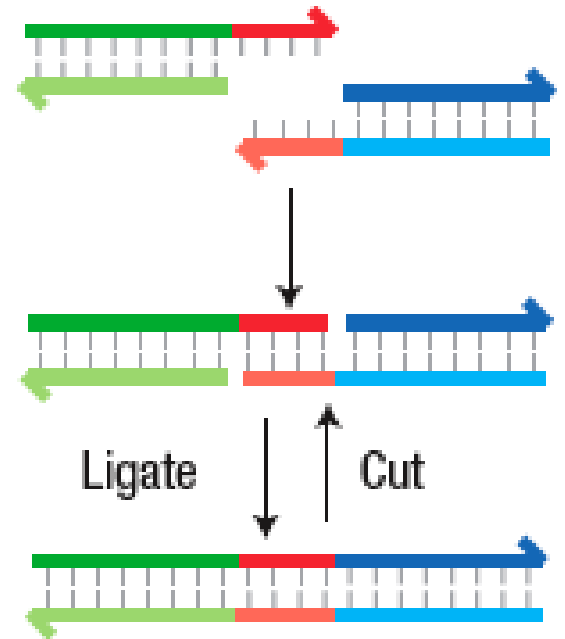
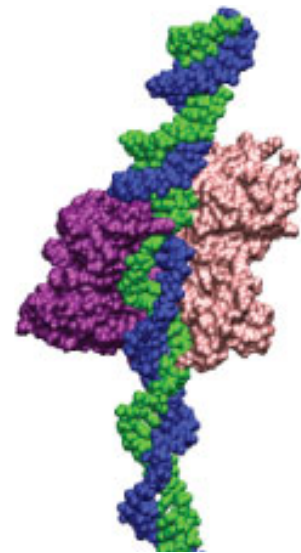
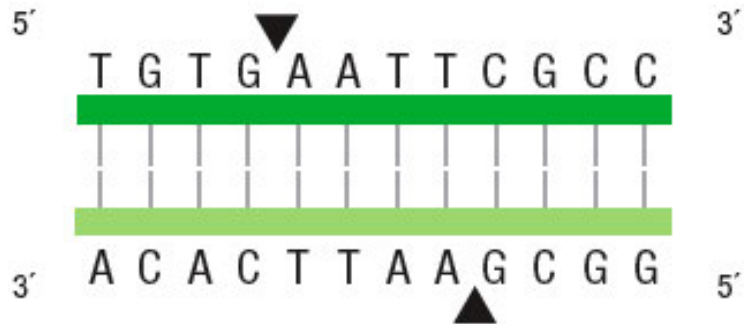
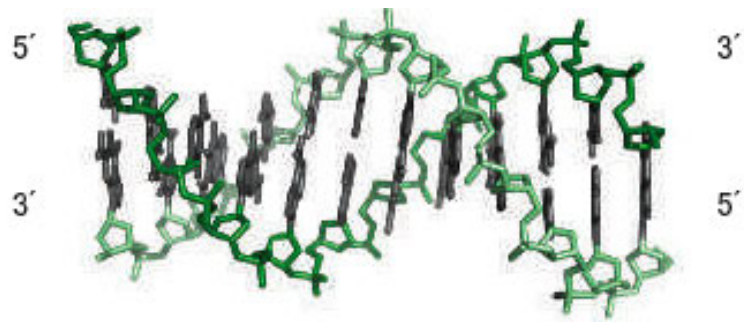
5'-AAGCTGGTTCAG-3'



Single stranded DNA

DNA technology

Use restriction enzyme to cut



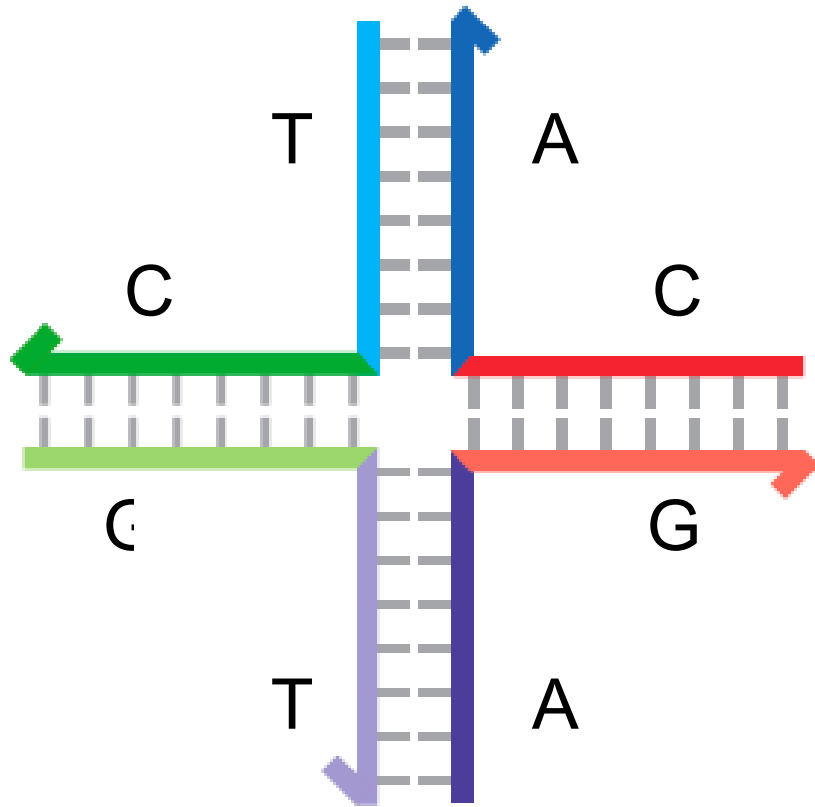
Use ligase to connect

Self-assembly of DNA structures

Recipe:

Mix: $T_n C_n, A_n C_n$

$G_n T_n, A_n G_n$

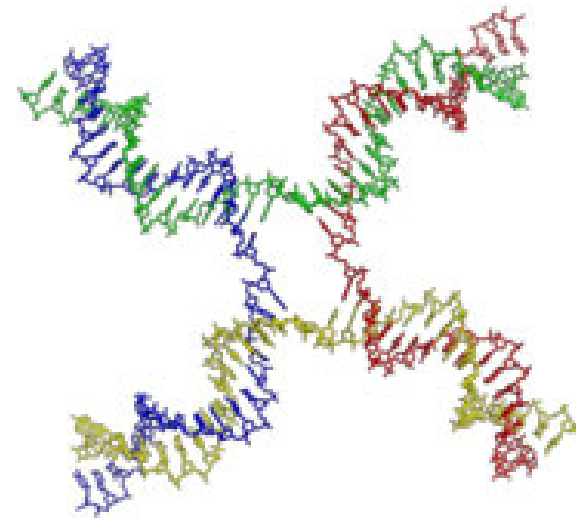


Holliday junction

Persistence length:

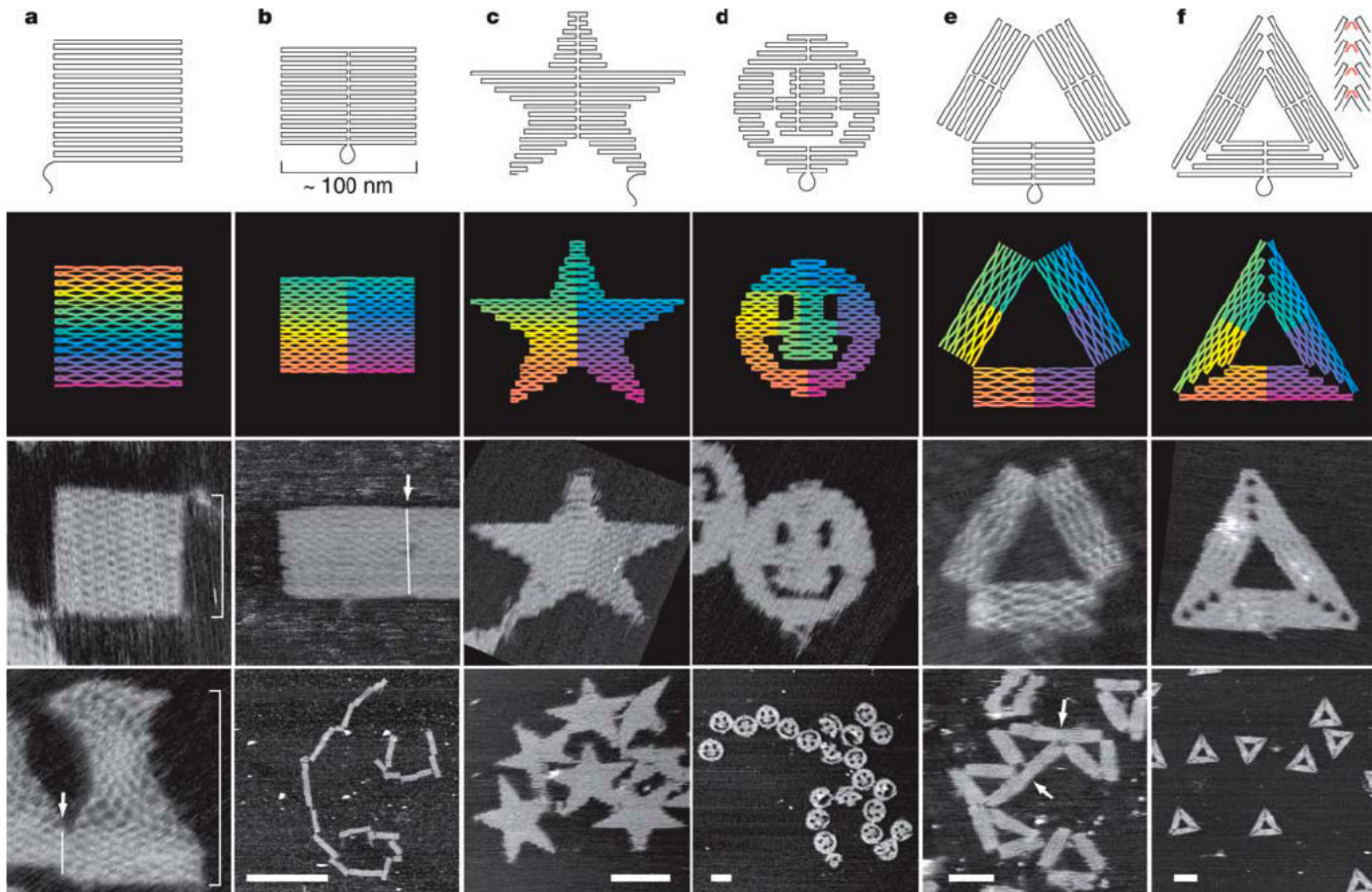
50 nm dsDNA

1.5 nm, ssDNA



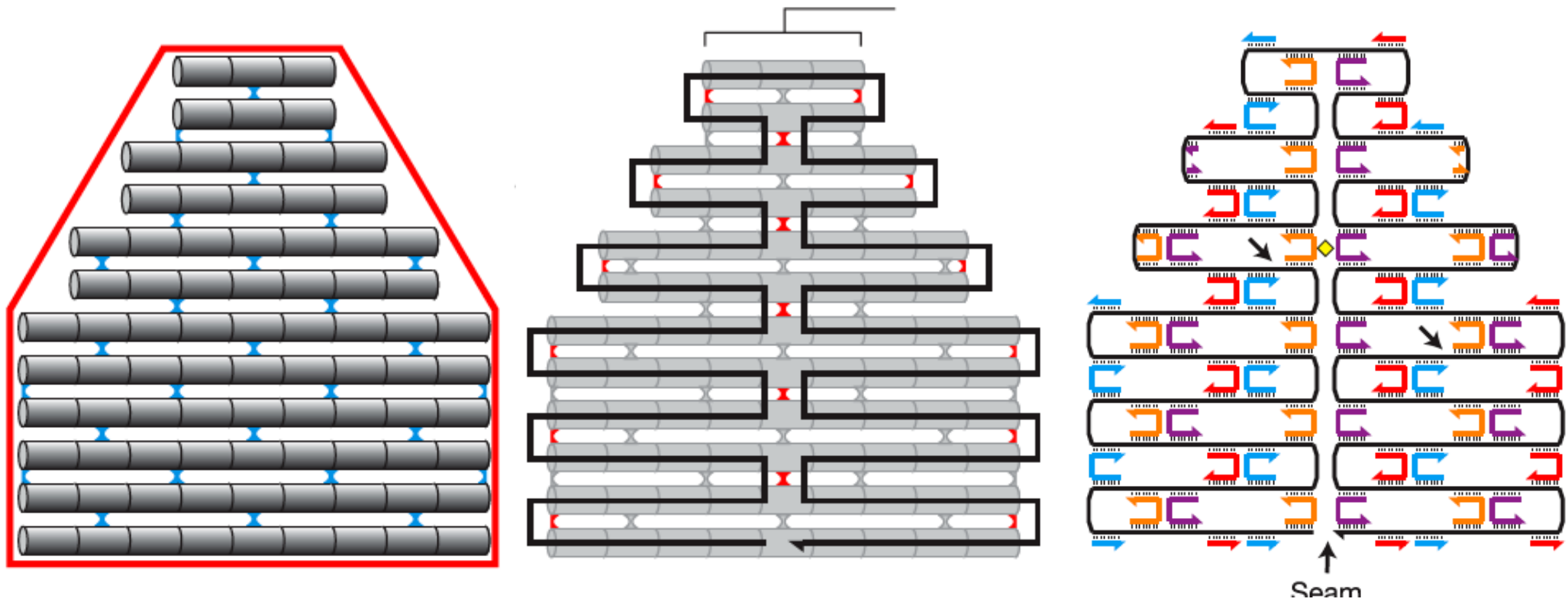
DNA sequence is designed chosen to promote hybridization of complimentary fragments and reduce non-specific association

How far we can take this?



DNA origami

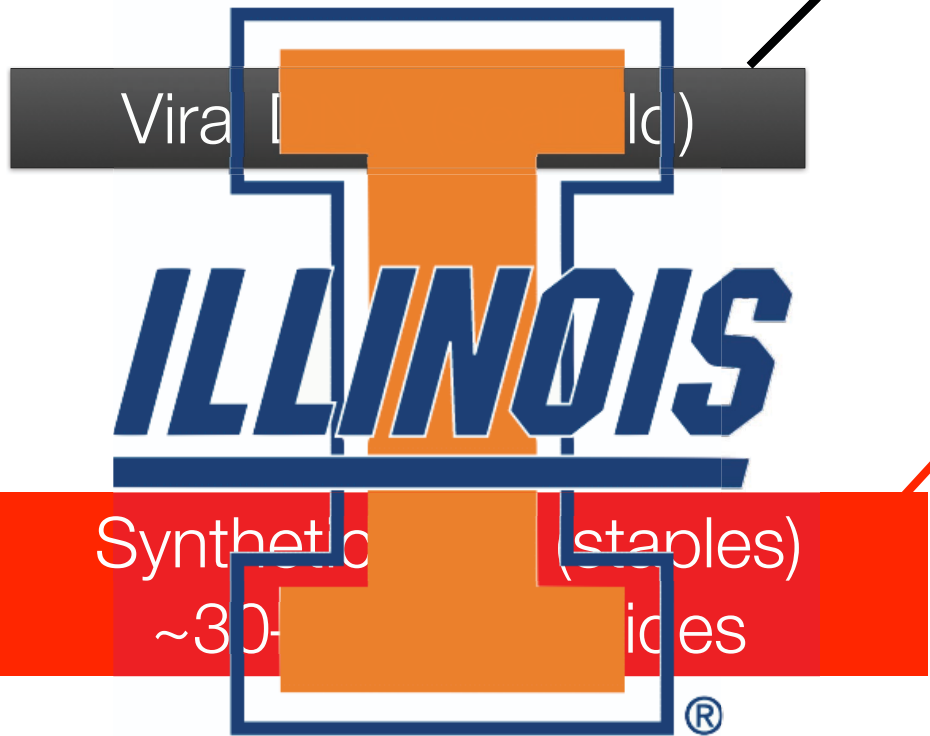
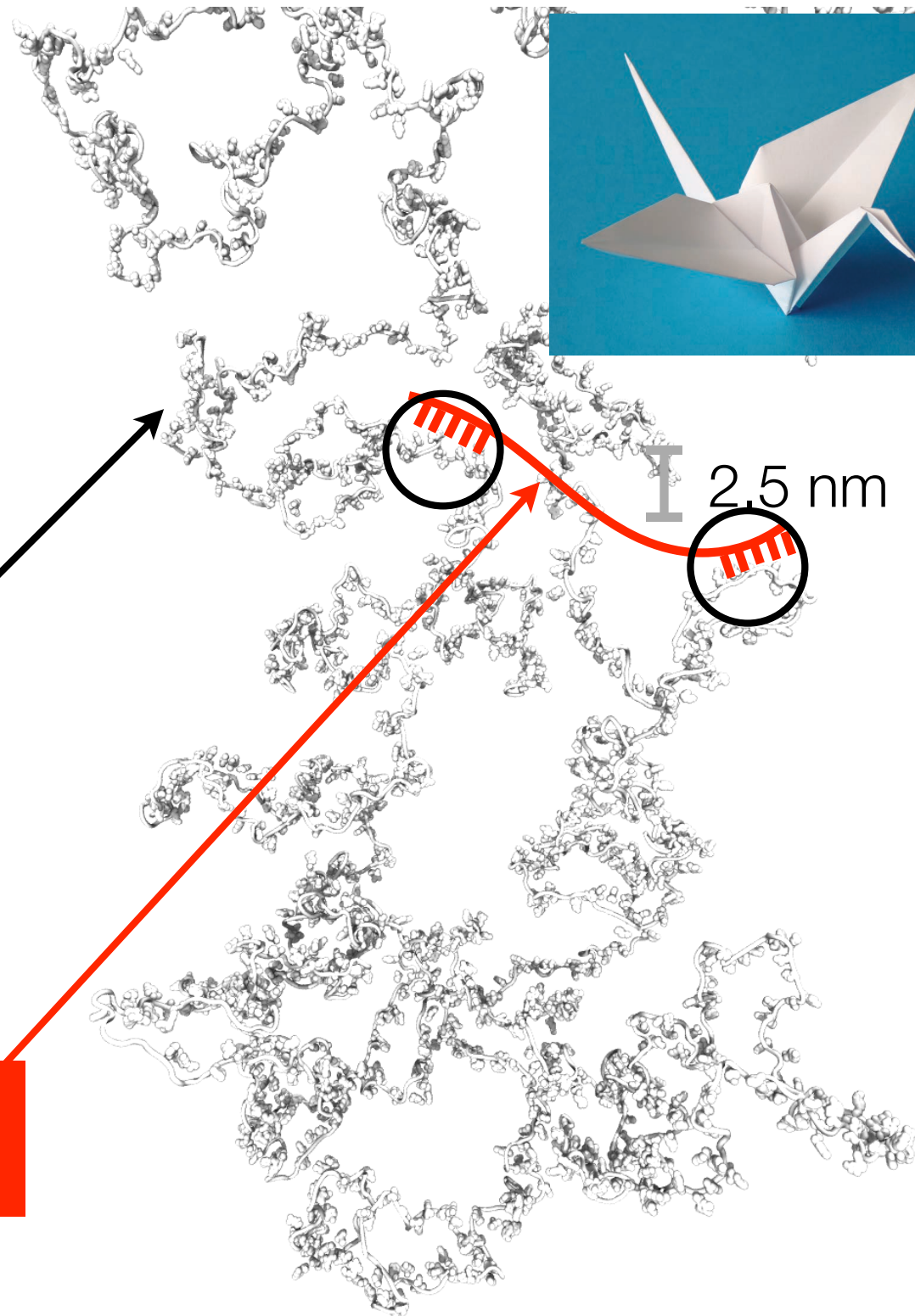
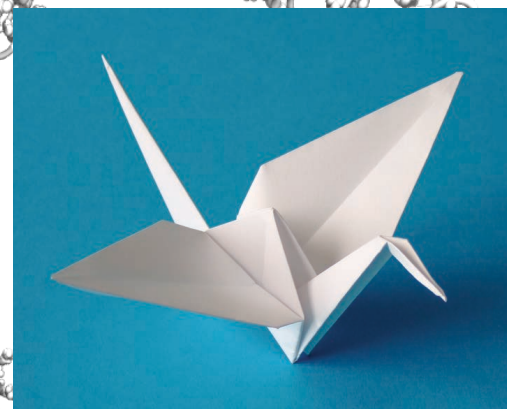
Idea: direct folding of a long single strand of DNA into desired shapes.



Paul Rothemund (2006), NATURE Vol 440:297

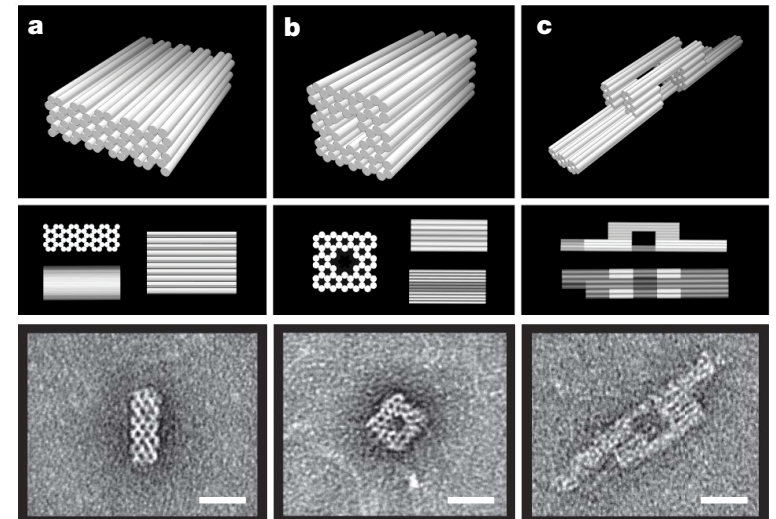
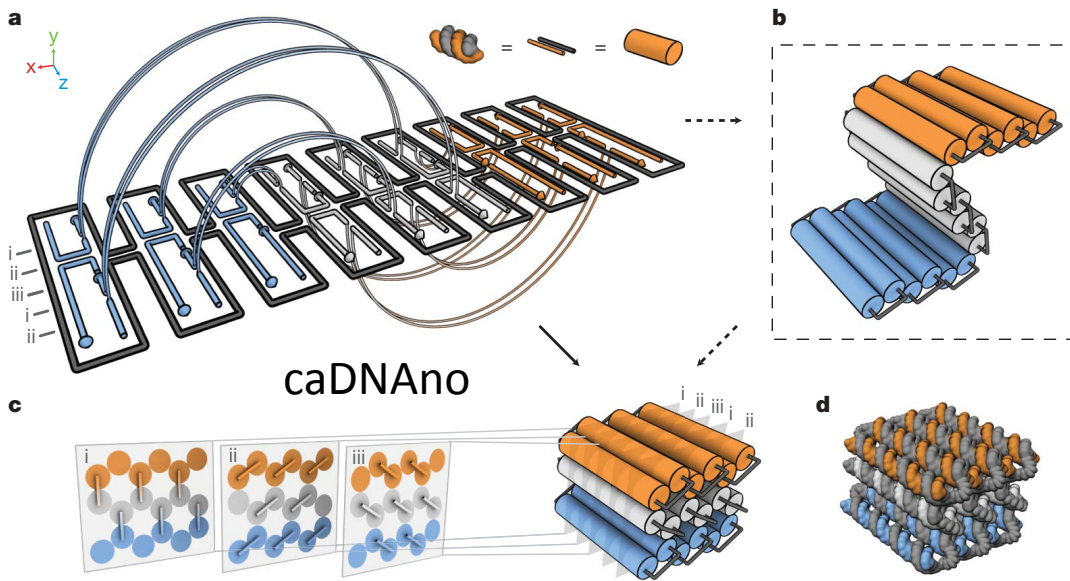
DNA origami

Building a structure with nanoscale precision by **folding** DNA

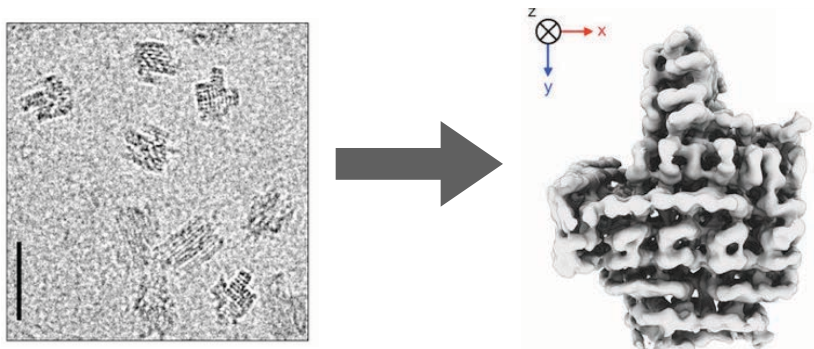


Design and characterization of DNA nanostructures

Computer-aided design of DNA origami with caDNAno (Shih group, Harvard U.)

