DNA Nanotechnology with Molecular Simulations

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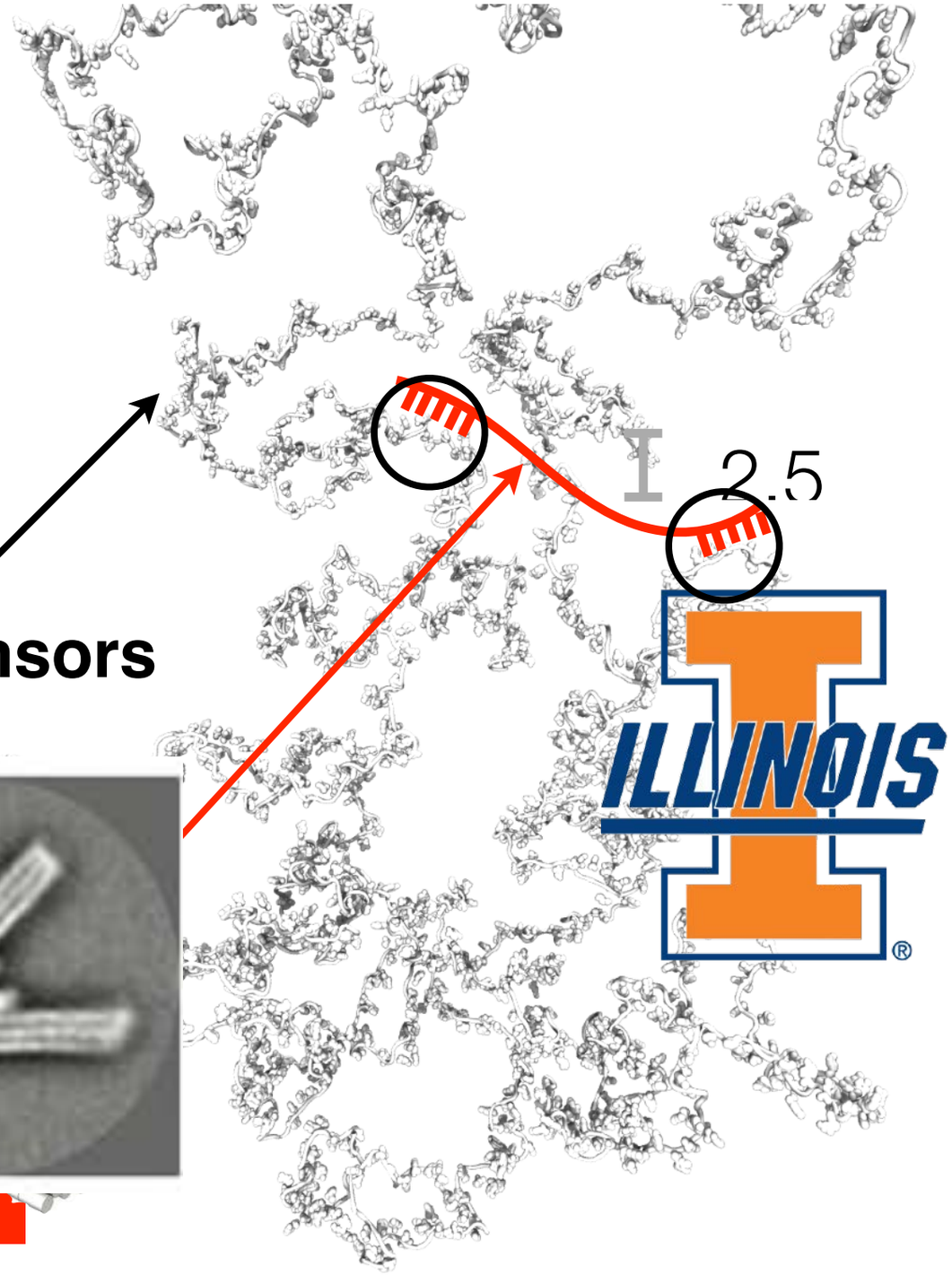
Department of Physics, University of Illinois