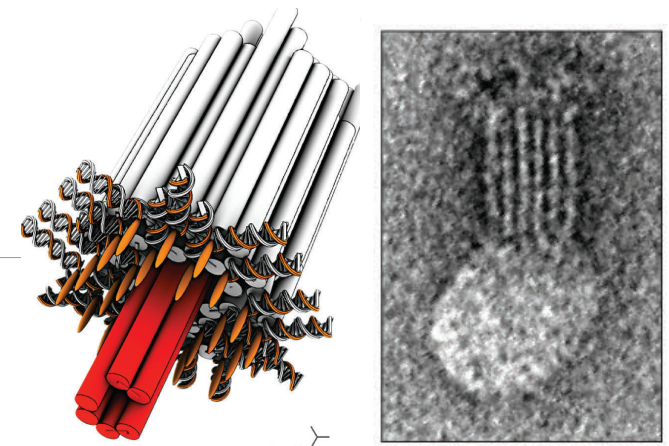


Transmission electron microscopy and/or atomic force microscopy validates the design

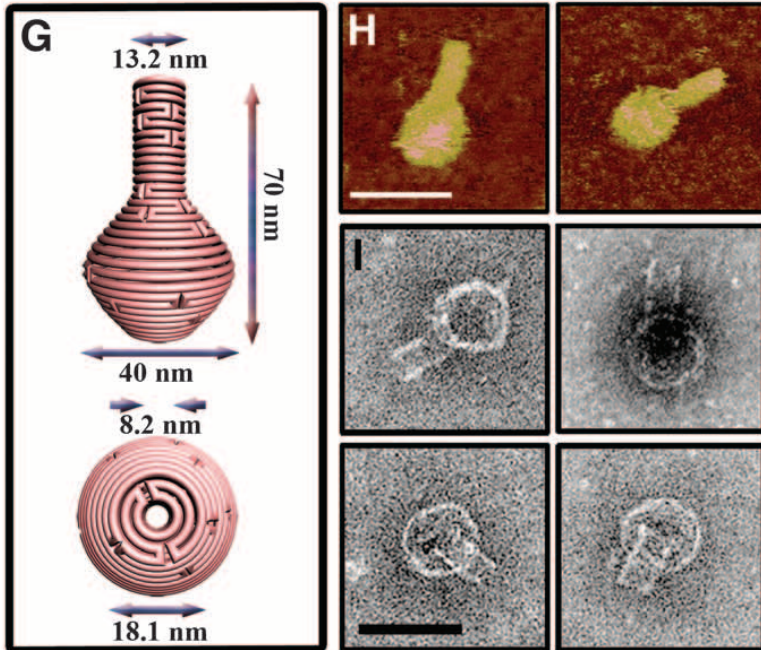


Cryo-EM reconstruction, the only experimentally derived structural model

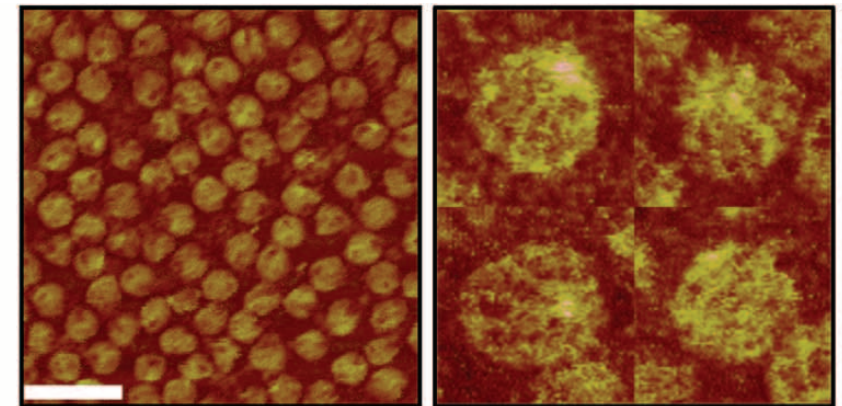
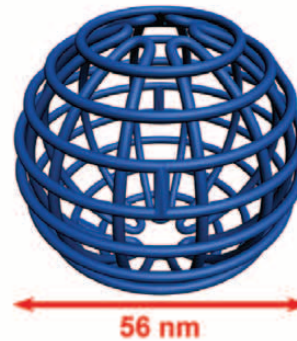
DNA origami structures



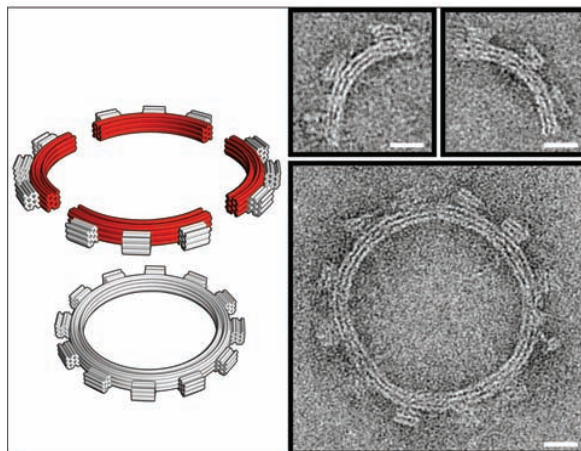
Dietz and coworkers, Science (2012)



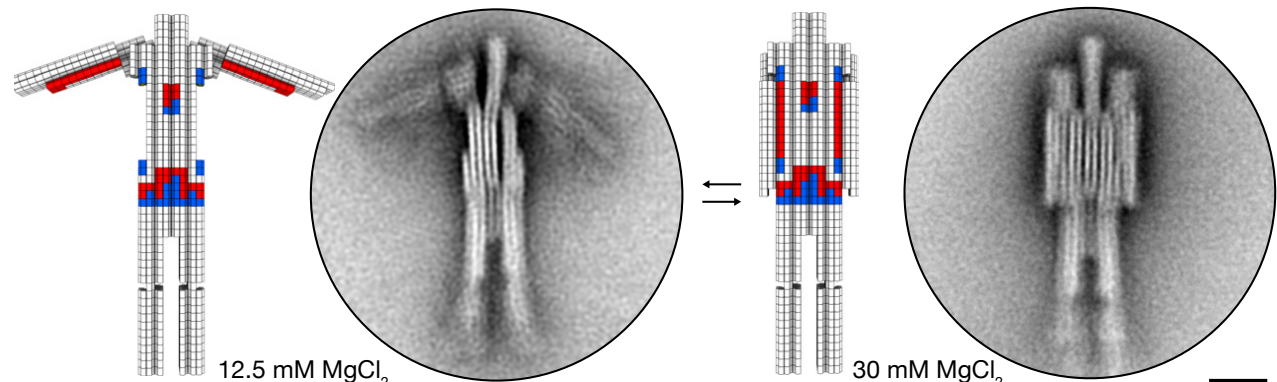
Yan and coworkers, Science (2011)



Yan and coworkers, Science (2013)



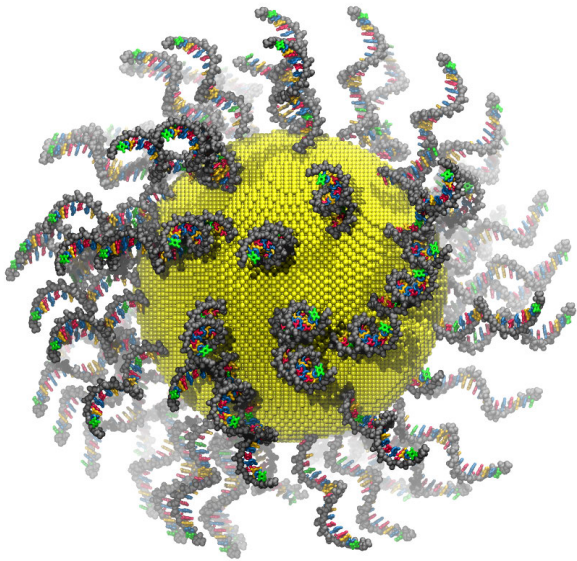
Shih and coworkers, Science (2009)



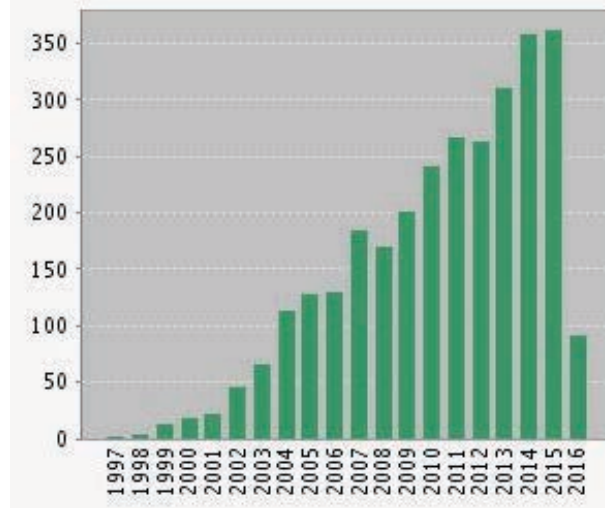
Dietz and coworkers, Science (2015)

DNA nanotechnology

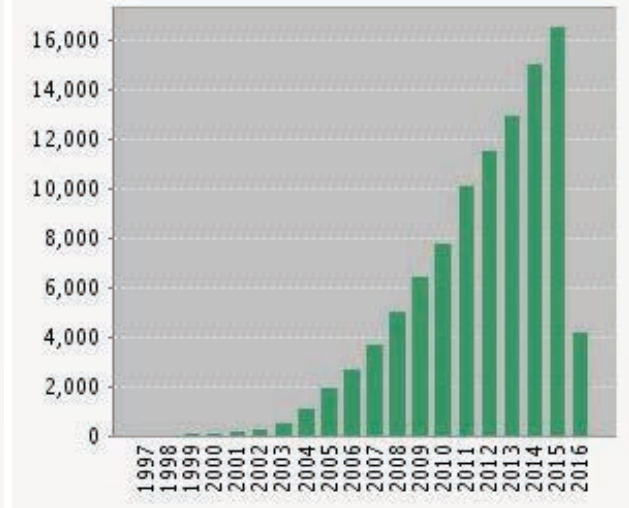
DNA-functionalized nanoparticle:



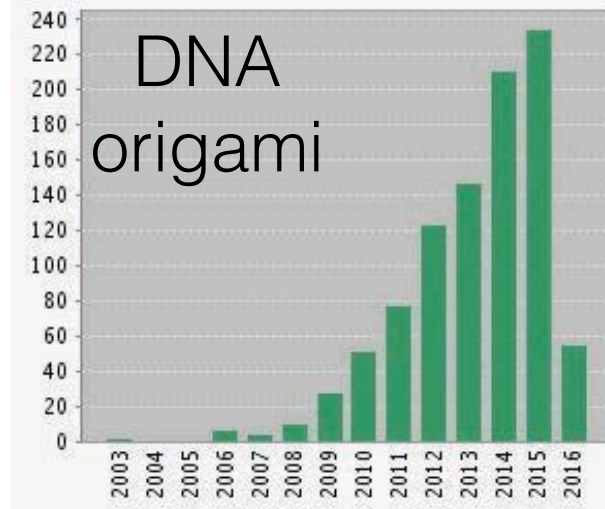
All-atom model build using
Functionalization Workbench



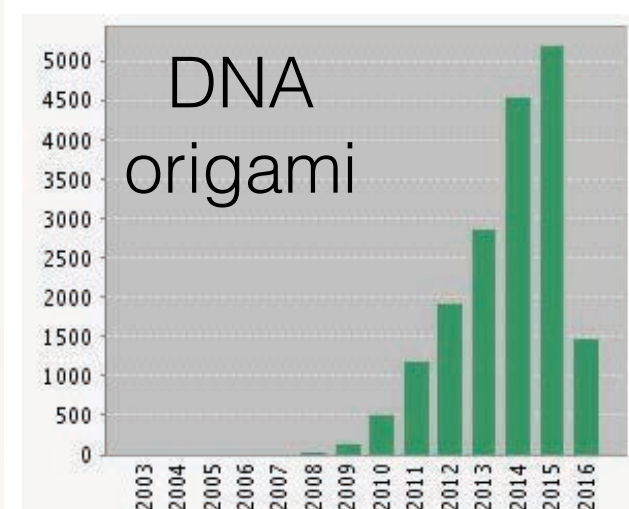
Number of papers published



Number of citations



DNA origami

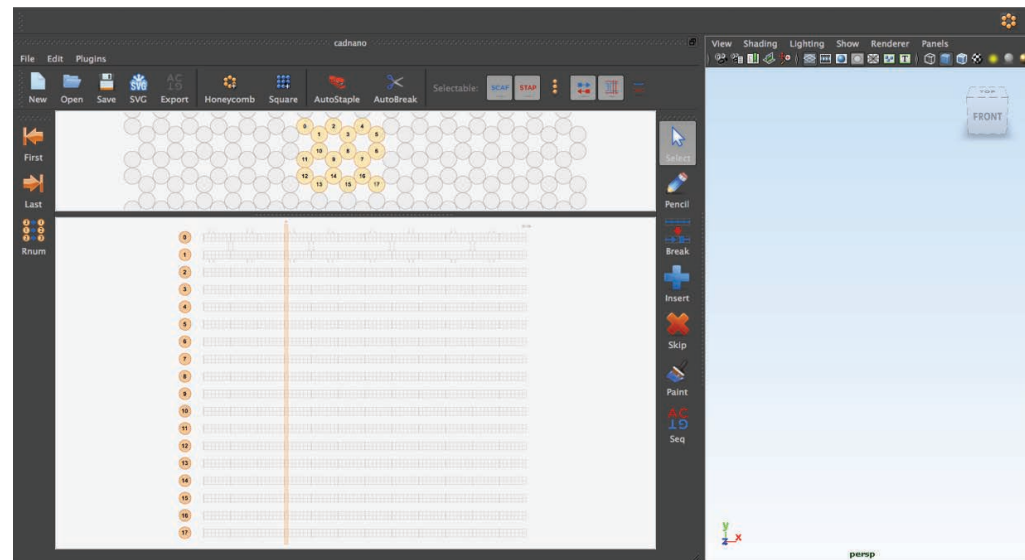
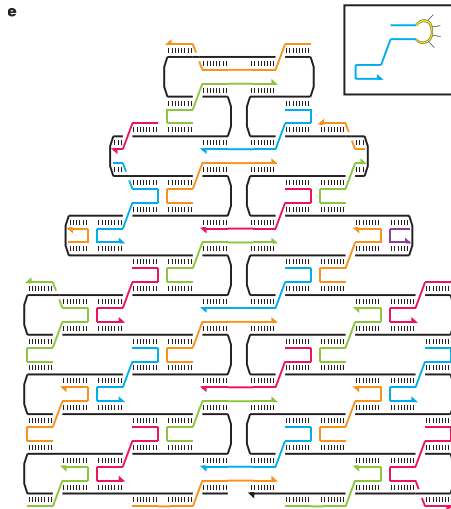


DNA origami

DNA nanotechnology is a rapidly expanding field

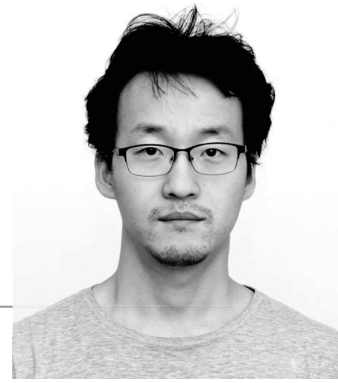
caDNAno

- Computer-Aided Design of DNA origami made by Shih group at Harvard.
- Designed as a plugin of Autodesk MAYA.
- Limited to a design of antiparallel DNA helices in a honeycomb or square lattice.

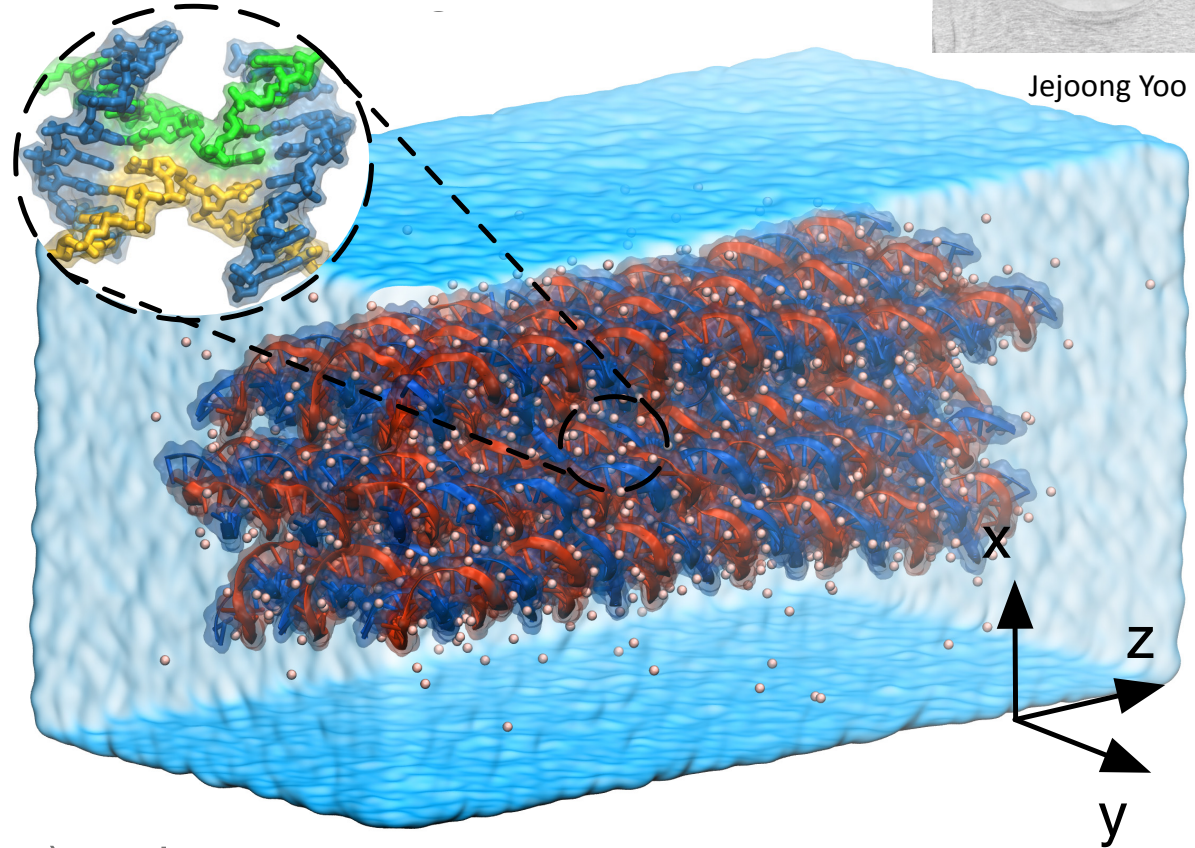
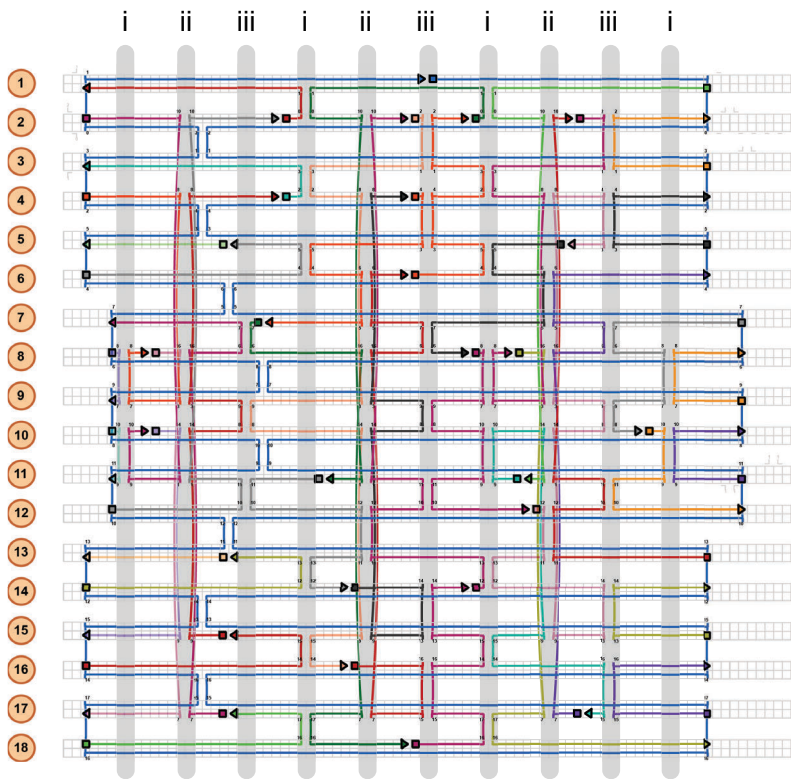


Douglas, S.M., Marblestone, A.H., Teerapittayanon, S., Vazquez, A., Church, G.M. & Shih, W.M., 2009, Rapid prototyping of 3D DNA-origami shapes with caDNAno, *Nucleic acids research*, 37(15), pp. 5001-6.

From caDNAno to all-atom



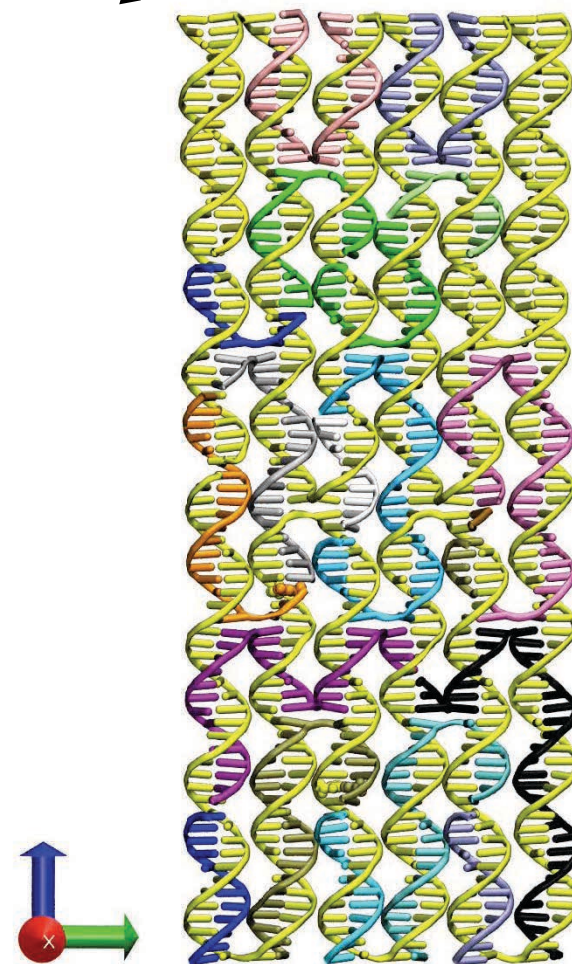
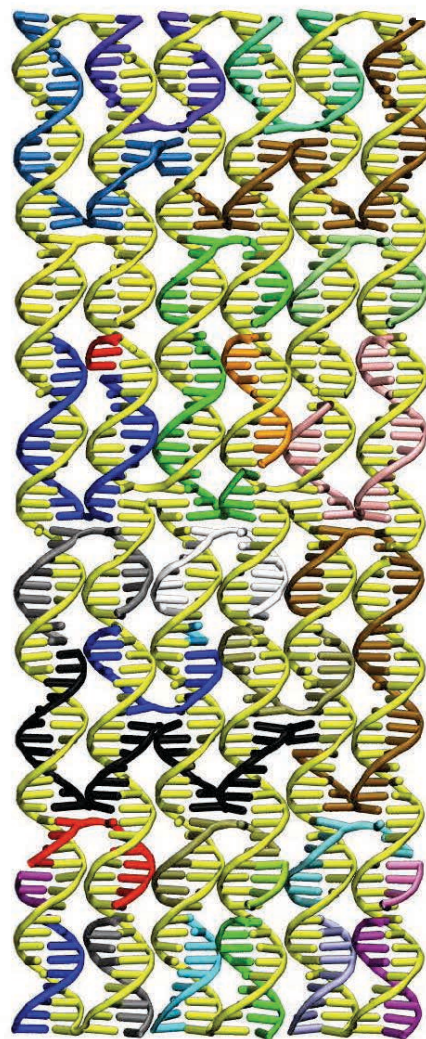
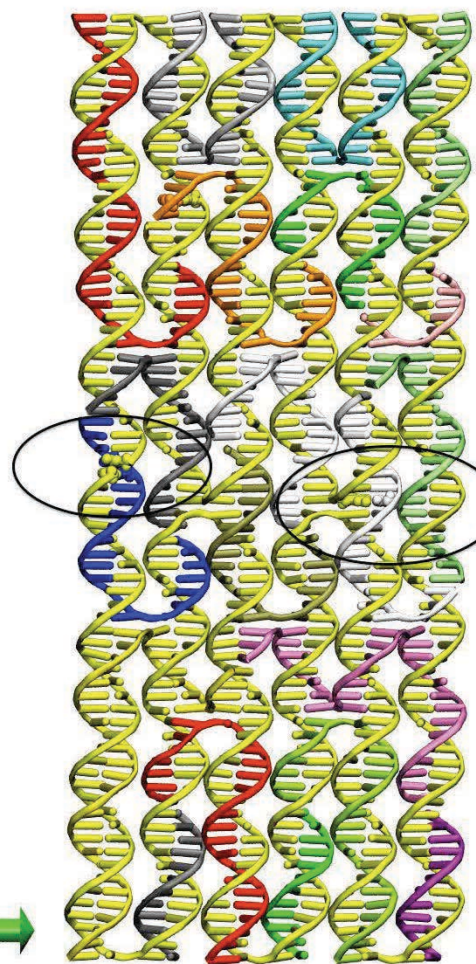
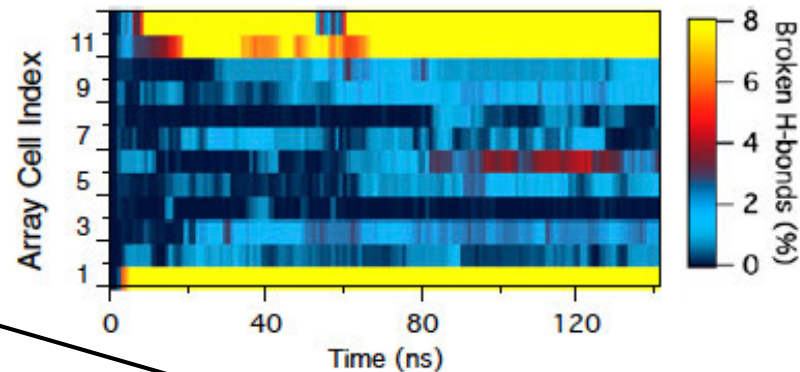
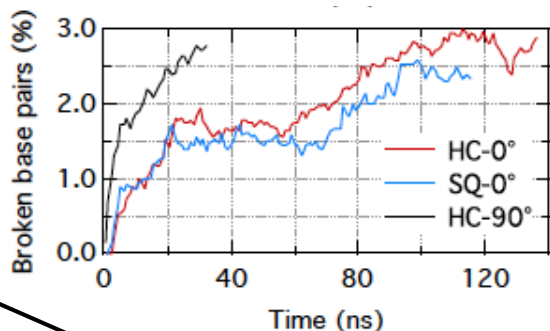
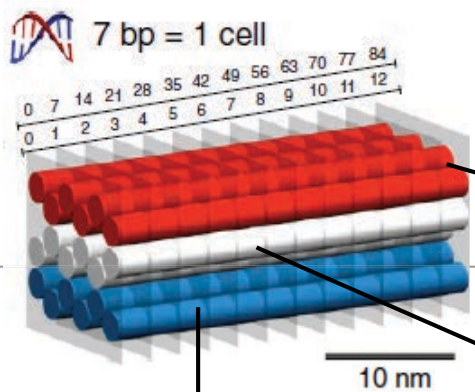
Jejoong Yoo



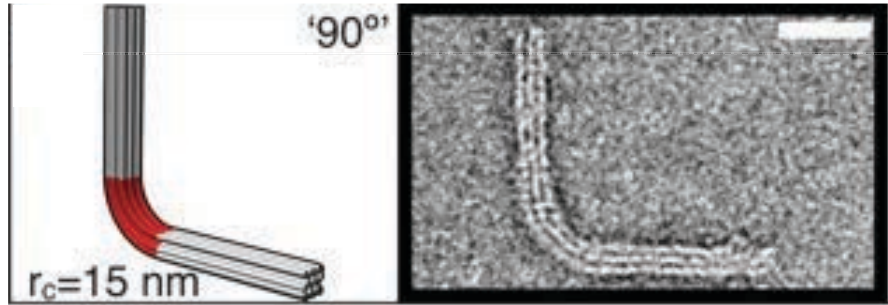
- caDNAno returns topology (json) and sequence (csv) information.
- **cadnano2pdb.pl** combines json and csv files into a PDB file.

- * CHARMM36 force field
- * Explicit water
- * [MgCl₂] ~ 10 mM
- * NAMD
- * 1 to 3M atoms
- * 500 to 1,000 CPUs

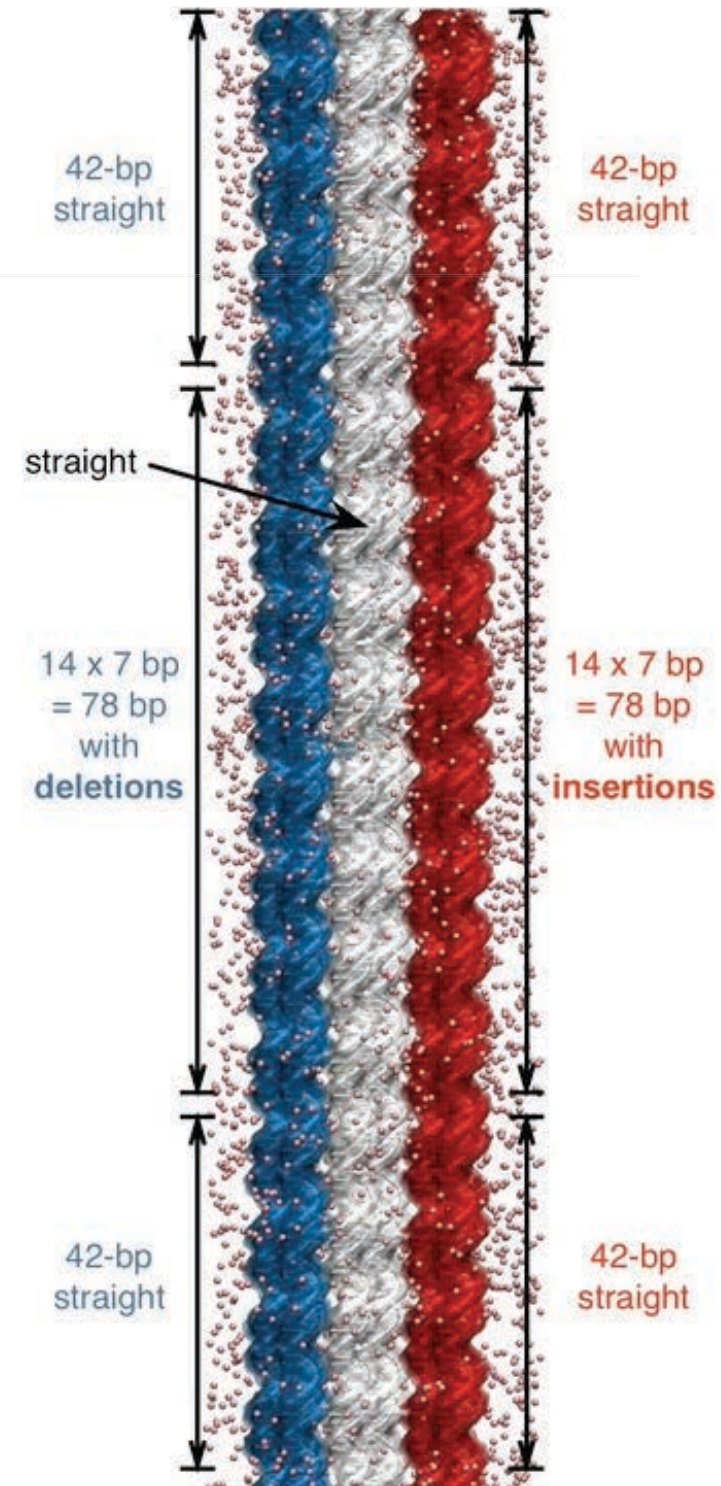
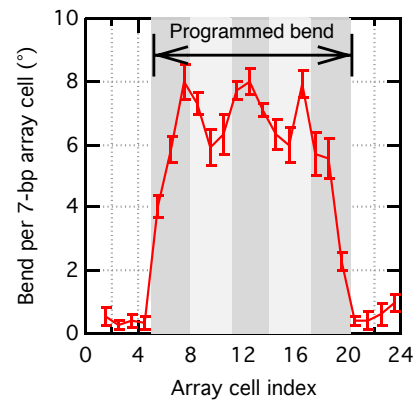
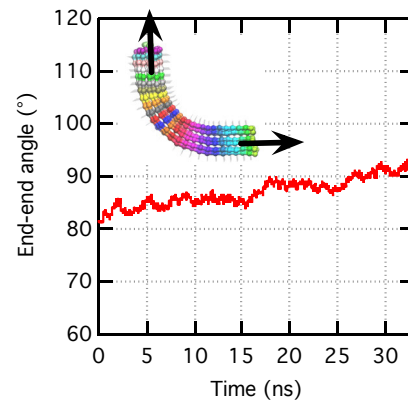
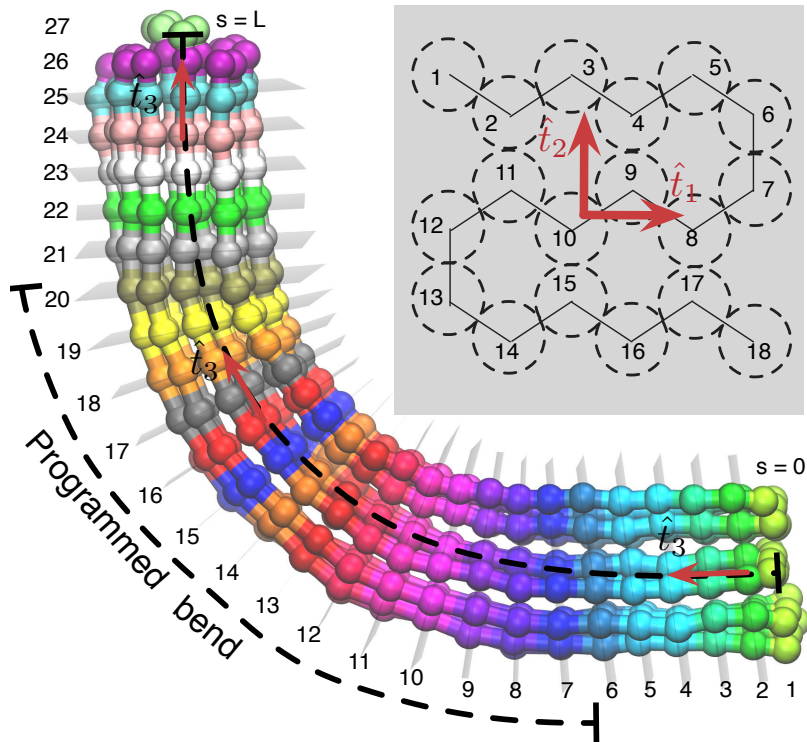
Structural dynamics



All-atom MD simulation of L-shape DNA origami



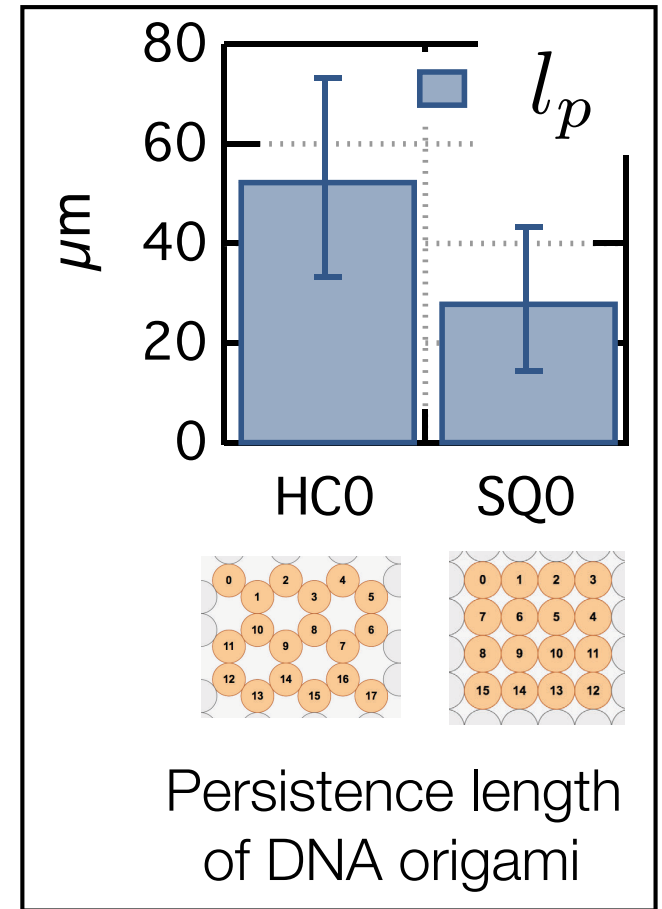
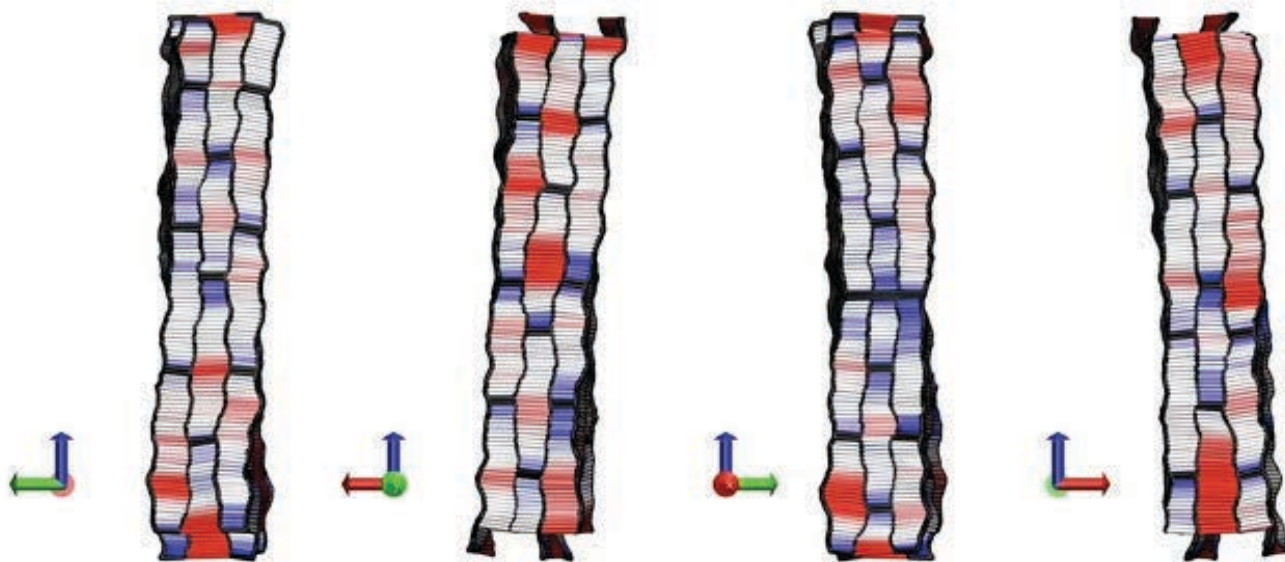
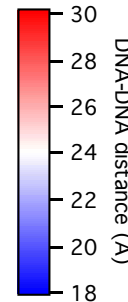
Experiment
Dietz, H. et al,
Science, 325



Structural fluctuations reveal local mechanical properties

MD trajectories allow us to compute natural bending and torsion as well as persistence length

- Inter-DNA distance in color map
- Chicken wire frame represents center line of helices & junction

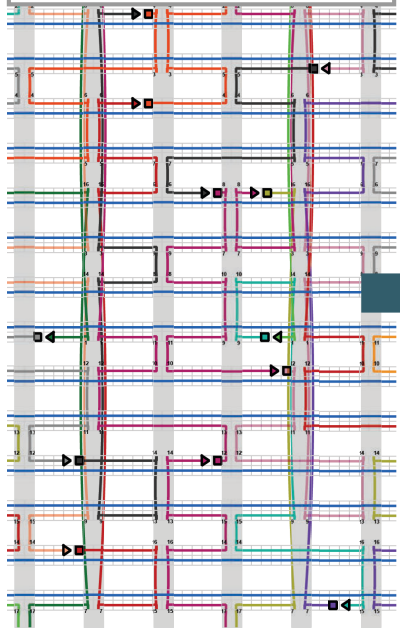


Our simulations predict higher rigidity for honeycomb-lattice design.

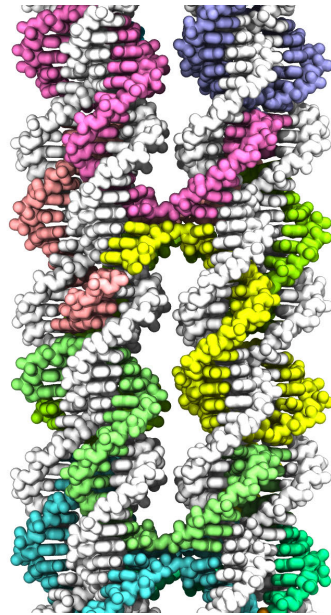
DNA structure converters

Structure web server (cadnano2pdb)
<https://nanohub.org/resources/cadnanocvrt>

Cadnano design



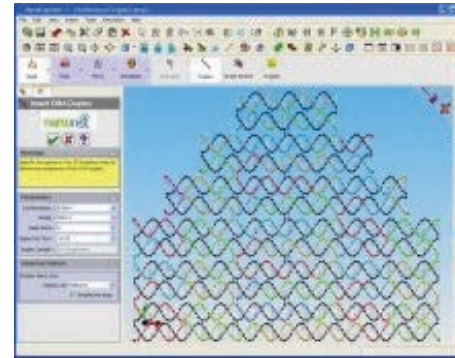
All-atom structure



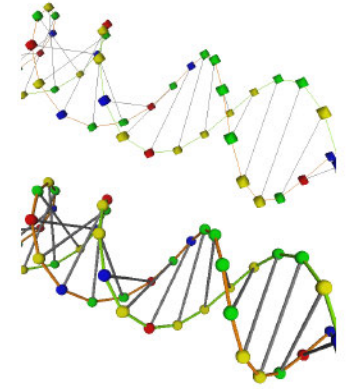
The web server provides all-atom structure with optimized conformations.

**131 unique user / 1,522 simulation runs
(since 2014)**

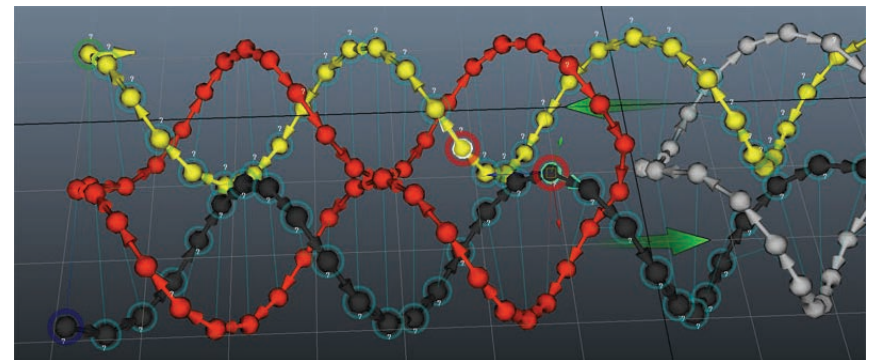
Further developments requested by experimentalists:



Nanoengineer
(done)

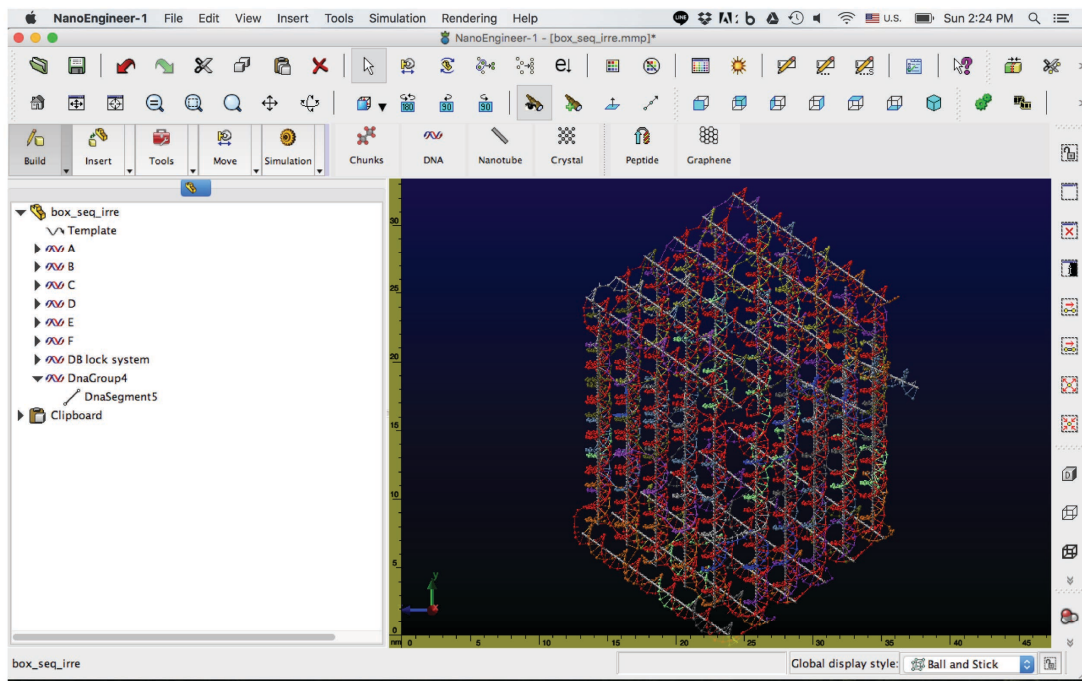


Tiamat
(in progress)



vHelix (planned)

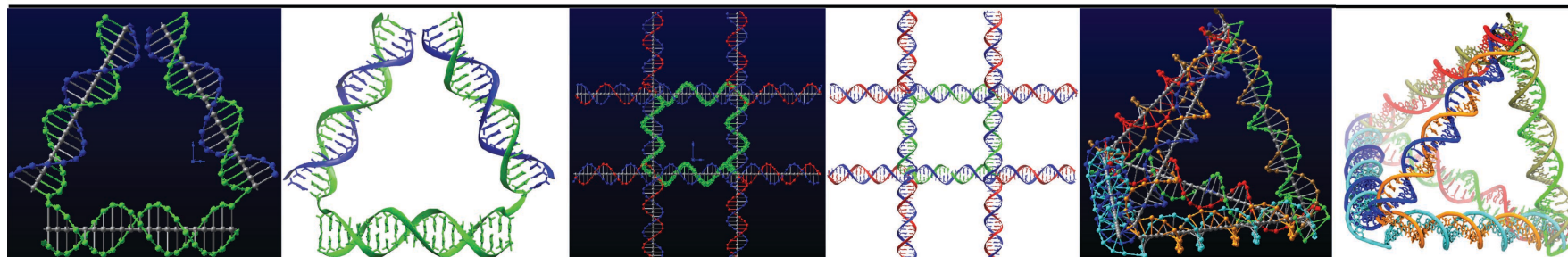
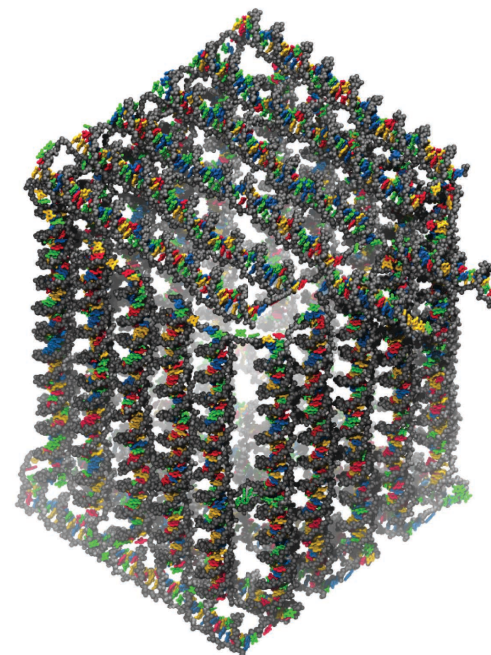
NanoEngineer-1



All-atom PDB

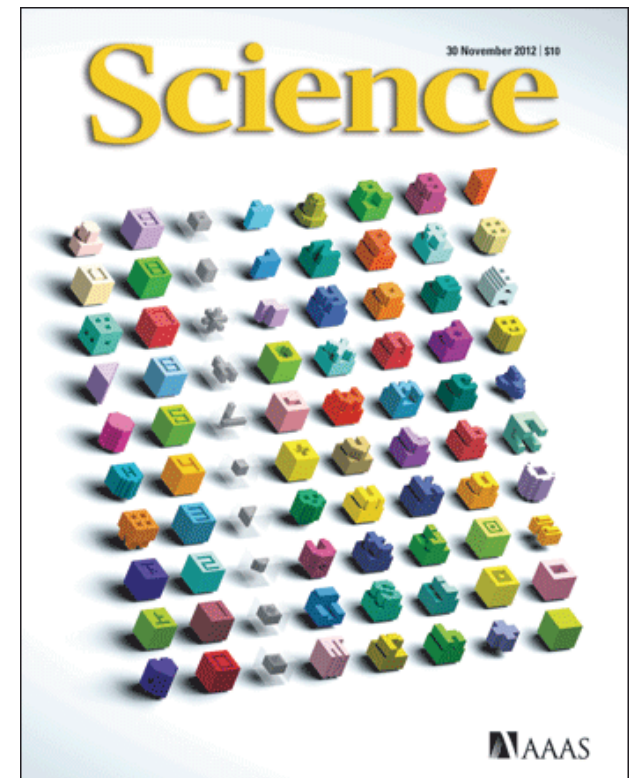
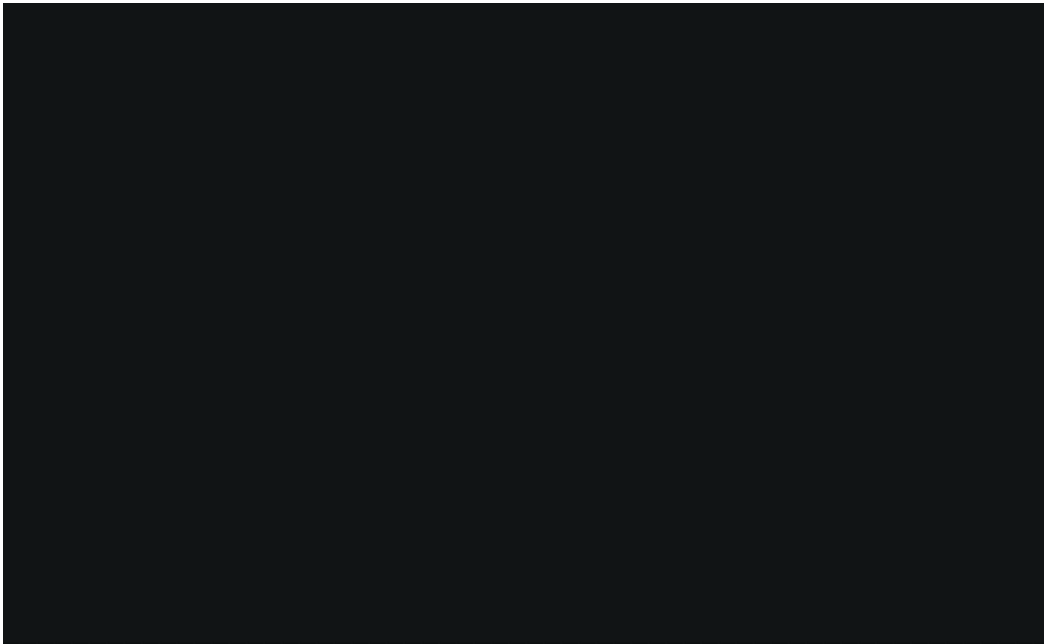
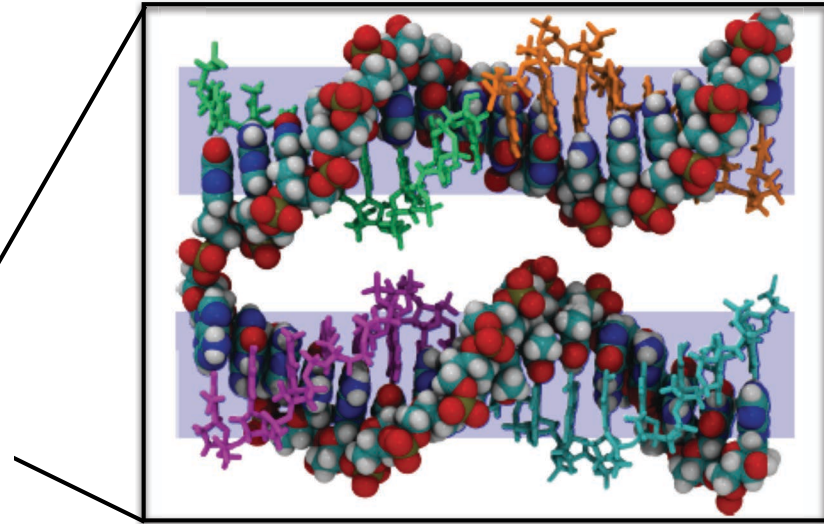
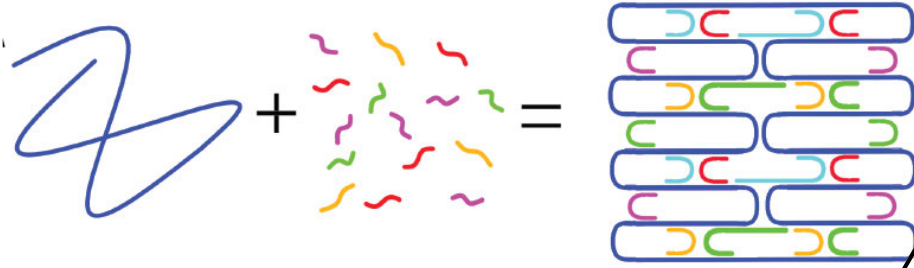


Chen-Yu Li

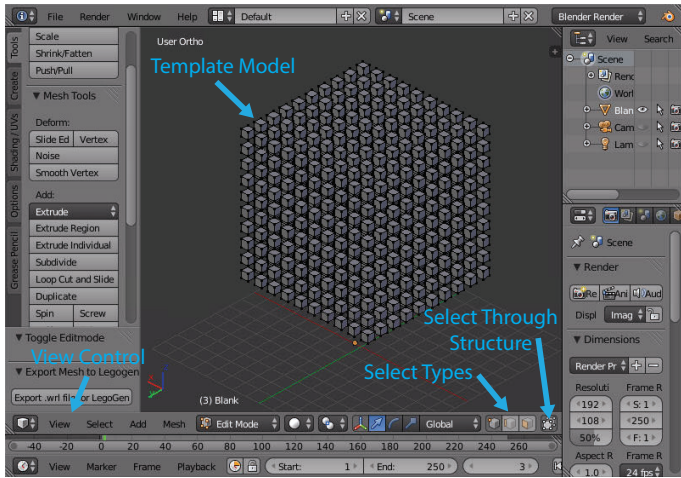


Tiled DNA nanostructures

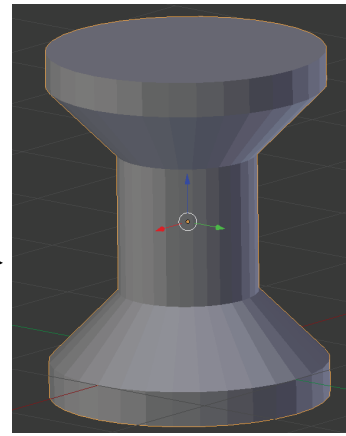
DNA
Origami



LegoGen workflow @ nanoHub.org

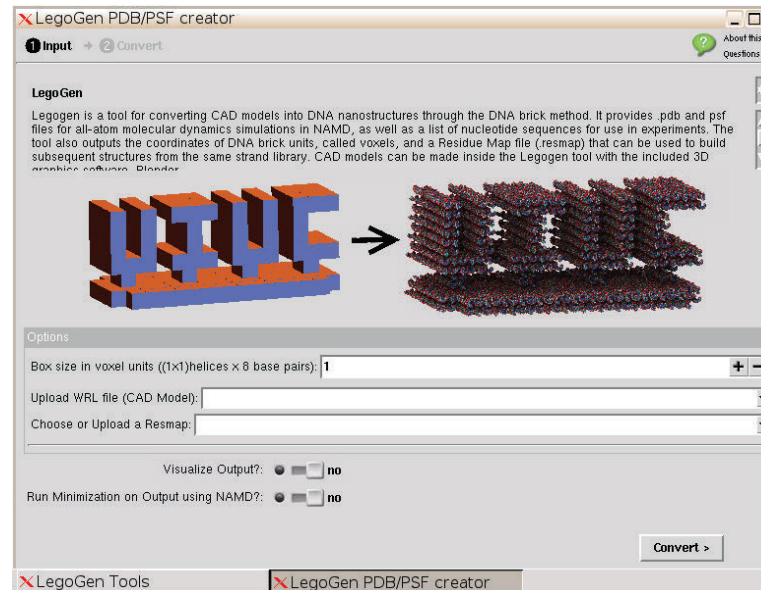


Blender (@nanoHub)



Carved shape

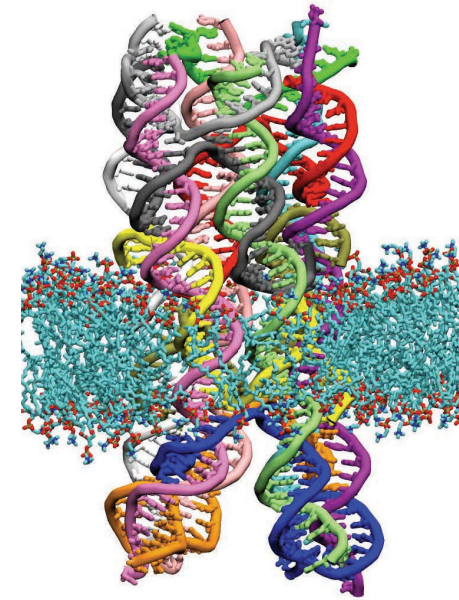
Converter



```

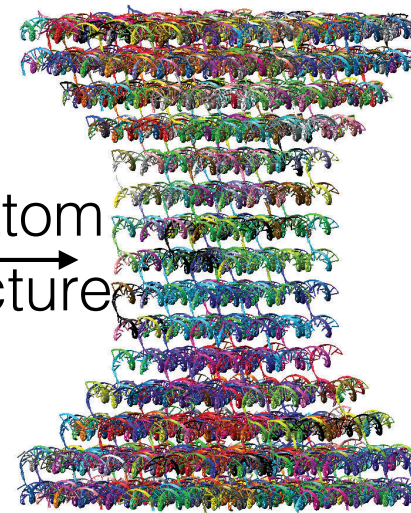
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2 AATGGGCGAAAAACCGTCTGGACTCCAGTT
3 ATCGTCATAAATATTCGGTGCCAGCAGGGTGGAGG
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5 TCAAAAATCATGAGTGAGATTAGCAAACGCCAGGGCT
6 TATCGCGTTTTTTCGGGAGAGAGTTACATTTTCGACG
7 GCGGATCCCTGACTATTATAGT
8 AGTAAGCAAACCTGGGCGCCTGCATTACTGTGTGA
9 TTTAAGCCCCAGCCAAAAGAACAGCCAGCTTTC
10 AATTGCTGAAAGAGGAAGGGCAAAGACTAAC
11 GAGTGTGTGCCAGTTTGAATTATAAATAGGCG
12 TTAGTAGGTTTGATAAGAGGTCATTTTAATTCTGA
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14 GCAACTAATGAAAAGGTGGCA
15 CCGGAAACCAG
    
```

Experimental realization

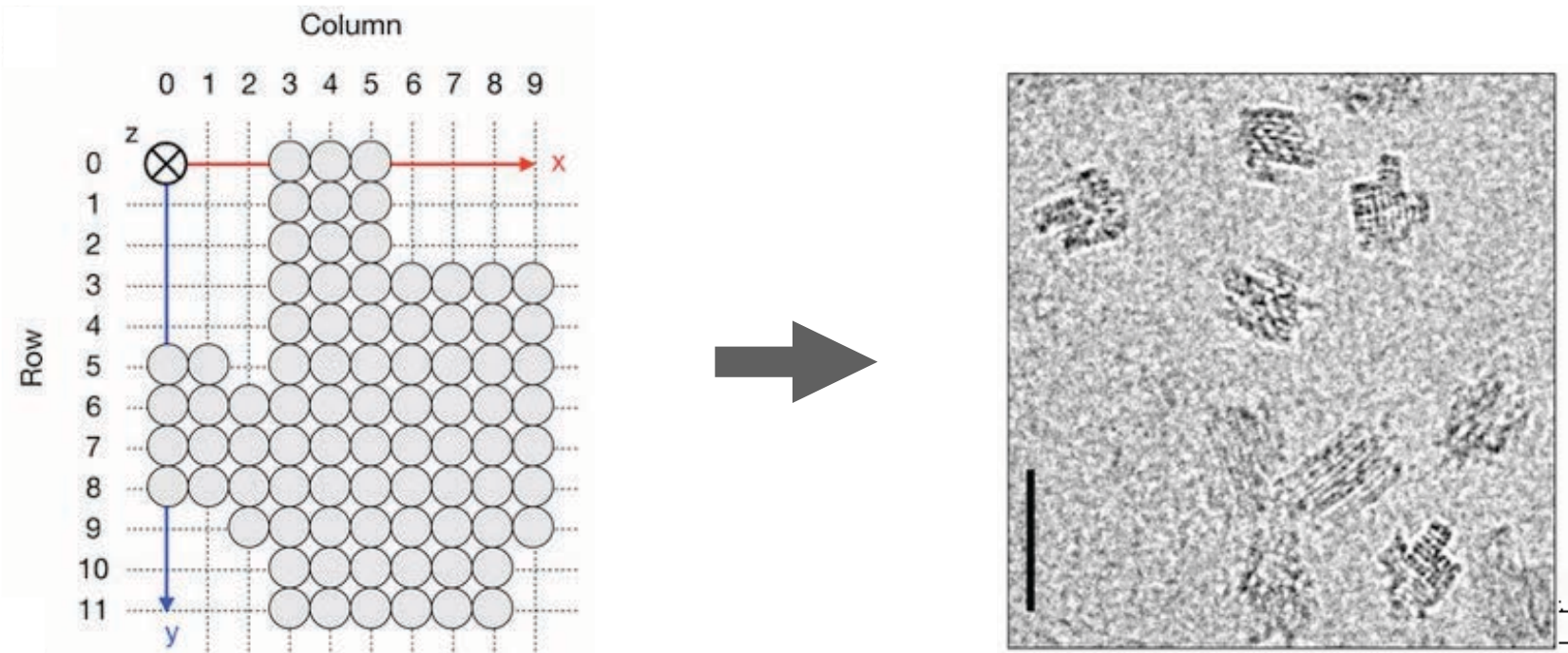


MD simulation

All-atom structure

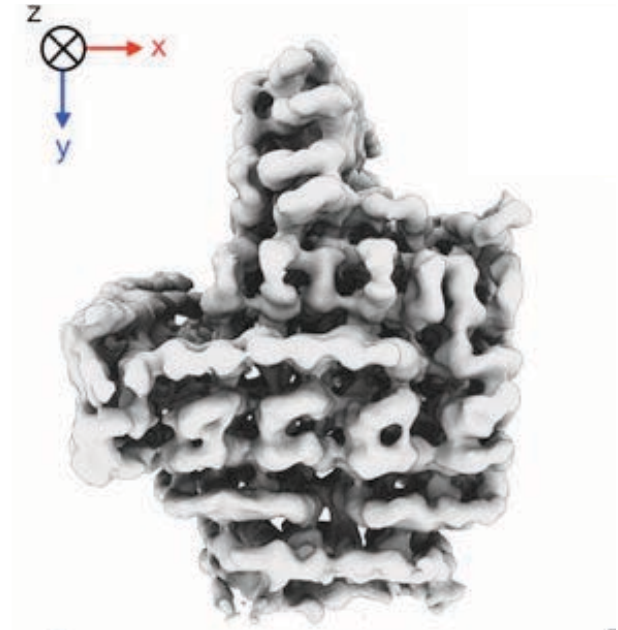
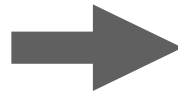
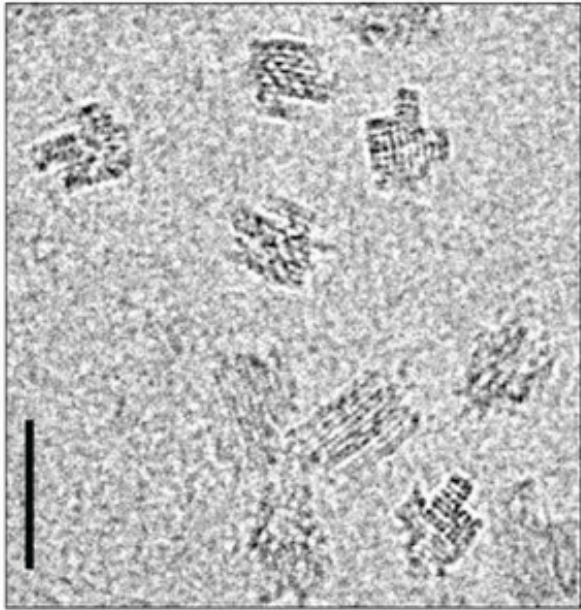


Cryo-EM reconstruction versus all-atom simulation



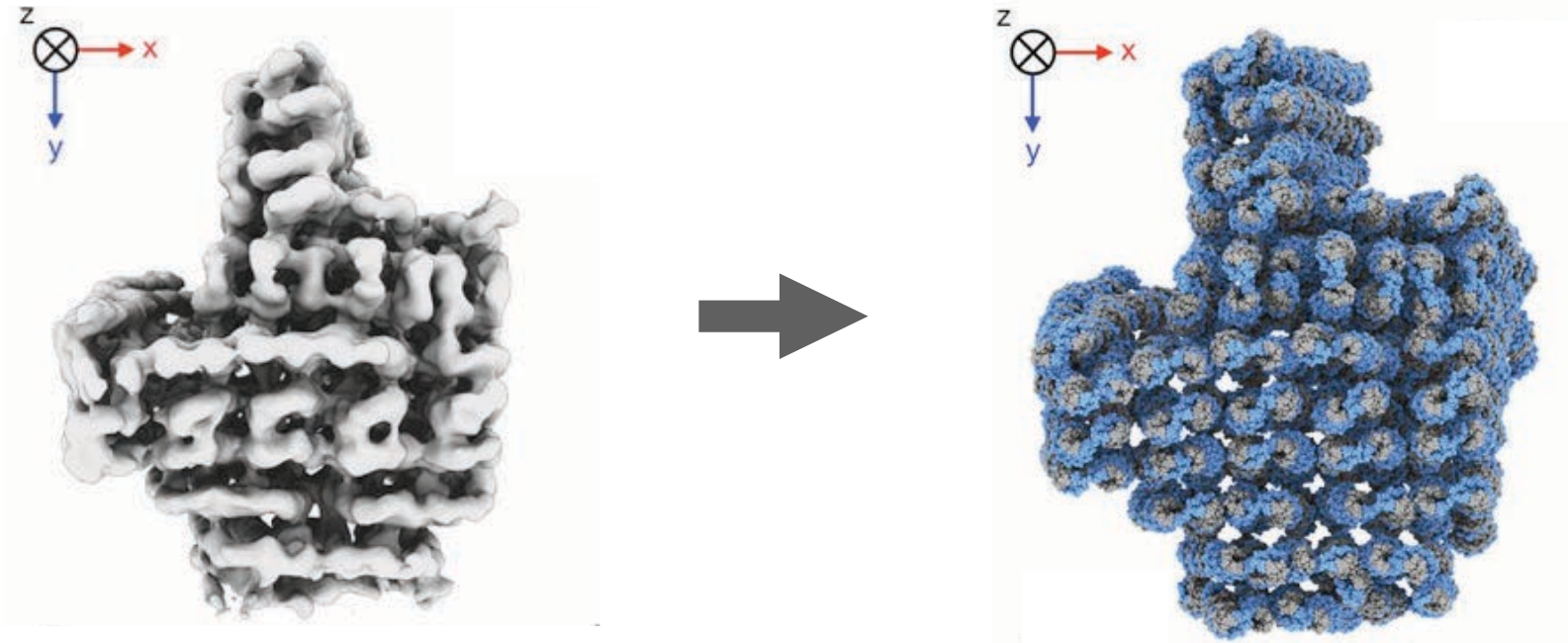
Bai *et al*, PNAS 109:20012 (2012)

Cryo-EM reconstruction versus all-atom simulation



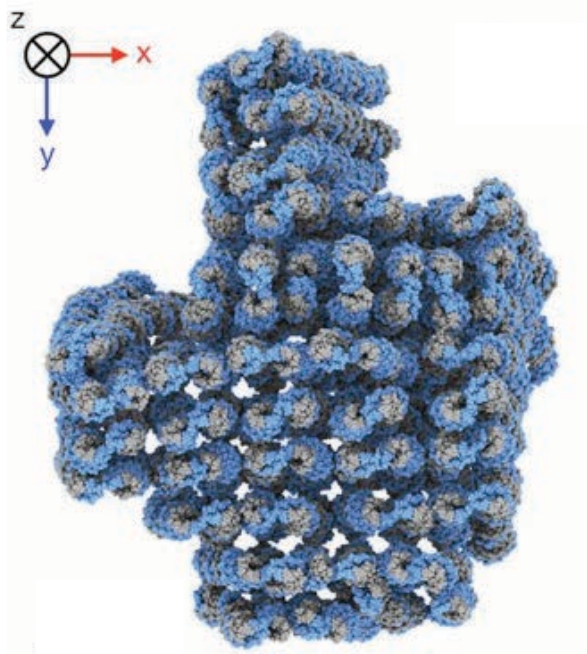
Bai *et al*, PNAS 109:20012 (2012)

Cryo-EM reconstruction versus all-atom simulation

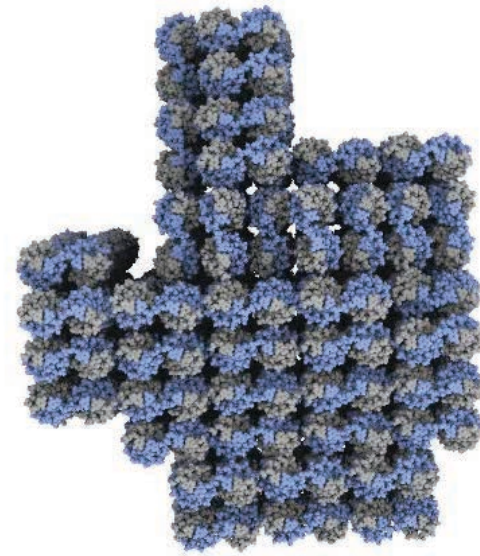


Bai *et al*, PNAS 109:20012 (2012)

MD simulation of the cryo-EM object starting from a caDNAano design

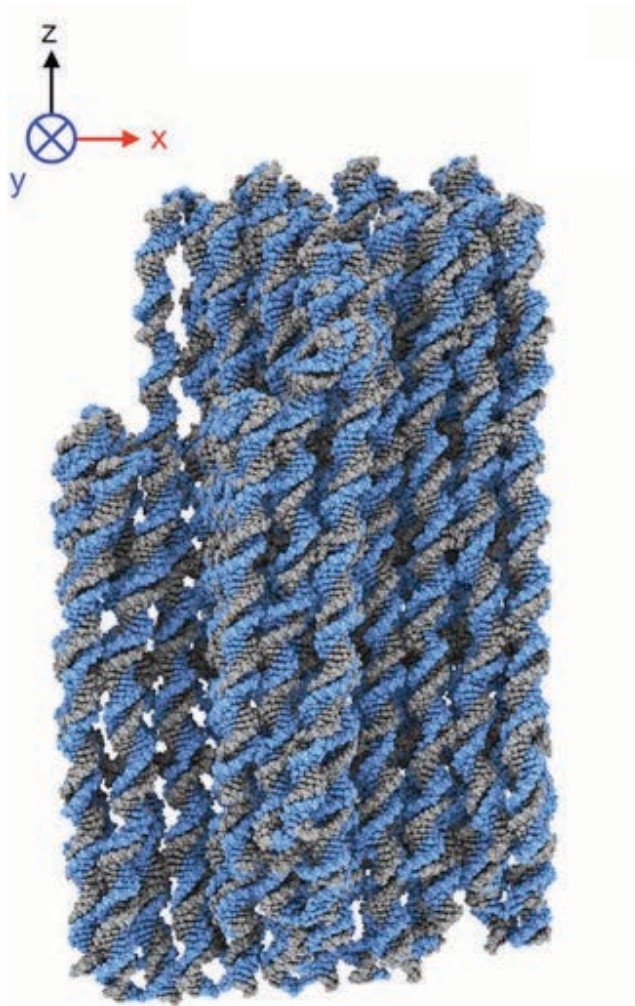


Bai *et al*, PNAS 109:20012 (2012)

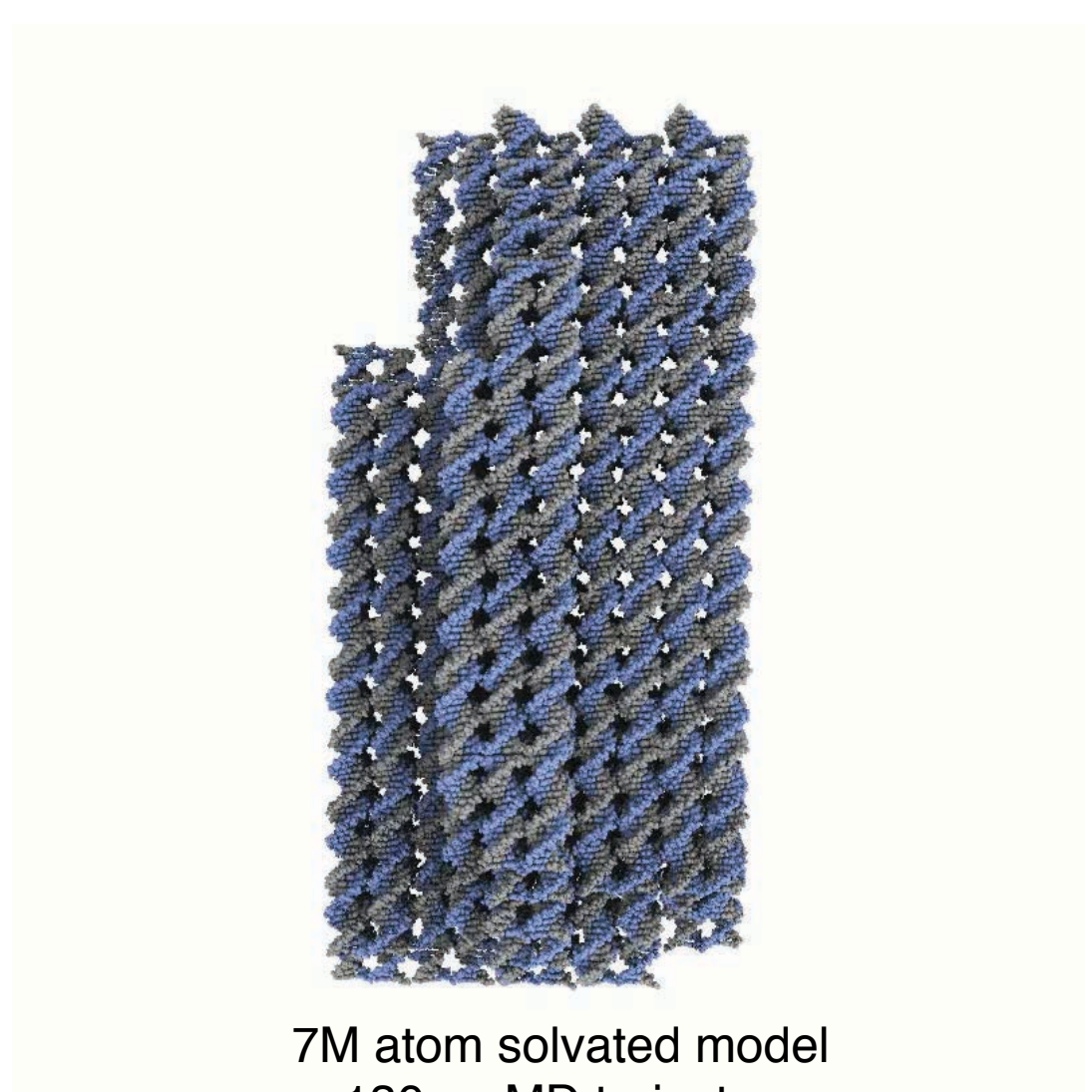


7M atom solvated model
130 ns MD trajectory

MD simulation of the cryo-EM object starting from a caDNAano design

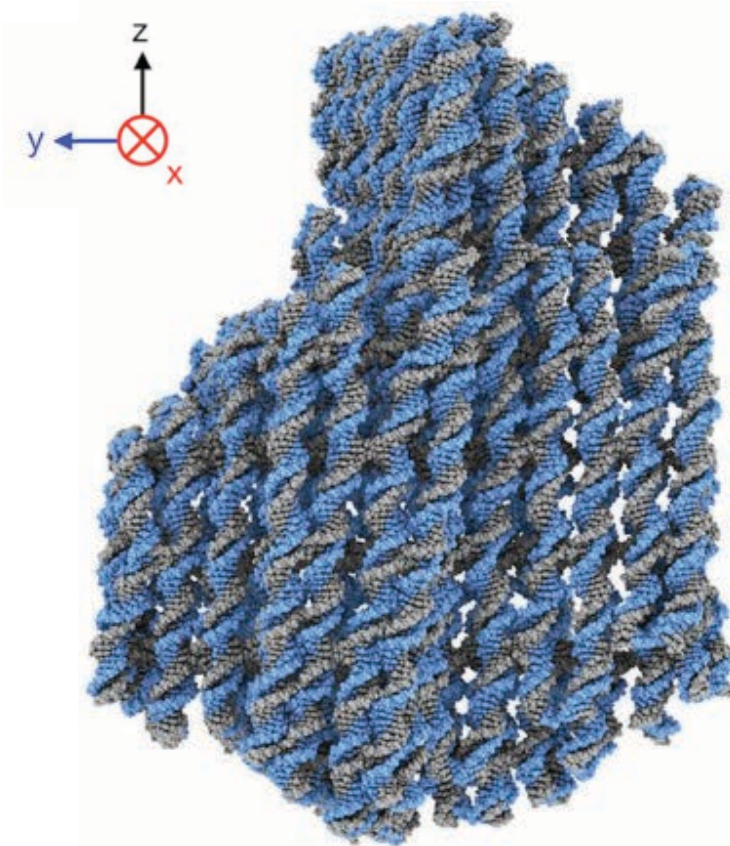


Bai *et al*, PNAS 109:20012 (2012)

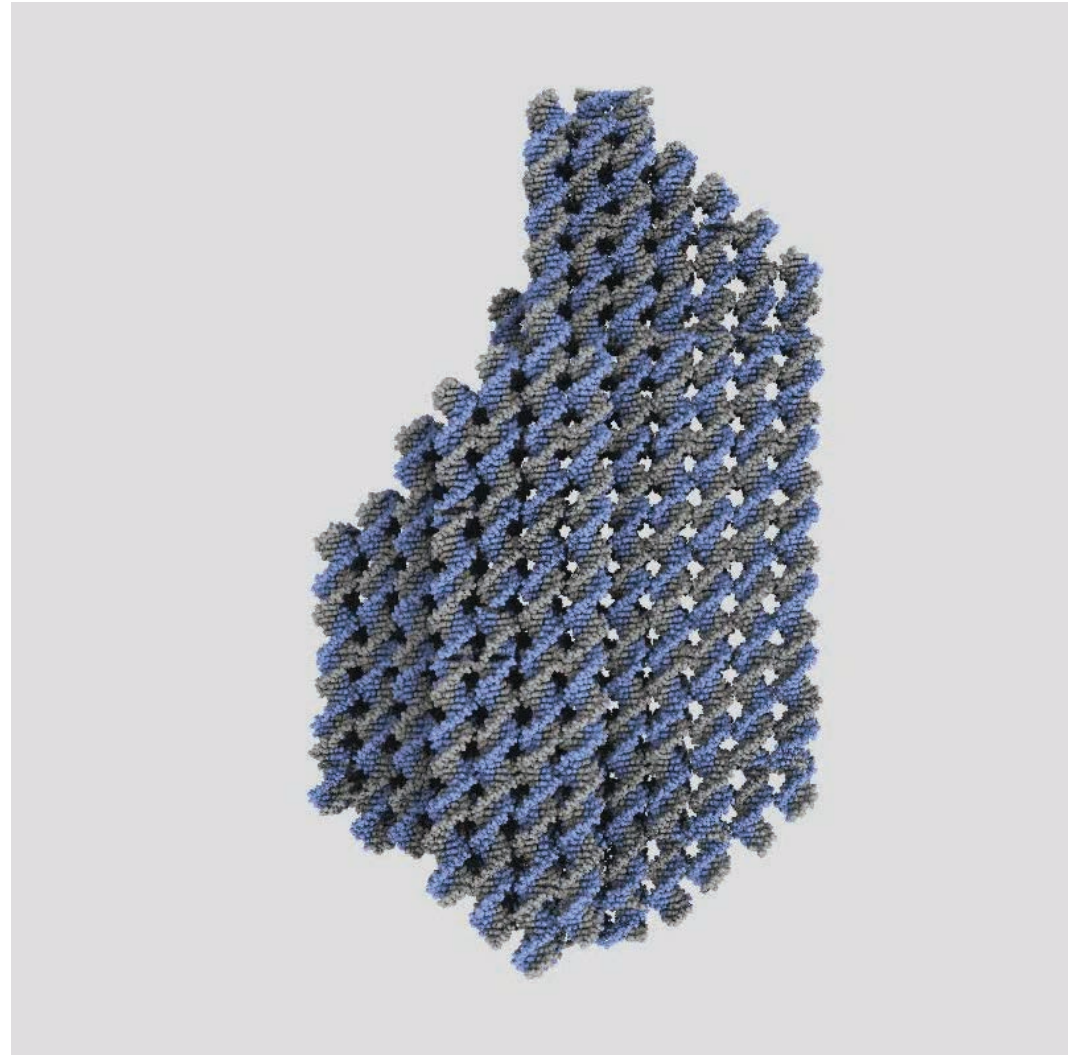


7M atom solvated model
130 ns MD trajectory

MD simulation of the cryo-EM object starting from a caDNAano design



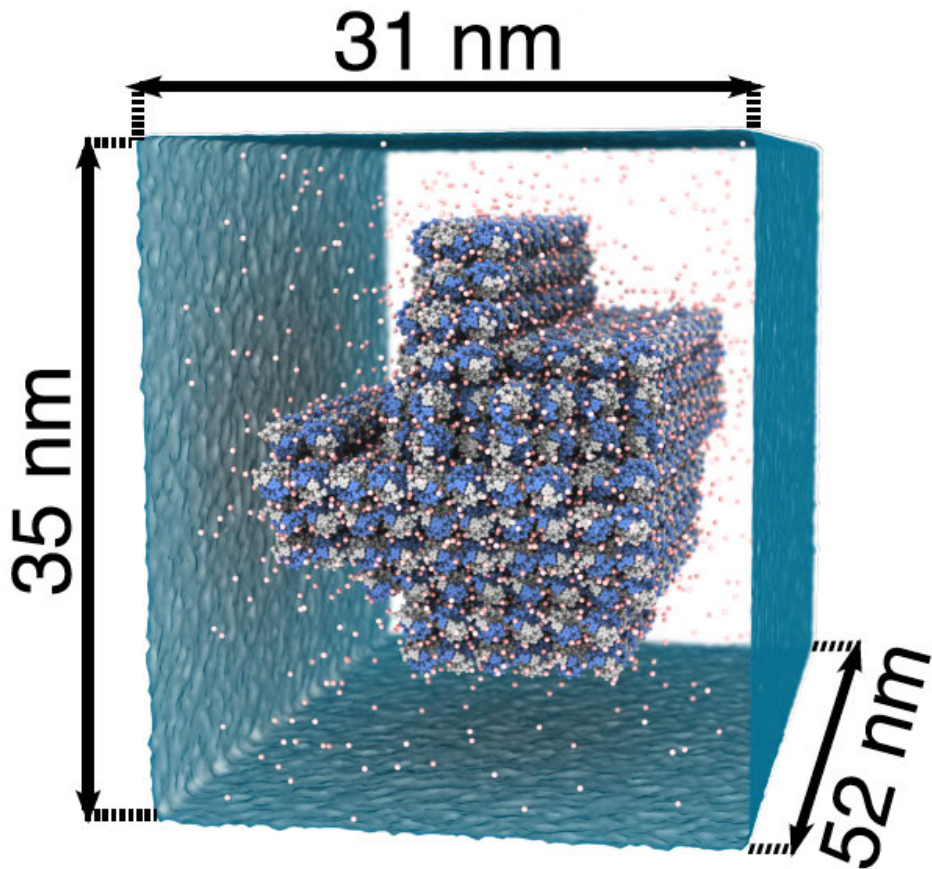
Bai *et al*, PNAS 109:20012 (2012)



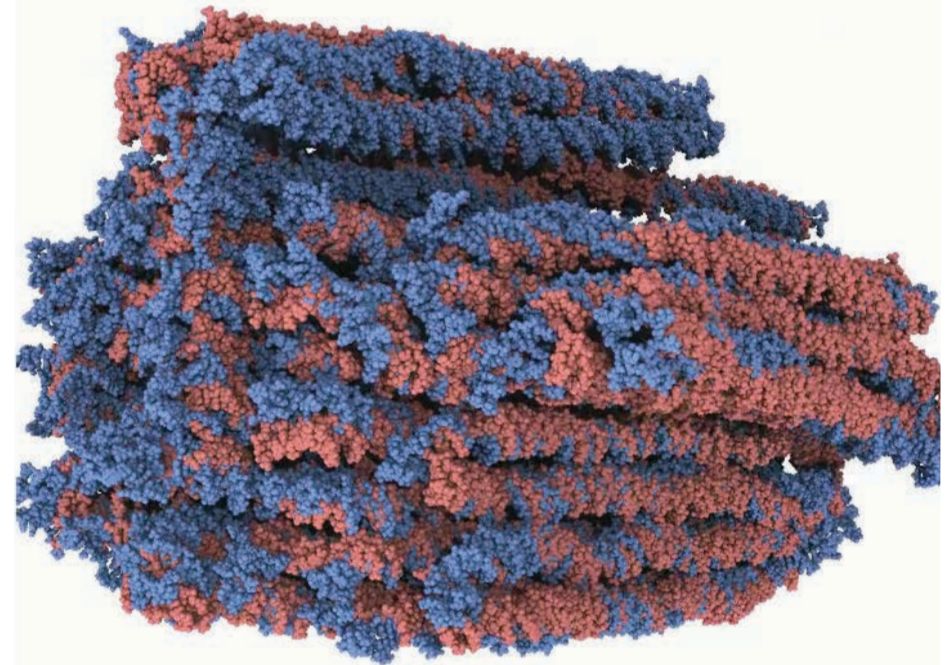
7M atom solvated model
130 ns MD trajectory

Direct comparison with cryo-EM reconstruction

Simulation on Blue Waters
(UIUC)



Time scale: 200 ns / Size $\sim 7,000,000$ atoms

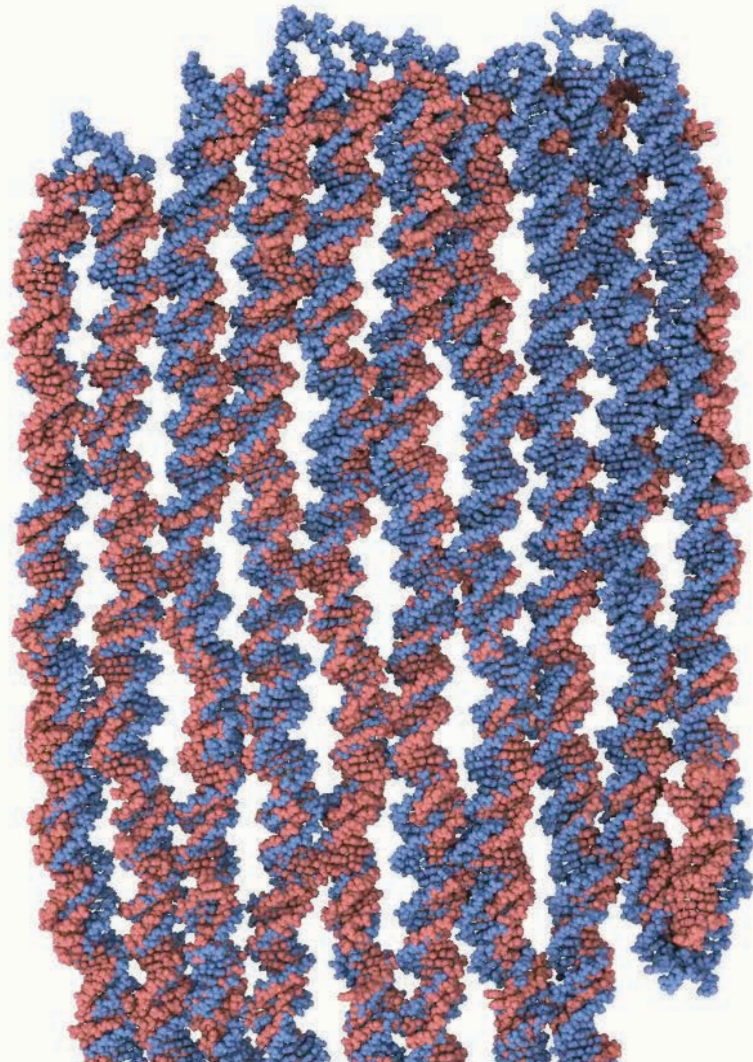


Maffeo, Yoo & Aksimentiev, *NAR* 44: 3013

Cryo-EM, Dietz group, *PNAS* (2012)

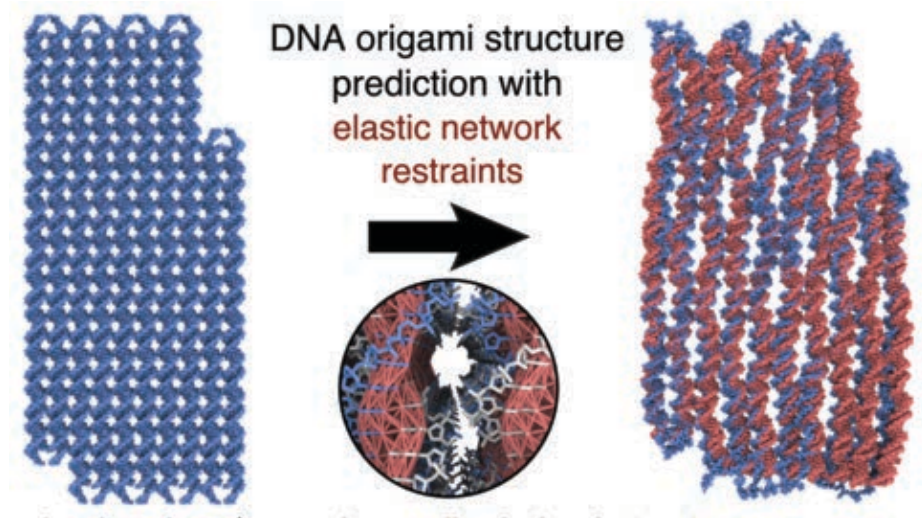
<http://bionano.physics.illinois.edu/origami-structure>

De novo prediction of DNA origami structure



Cryo-EM, Dietz group, *PNAS* (2012)

Maffeo, Yoo & Aksimentiev, *NAR* 44: 3013



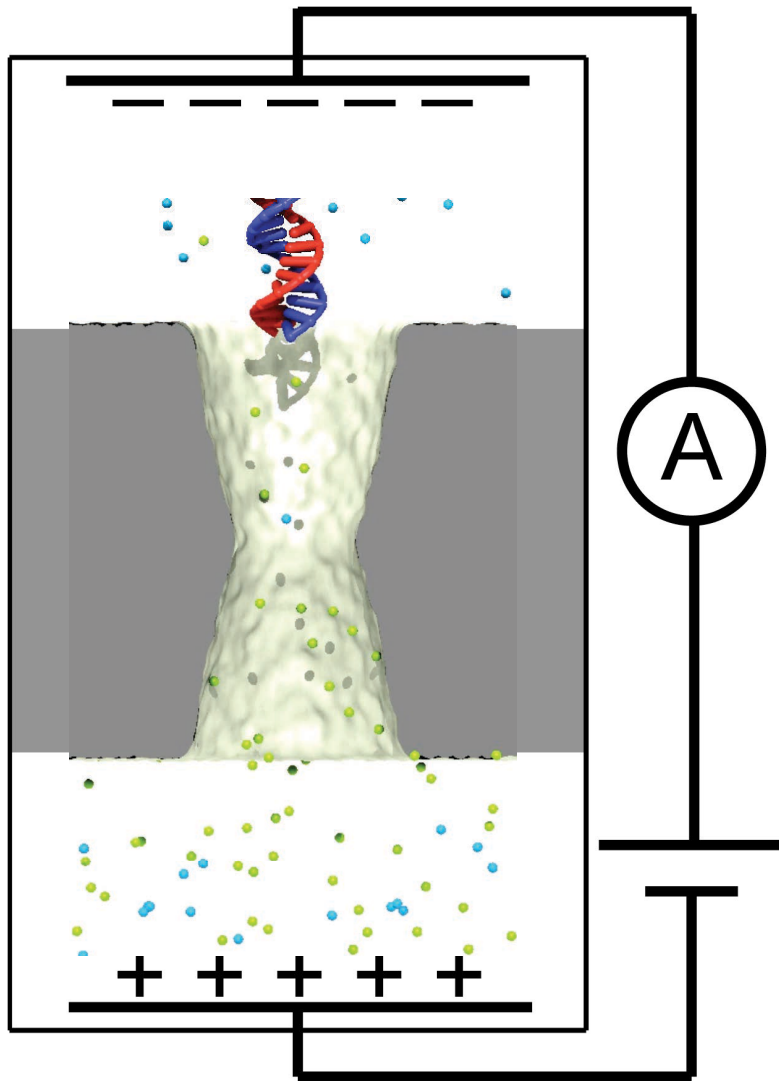
Modest computational cost
(10 hours single workstation)

Server implementation has been
requested by experimentalists

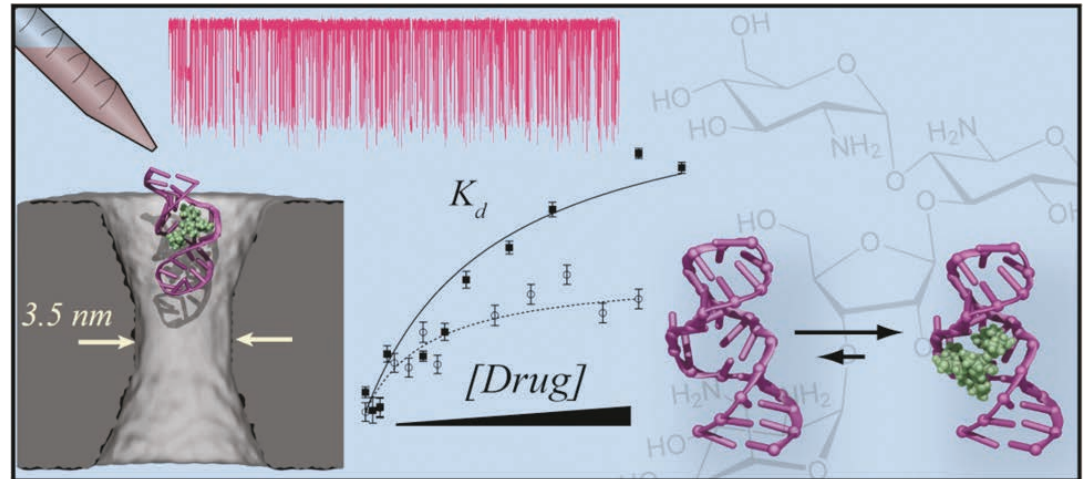
nanoHub implementation pending

<http://bionano.physics.illinois.edu/origami-structure>

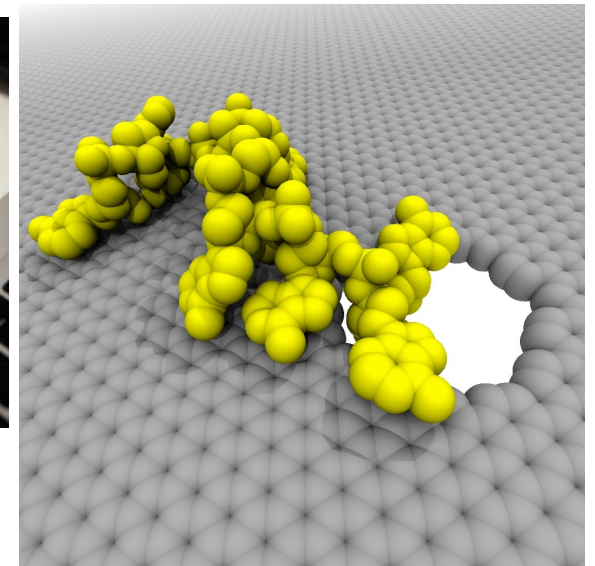
Nanopores for DNA and protein sequencing and drug design



current transients indicate
passage of DNA

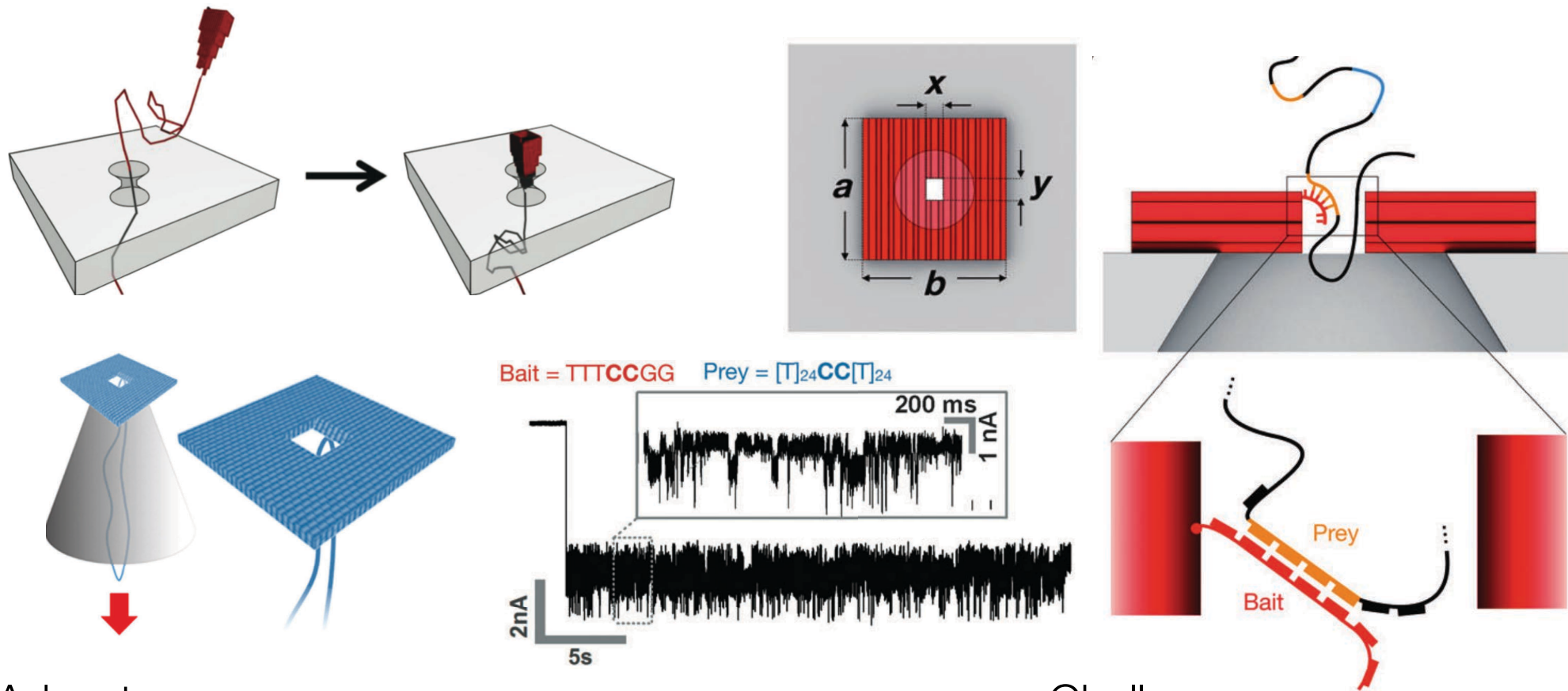


MinION
(Oxford Nanopore
Technologies)



Graphene nanopore

The hybrid DNA origami/solid-state nanopore



Advantages:

- subnanometer control over pore geometry
- functionalization with auxiliary components

Challenges:

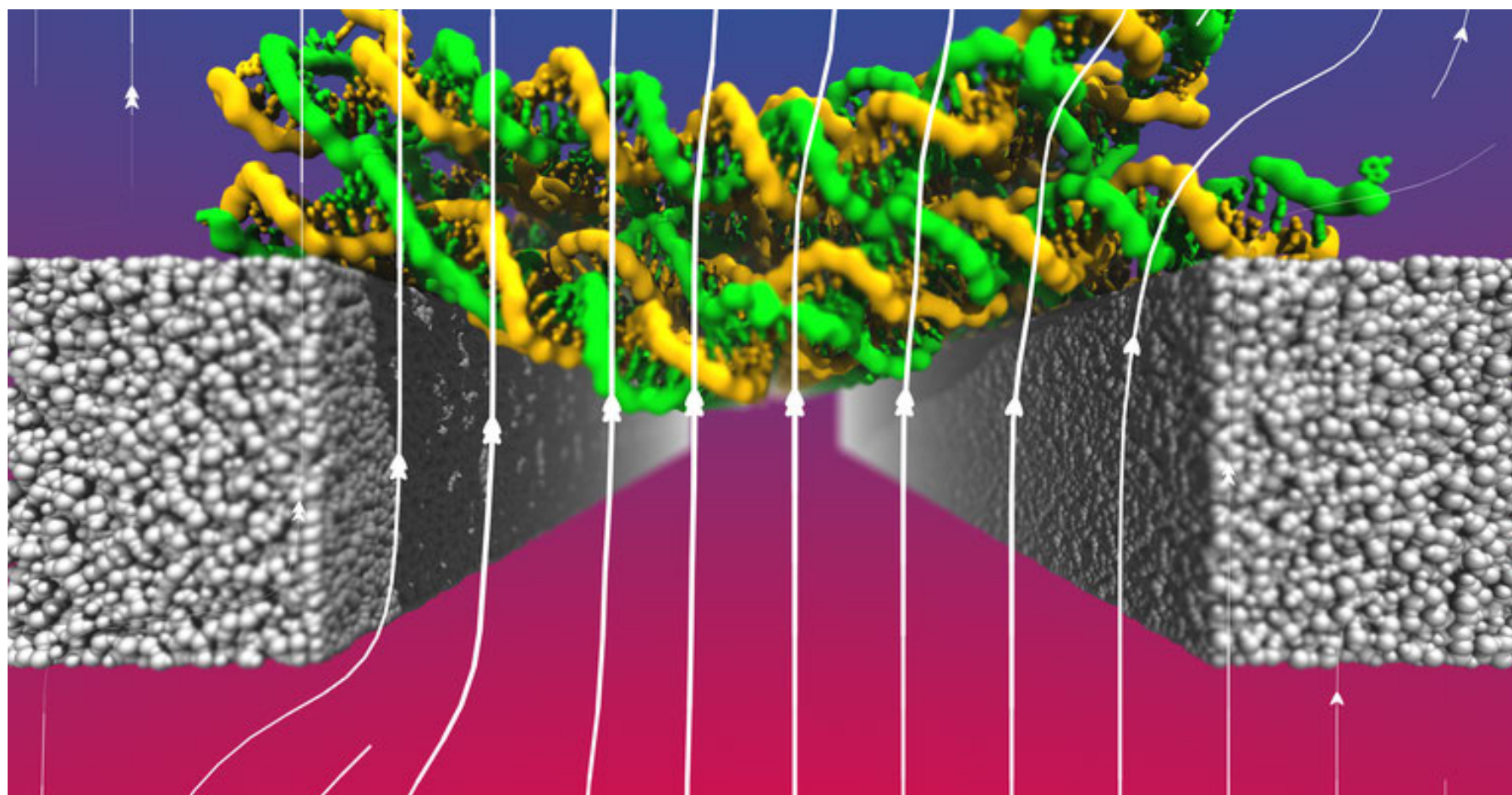
- leakage current
- structural integrity

Nicholas A. W. Bell et al. *Nano Lett.* 2012 12 (1), 512-517
Hernández-Ainsa, S. et al. *Nano Lett.* 2014, 14, 1270-1274.
Wei, R. et al. *Angew. Chem., Int. Ed.* 2012, 51, 4864-4867.

Ionic conductivity and structural deformation of DNA origami **nanoplate**



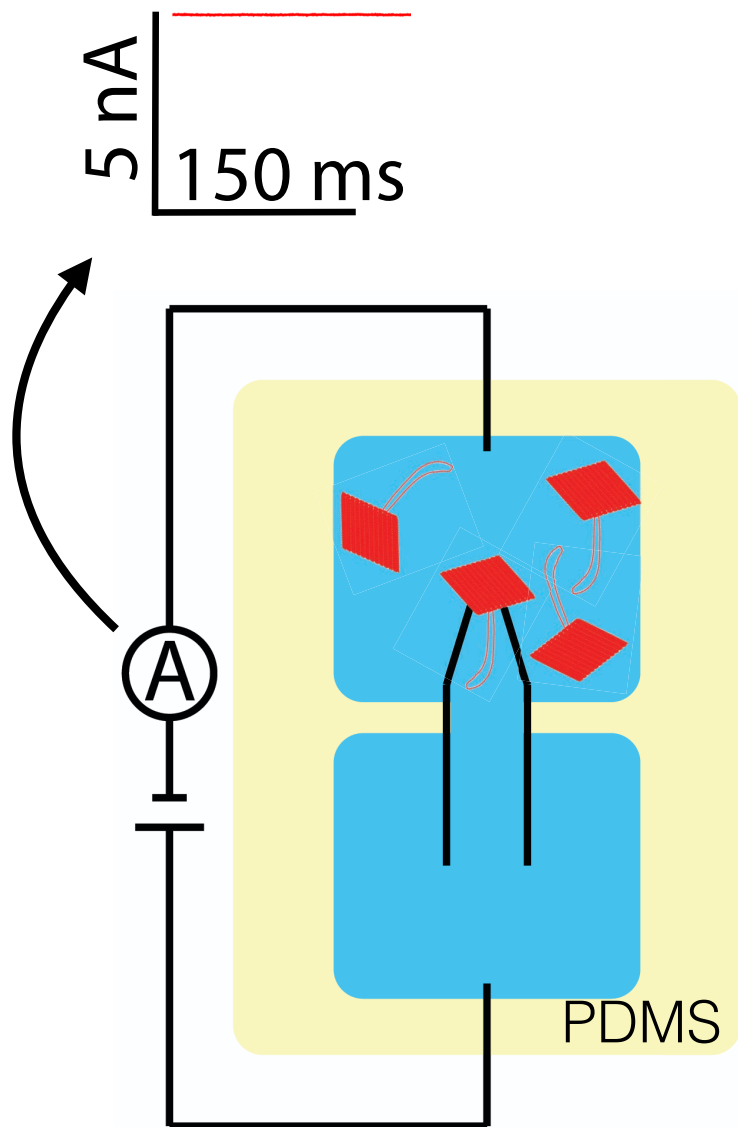
Prof. Ulrich Keyser



is.
only MD simulation can

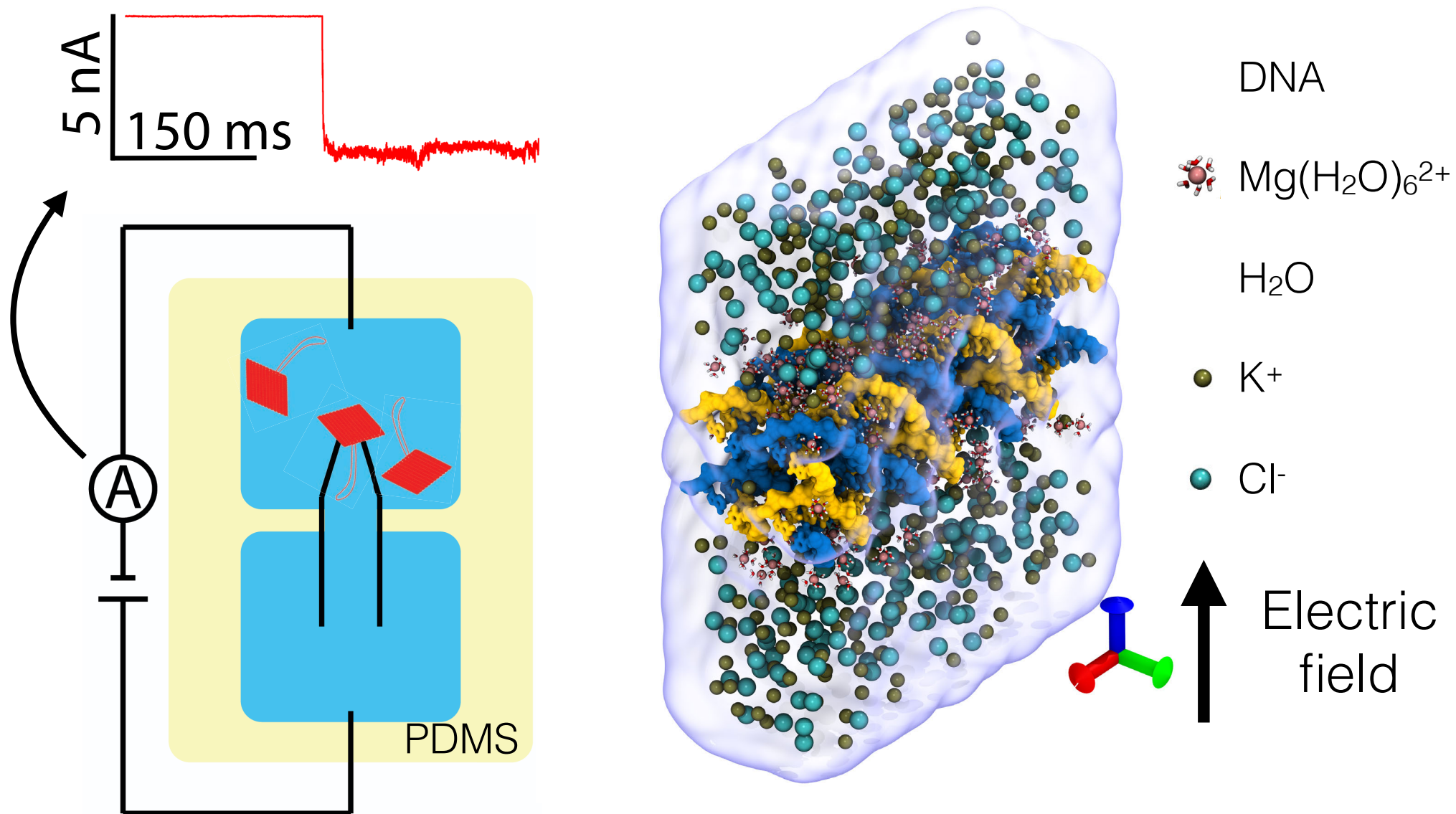
Li, Hemmig, Kong, **JY** et al, *ACS Nano* (2015)

MD simulation of DNA origami conductivity



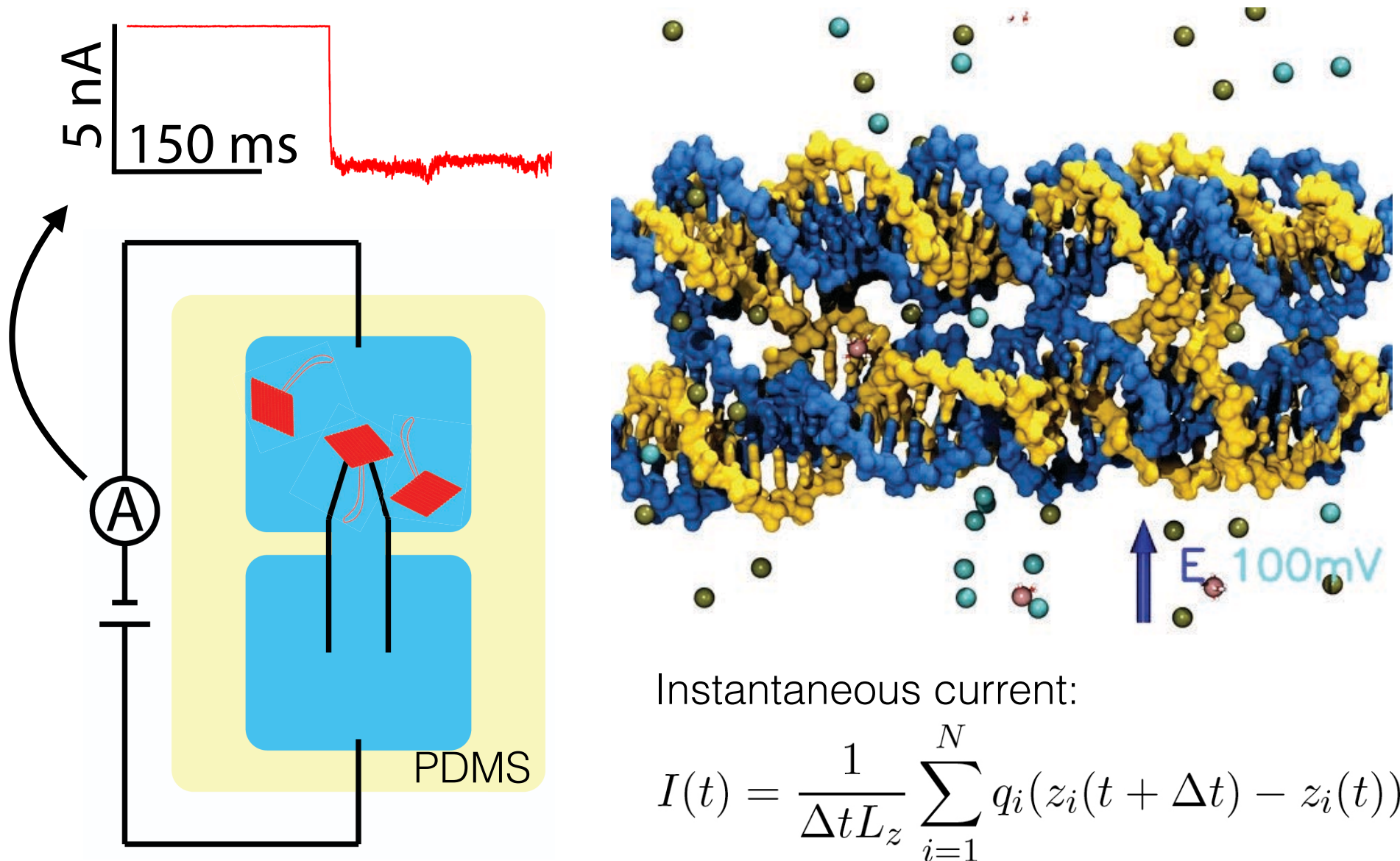
The screenshot displays the nanoHUB website interface. The top part shows the 'caDNAno' tool, which includes a 2D layout of DNA origami and a 3D visualization of the structure. Below this, the 'all-atom' simulation window is shown, displaying a detailed view of the DNA double helix structure. The nanoHUB logo and navigation menu are visible at the top of the browser window.

MD simulation of DNA origami conductivity



Li, Chen-Yu et al. *ACS Nano* 9:1420-1433 (2015)

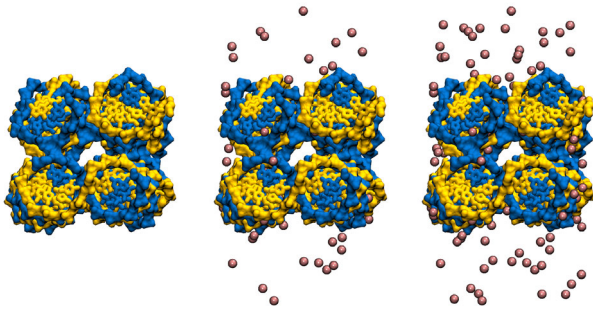
MD simulation of DNA origami conductivity



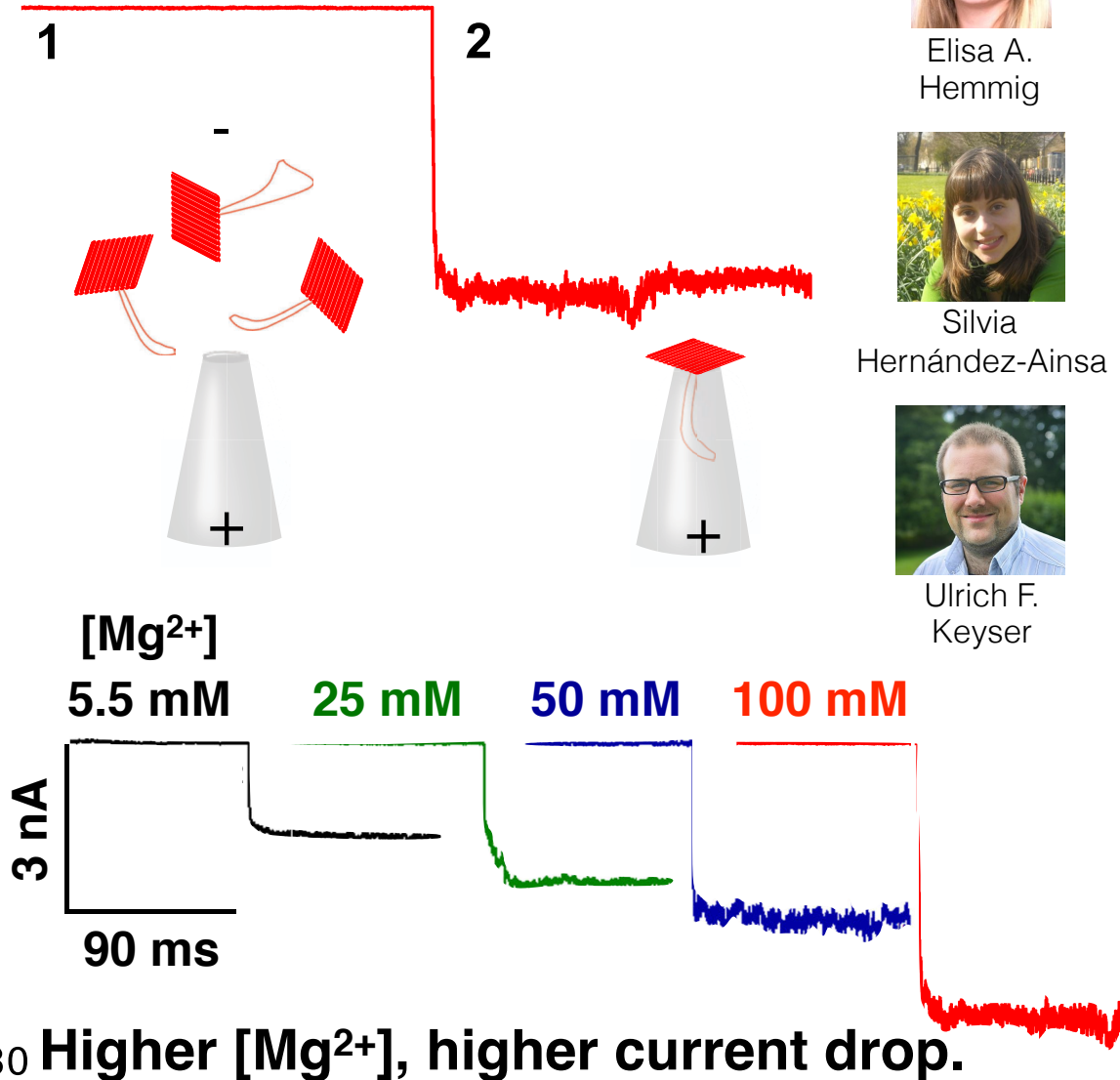
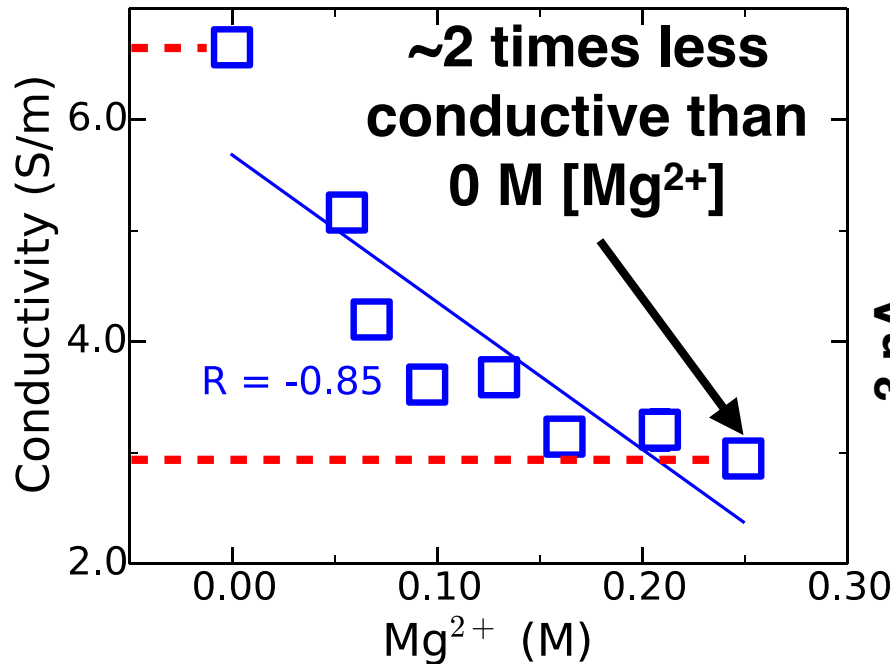
Effect of Mg^{2+}

Higher $[Mg^{2+}]$ makes DNA origami less conductive.

SQ2, m13 sequence



$[Mg^{2+}]$



Elisa A. Hemmig



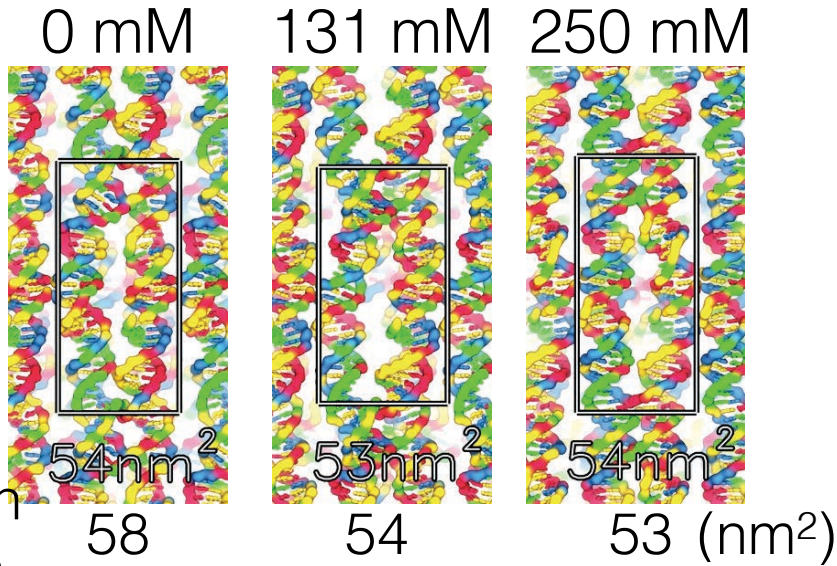
Silvia Hernández-Ainsa



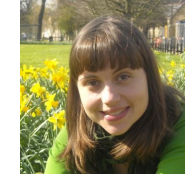
Ulrich F. Keyser

Mg²⁺ makes DNA origami more compact, by screening the DNA-DNA repulsion.

Mechanism of Mg²⁺



Elisa A. Hemmig

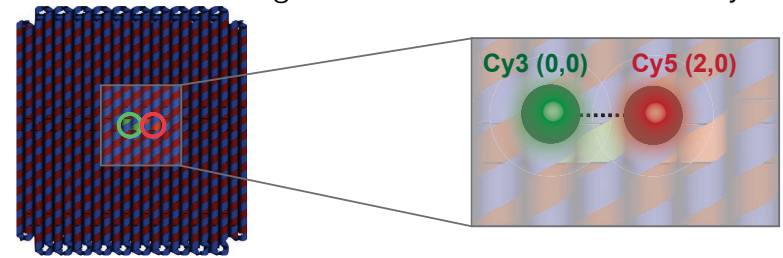


Silvia Hernández-Ainsa

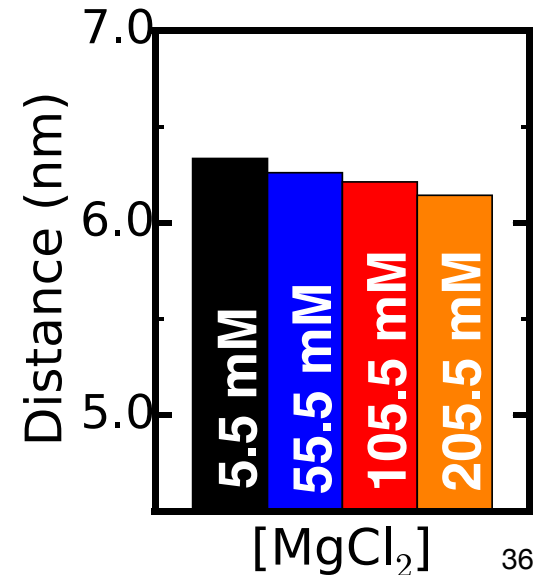
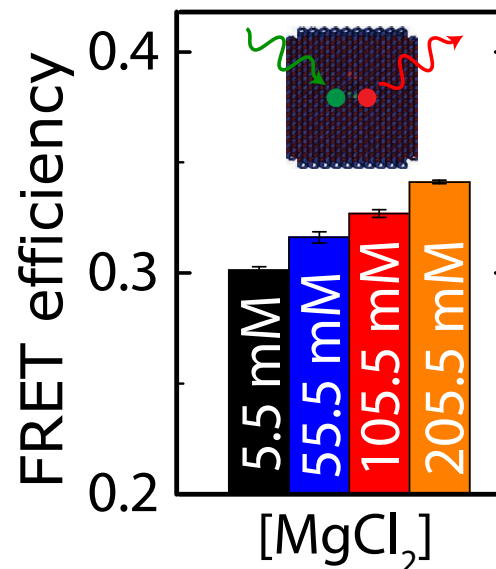
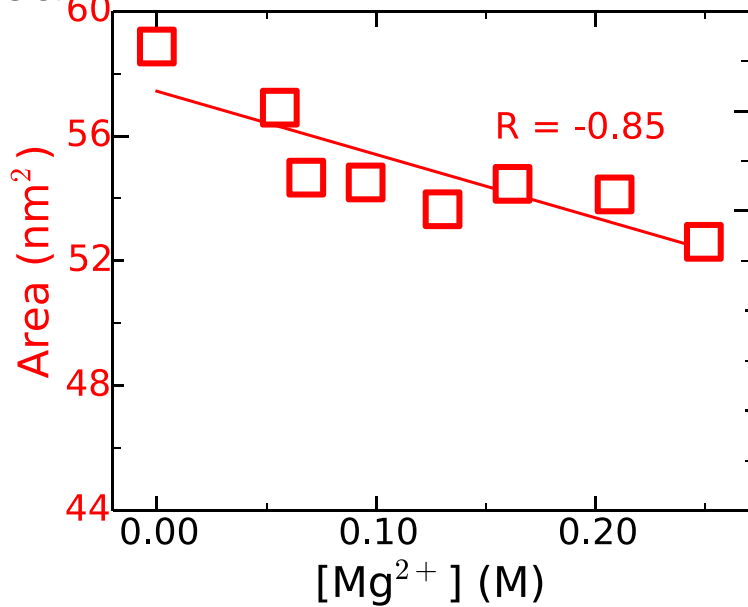


Ulrich F. Keyser

FRET:



Higher [Mg²⁺], lower inter-DNA distance



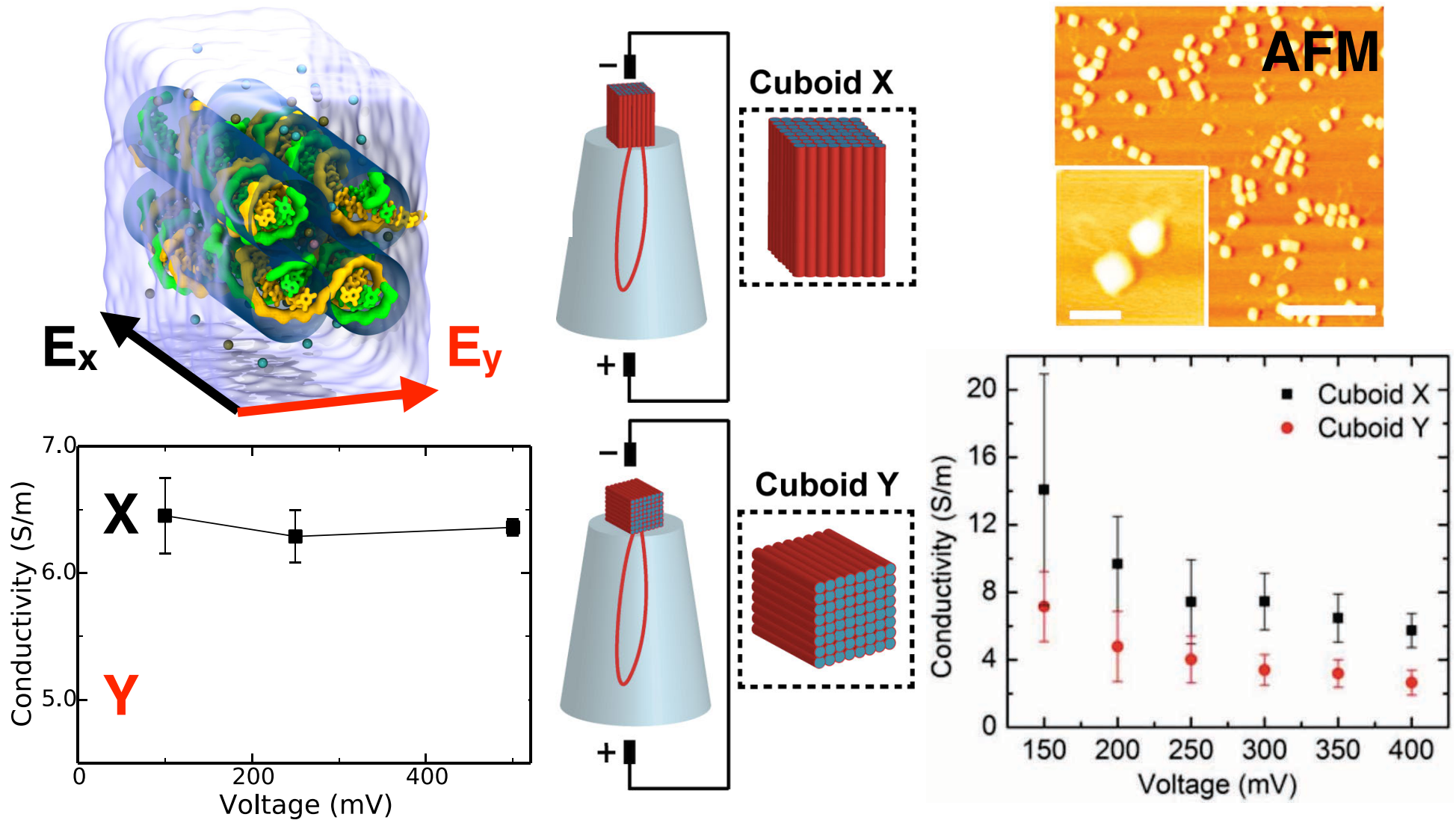
Anisotropic conductivity



Jinglin Kong

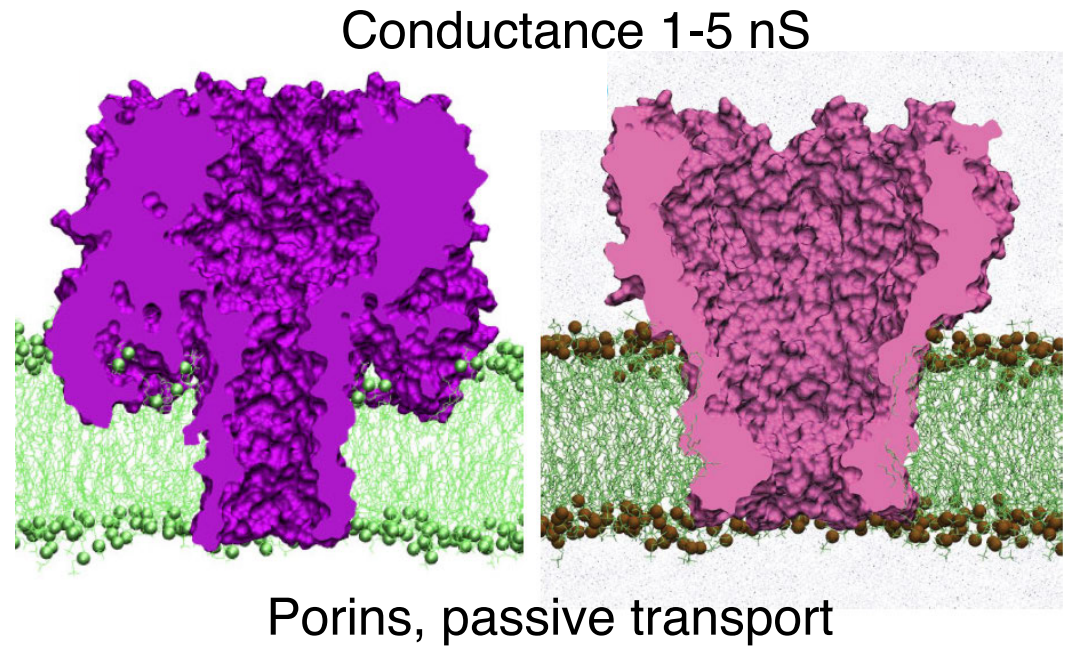
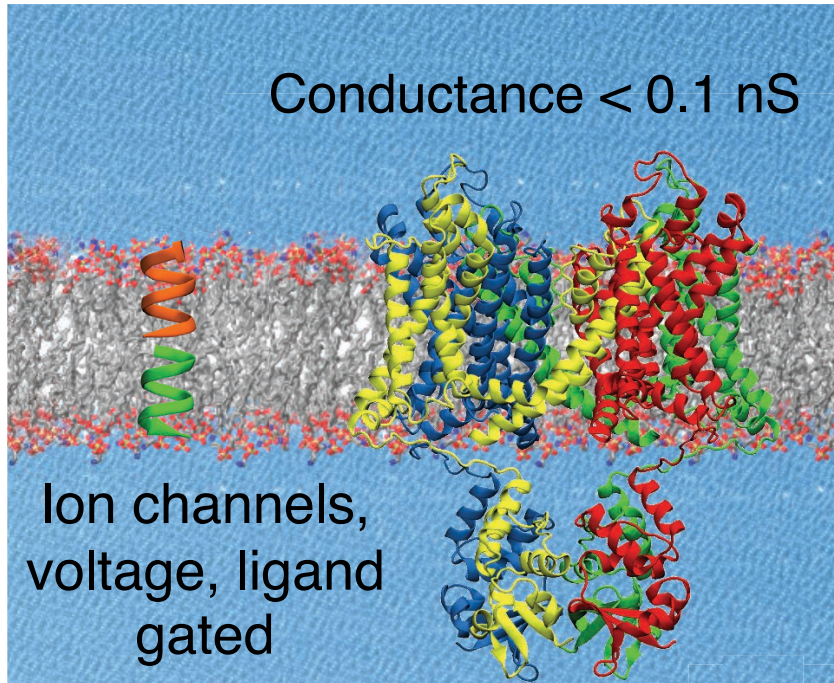


Ulrich F. Keyser

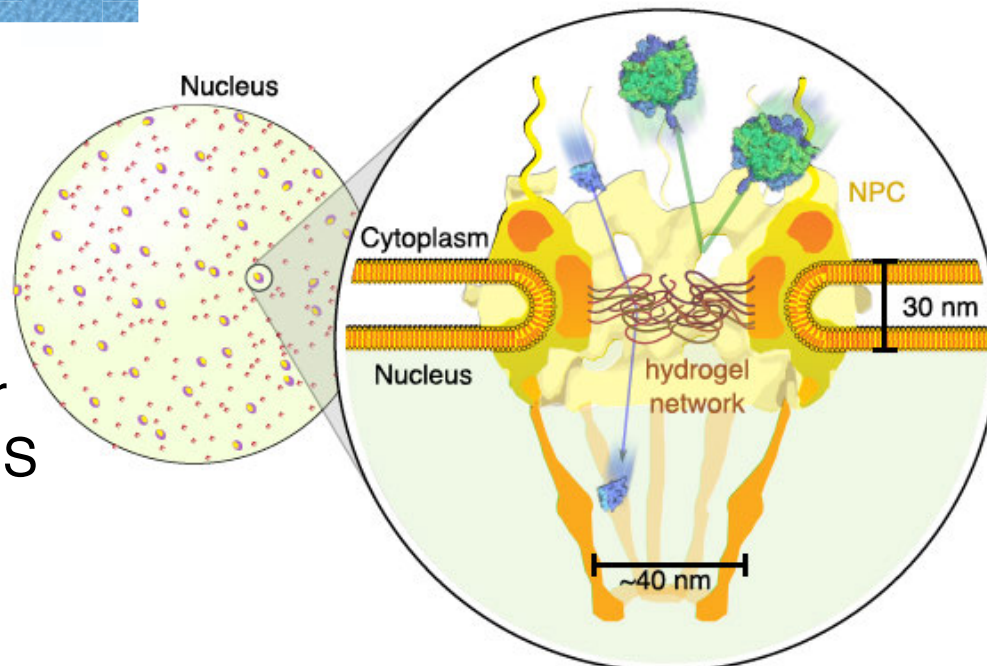


Li, Chen-Yu et al. *ACS Nano* 9:1420-1433 (2015)

Membrane channels



Nuclear pore complex
Selective cargo transport
Conductance 10 – 100 nS



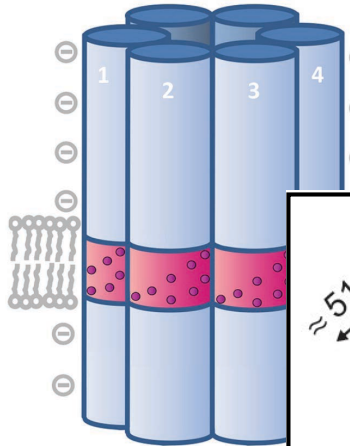
DNA Channels

6-helix

Conductance 0.4 - 1.5 nS

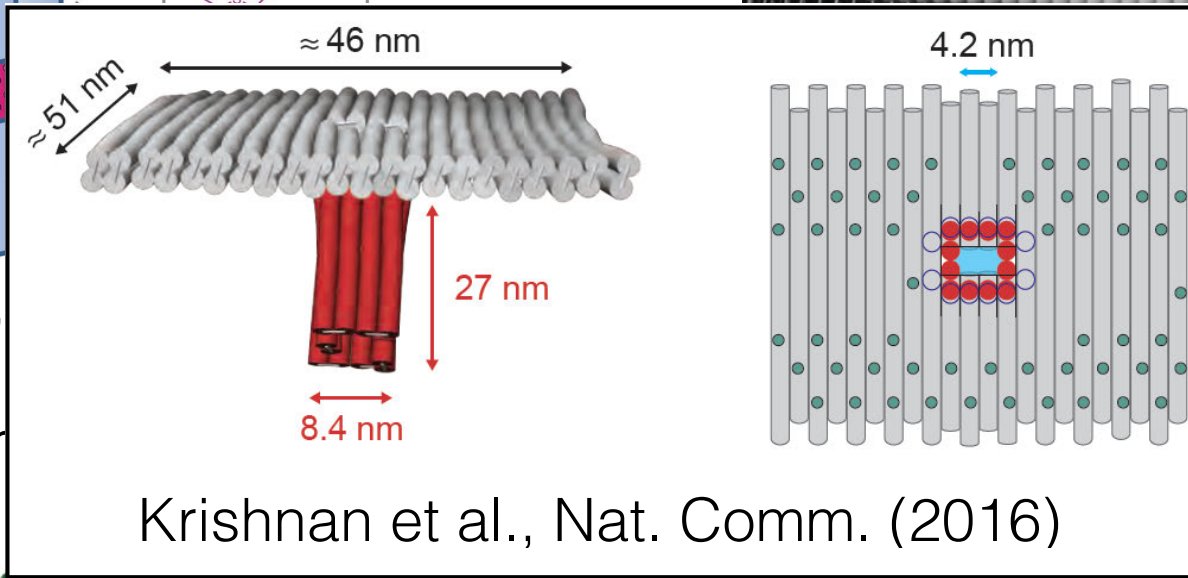
4-helix

Conductance $\sim 0.3-1.4$ nS



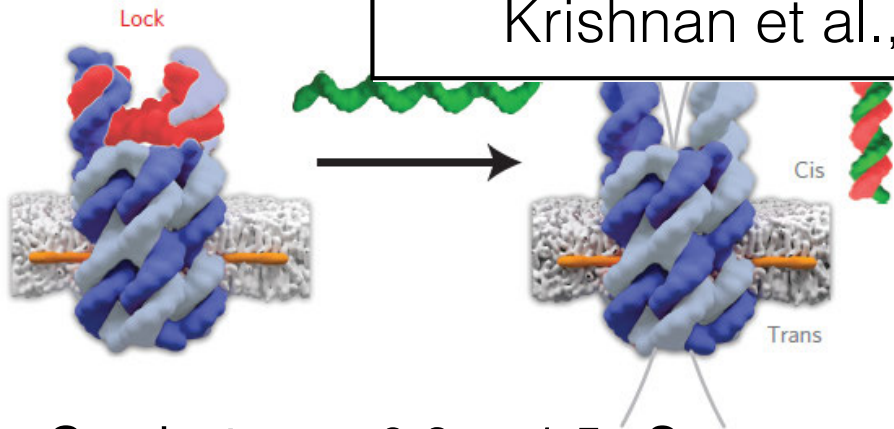
Burns, J. R. *et al.*,

Ligand gating



Krishnan *et al.*, *Nat. Comm.* (2016)

ett., **2015**, 15:3134



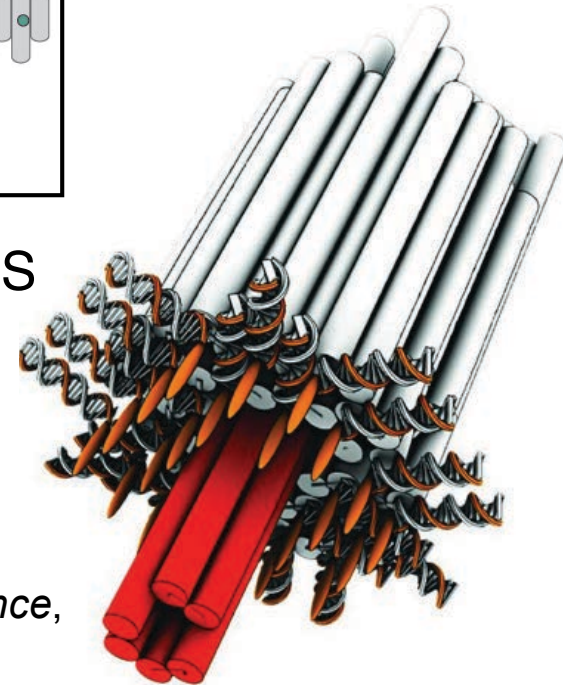
Conductance: 0.6 — 1.5 nS

Burns, J. R. *et al.*, *Nat. Nanotechnol.*, **2016**, 11:152

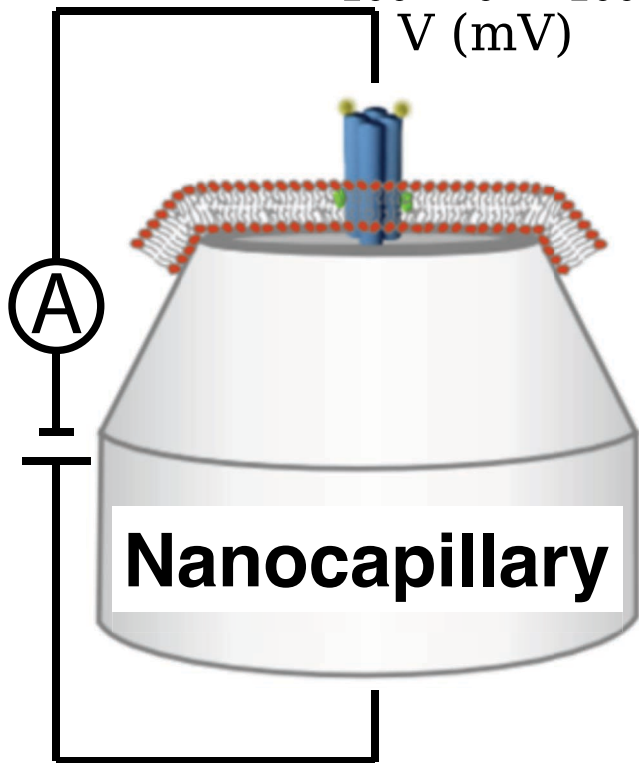
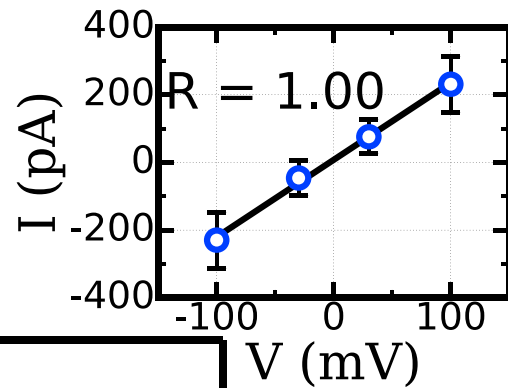
Conductance ~ 0.8 nS

ssDNA transport

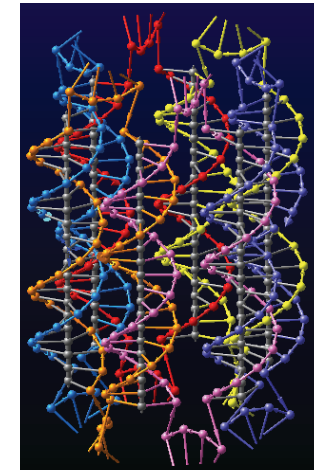
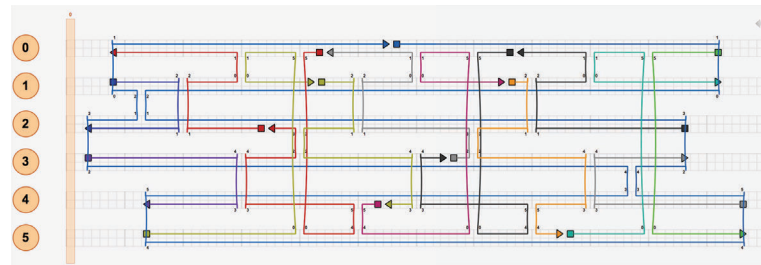
Langecker, M. *et al.*, *Science*, **2012**, 338, 932-936.



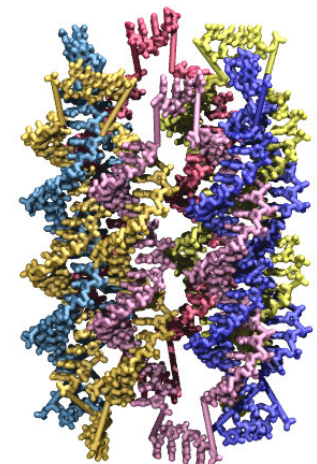
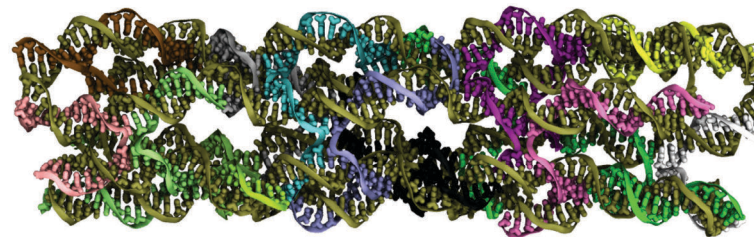
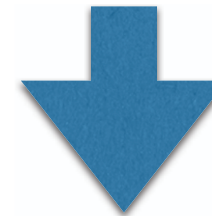
MD simulation of DNA channel conductivity



caDNAno or NanoEngineer-1



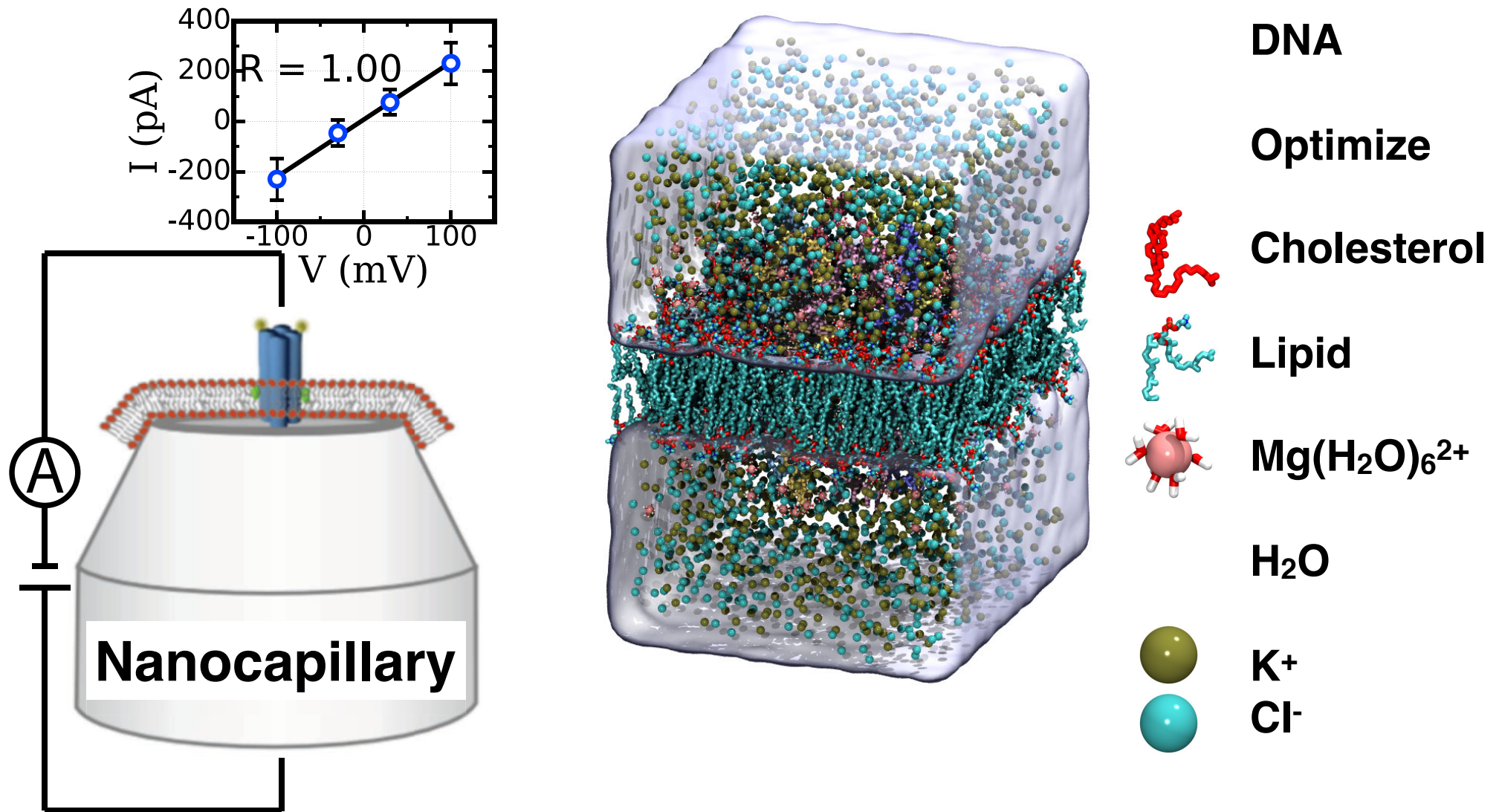
all-atom
coordinates



Göpfrich, Kerstin *et al.*,
Nano Lett., 2015, 15(5), 3134–3138.

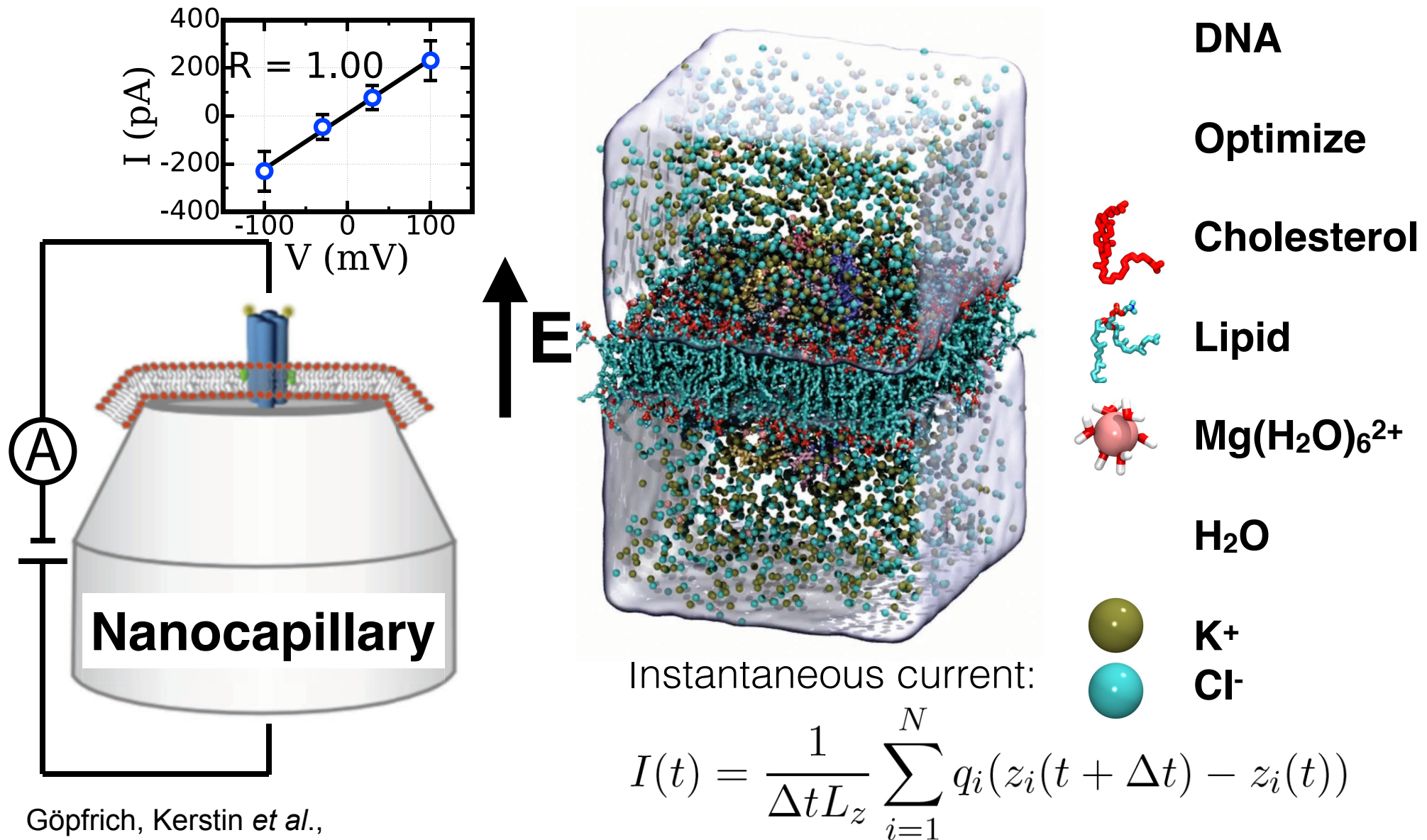
(Right) design from Burns, Jonathan R. *et al.*,
Nat. Nanotechnol., 2016, 11, 152–156.

MD simulation of DNA channel conductivity



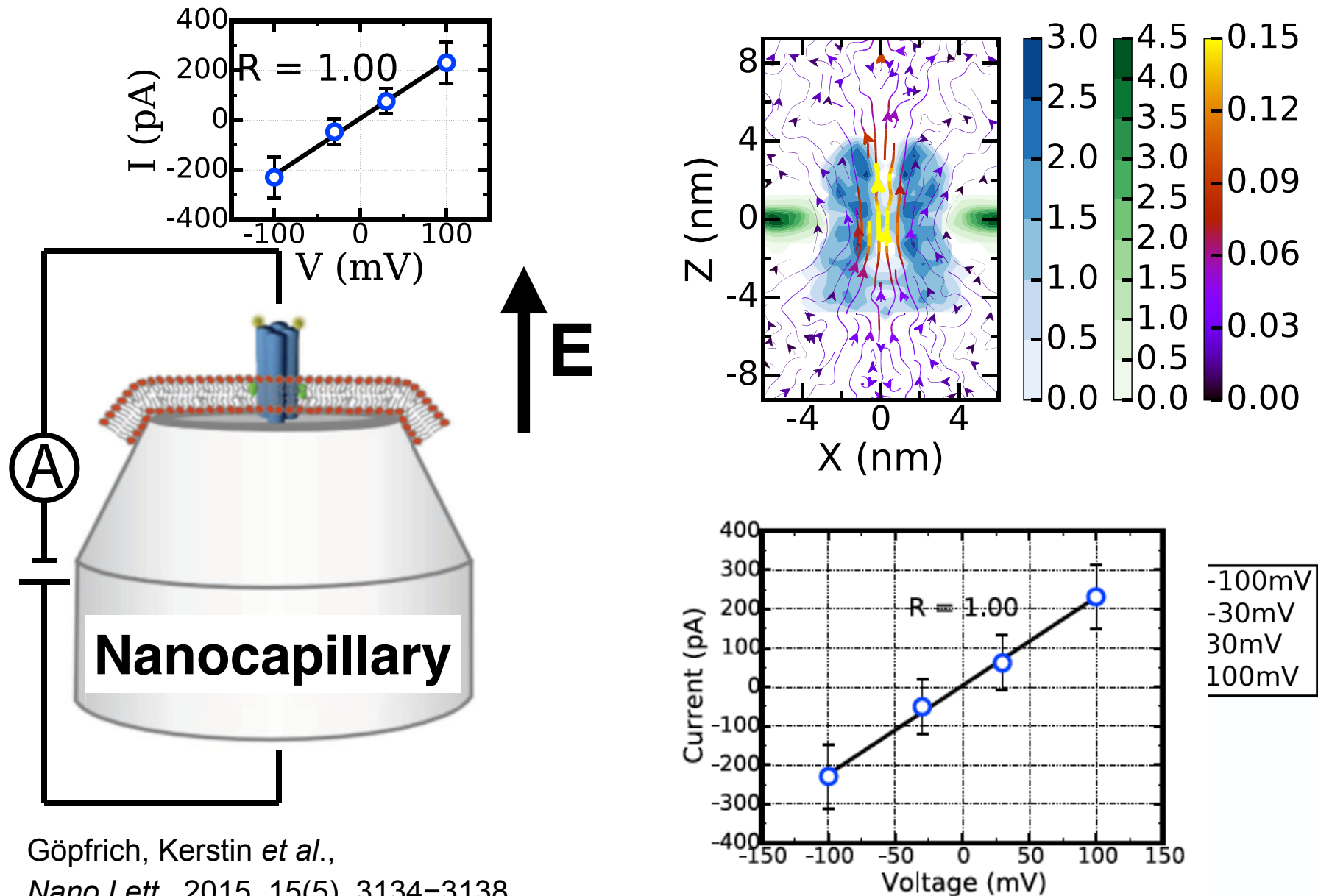
Göpfrich, Kerstin *et al.*,
Nano Lett., 2015, 15(5), 3134–3138.

MD simulation of DNA channel conductivity



Göpfrich, Kerstin *et al.*,
Nano Lett., 2015, 15(5), 3134–3138.

MD simulation of DNA channel conductivity

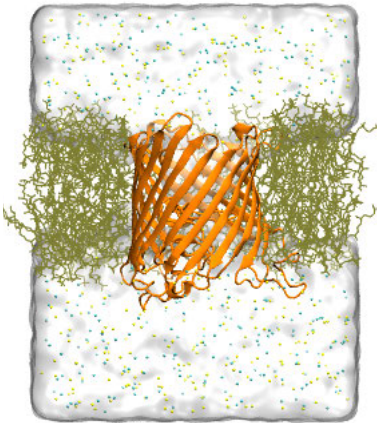


Göpfrich, Kerstin *et al.*,
Nano Lett., 2015, 15(5), 3134–3138.

Does the method work?

OprD

Bias: 80 mV

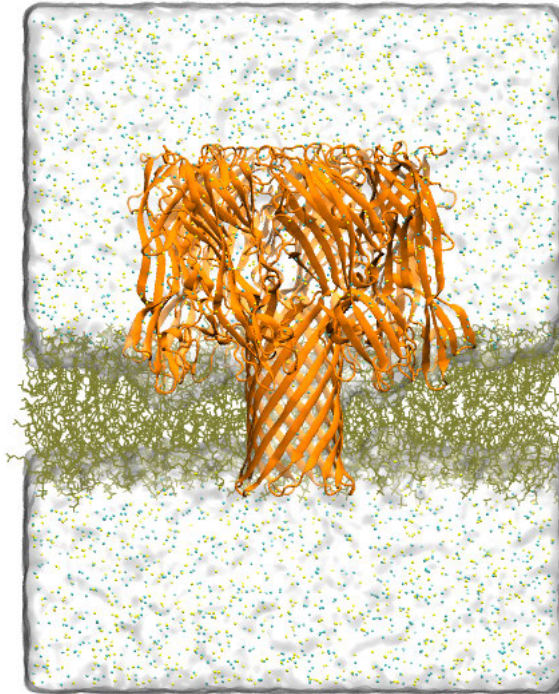


$$G_{MD} = < 0.05 \text{ nS}$$

$$G_{EXP} = 0.03 \text{ nS}$$

Alpha-hemolysin

Bias: 100 mV

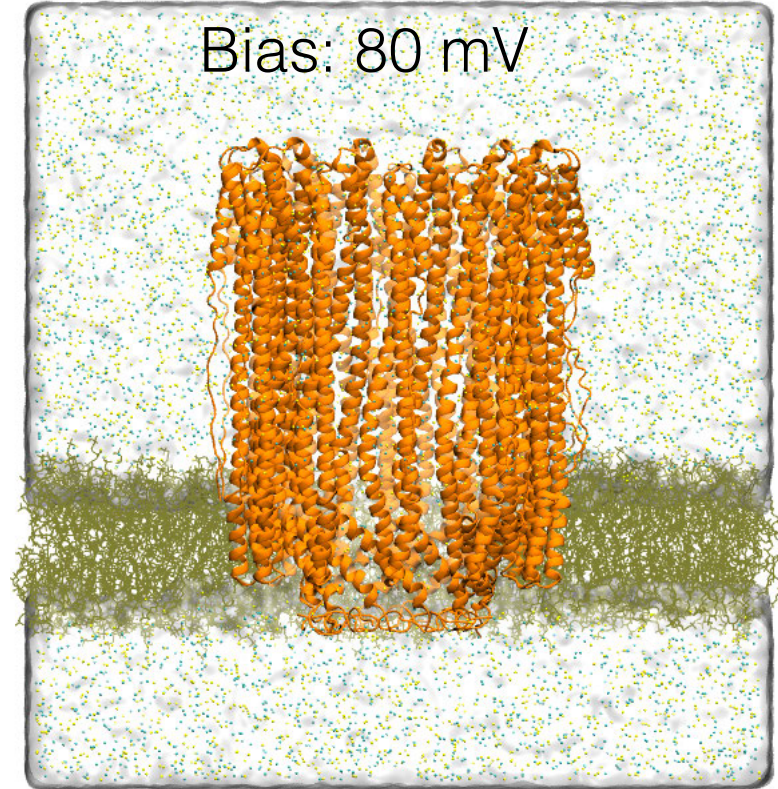


$$G_{MD} = 1.1 \text{ nS}$$

$$G_{EXP} = 1 \text{ nS}$$

ClyA

Bias: 80 mV

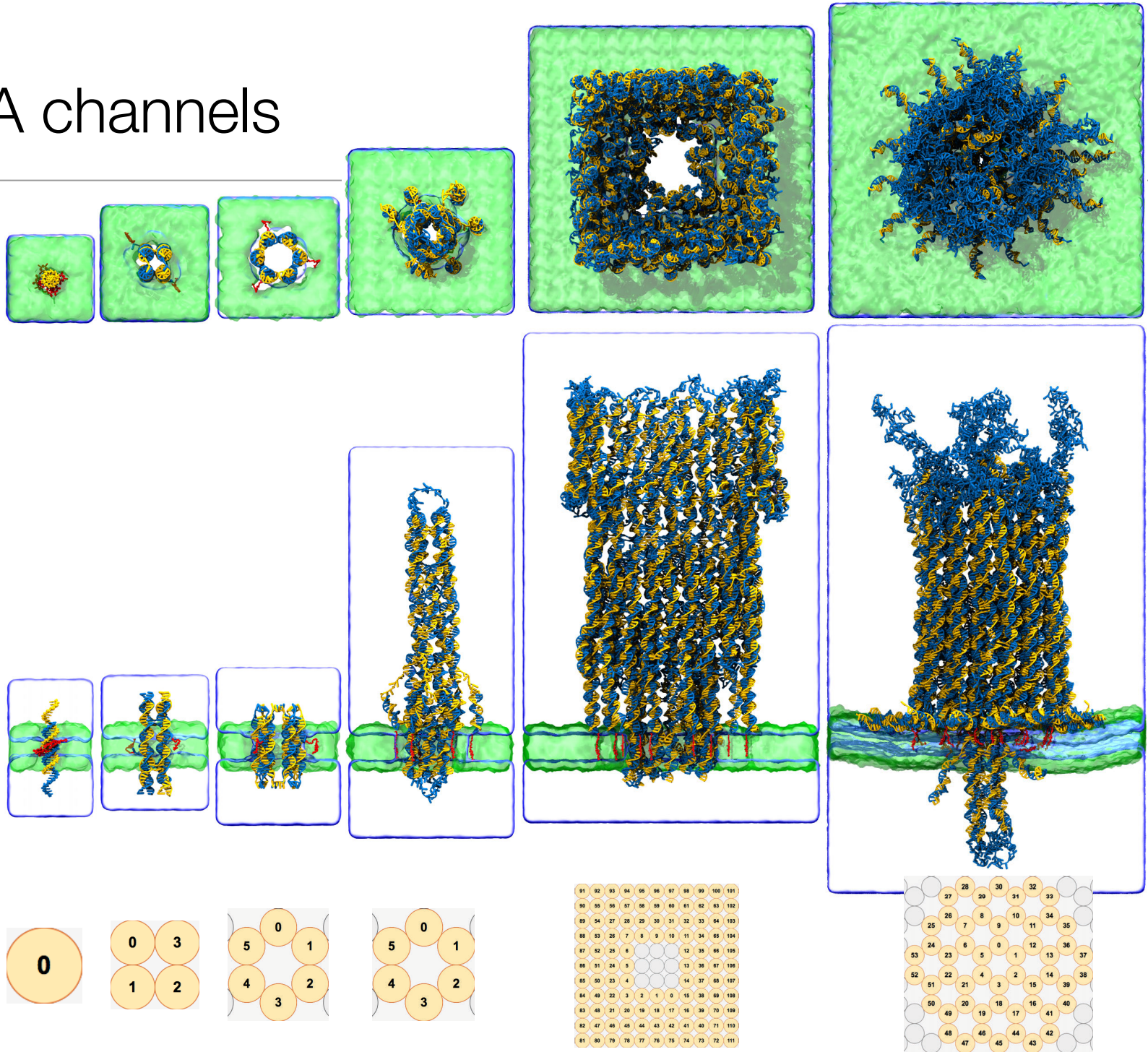


$$G_{MD} = 7.5 \text{ nS}$$

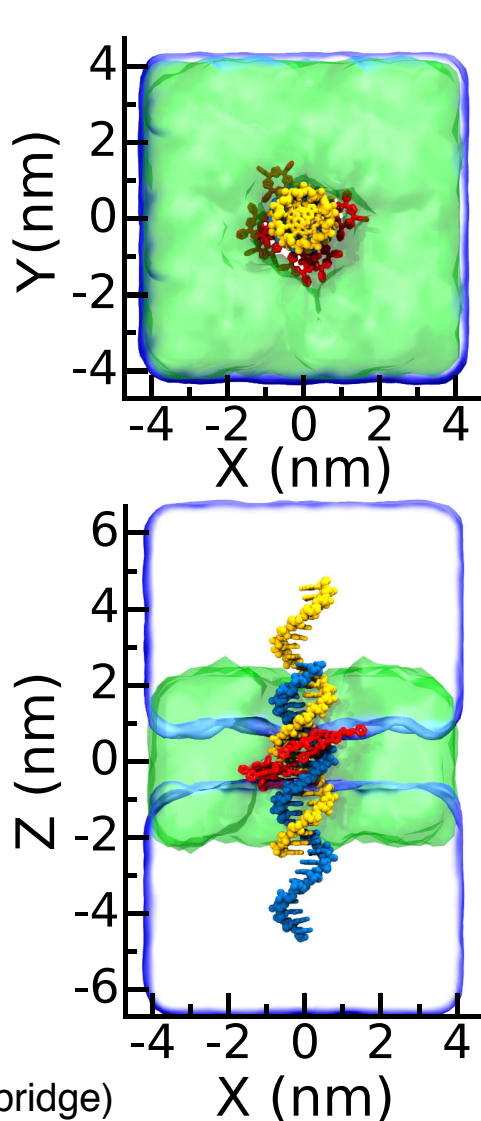
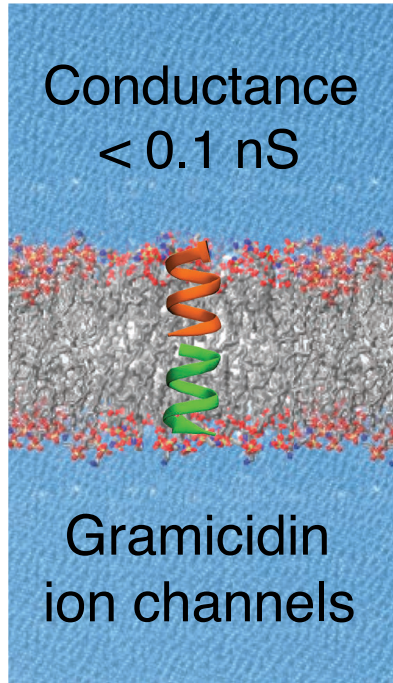
$$G_{EXP} = 11 \text{ nS}$$

Biophysical Journal 88:3745 (2005)

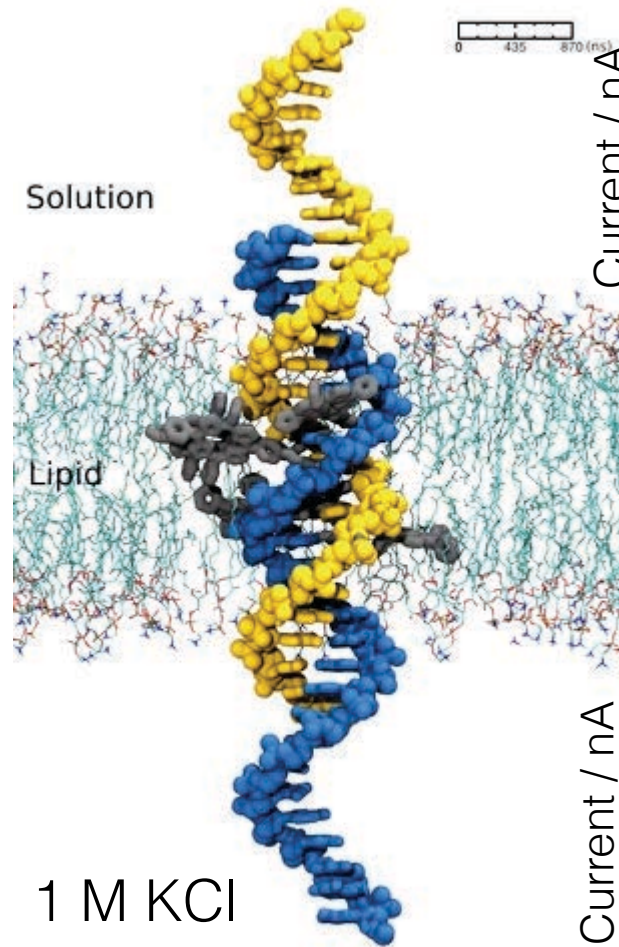
The DNA channels



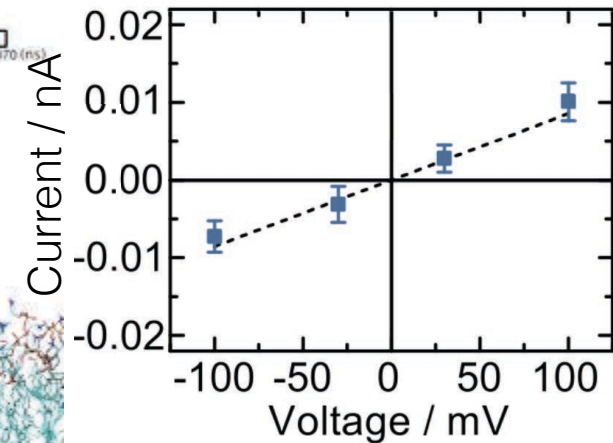
Small conductance DNA channel



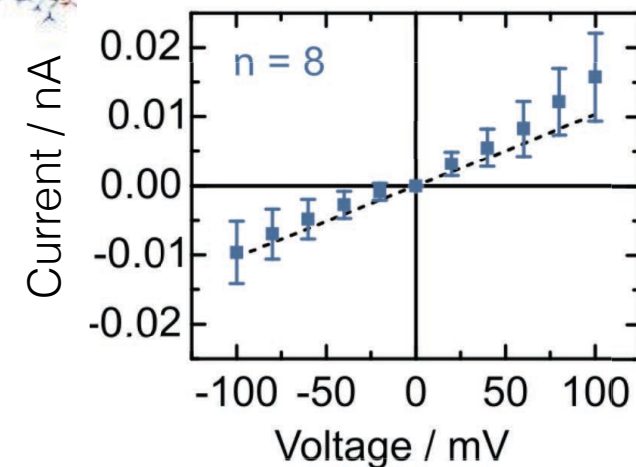
1 helix
140,000 atoms



1 M KCl



Conductance: ~ 0.1 nS



Experiment:

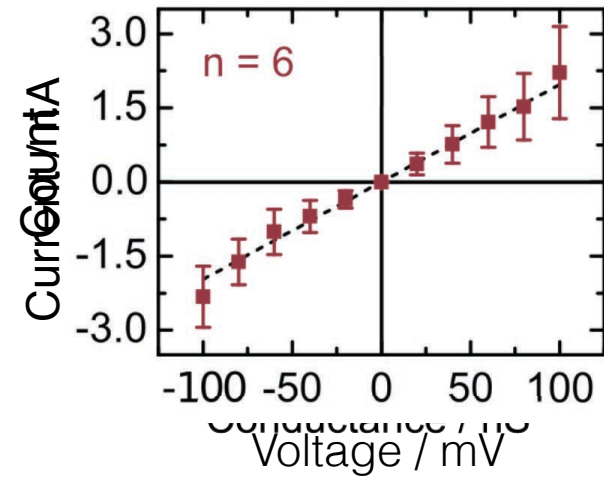
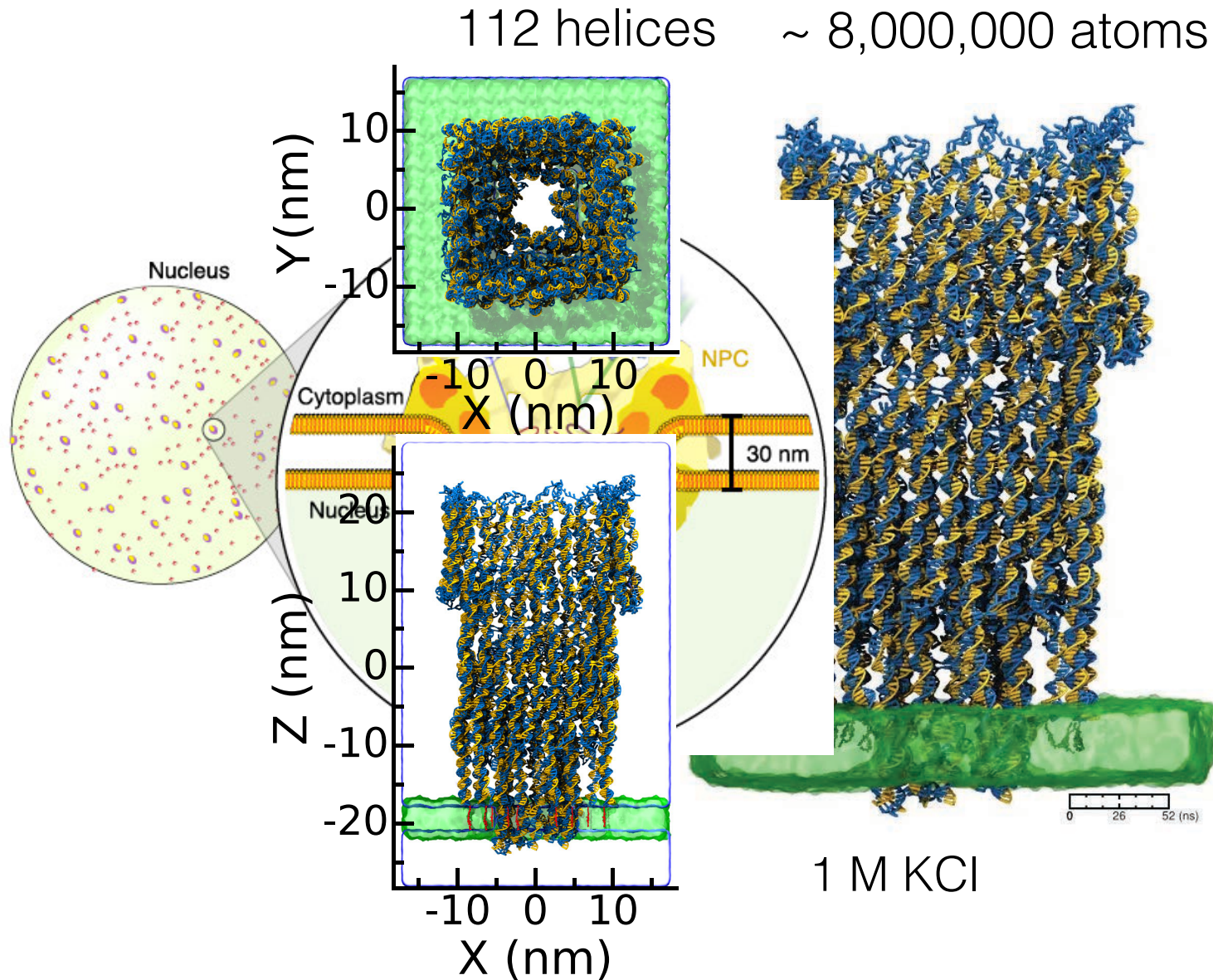
Ulrich Keyser (Cambridge)

Eugen Stulz (Southampton)

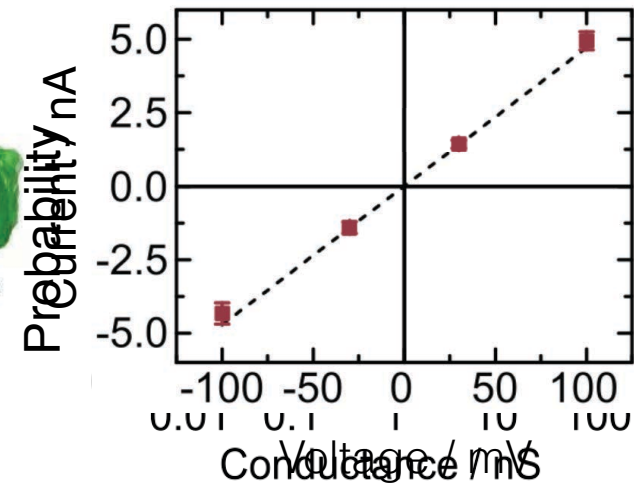
Mathias Winterhalter (Jacobs U)

Goepfrich, et al., Nano Lett 16: 4665 (2016)

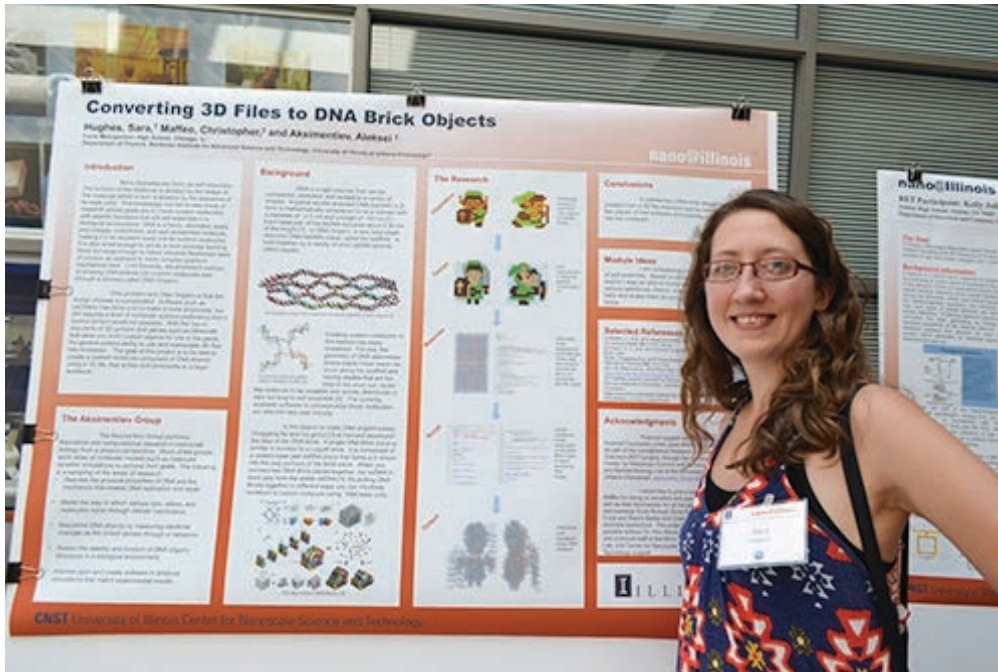
Large conductance DNA channel



Conductance: ~ 30 nS



Opportunities in Education and Outreach



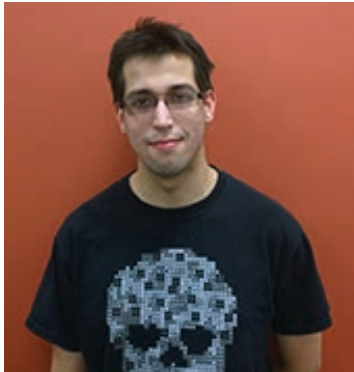
Sara Huges, high school teacher from Chicago (summer 2015)

Developed a lesson "Extending Molecular Geometry: Nanotechnology"



Web implementation enable exploration

Ongoing projects with local high school (Uni High, Urbana)



Michael Slone



Chen-Yu Li



Chris Maffeo

<http://bionano.physics.illinois.edu/dna-nanotechnology>