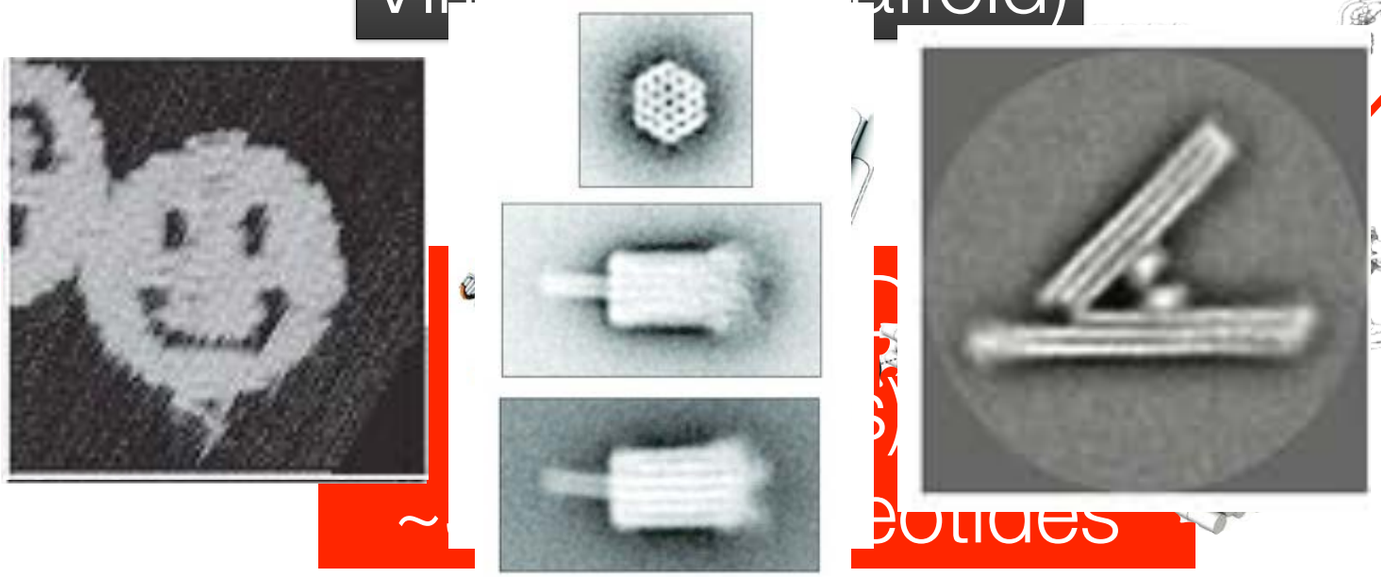
Structural DNA Nanotechnology

DNA origami: a method to **program self-assembly** of custom-shape 3D nanostructures

- Nanometer-scale precision
- High yield
- No expensive fabrication facilities



Custom shapes, channels, and sensors



Nadrian Seeman
Paul Rothemund

William Shih
Hendrik Dietz

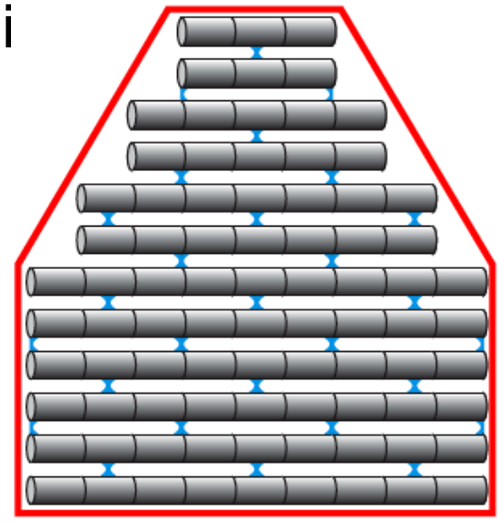
For illustration, an unfolding trajectory at a high temperature is played backward.

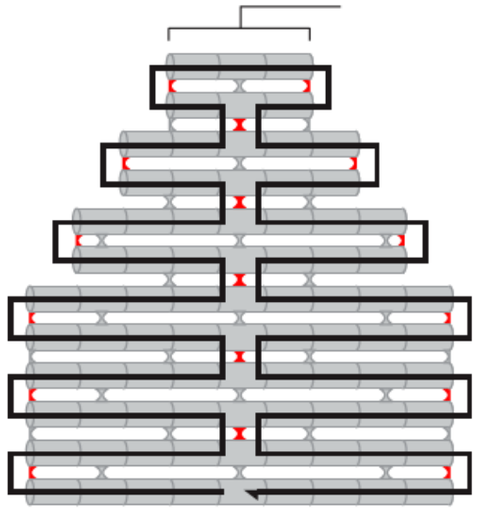
Computer models shaped the field

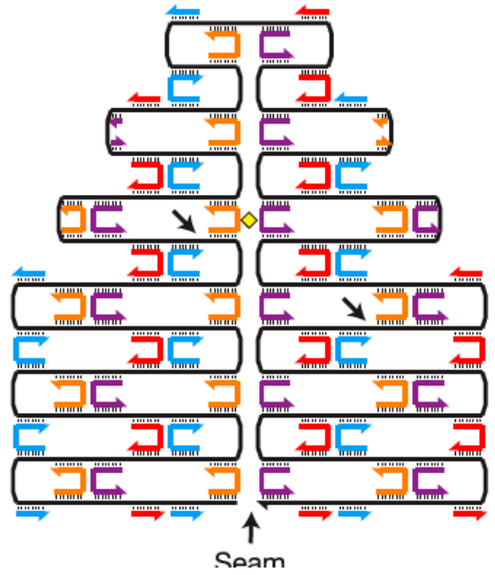
Paul Rothemund
(Caltech)

2D DNA origami

NATURE Vol 440:297
(2006)

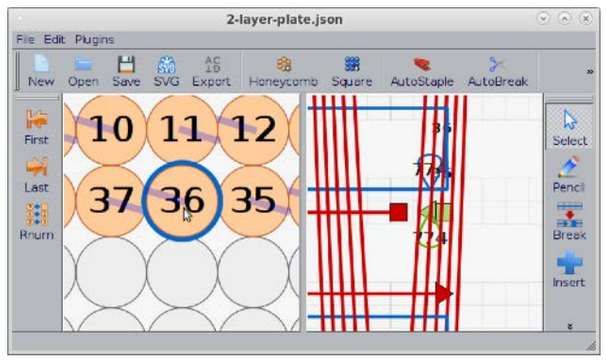


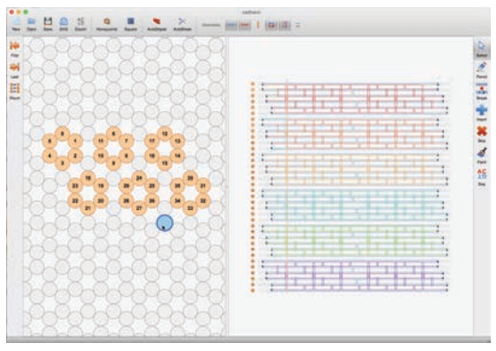


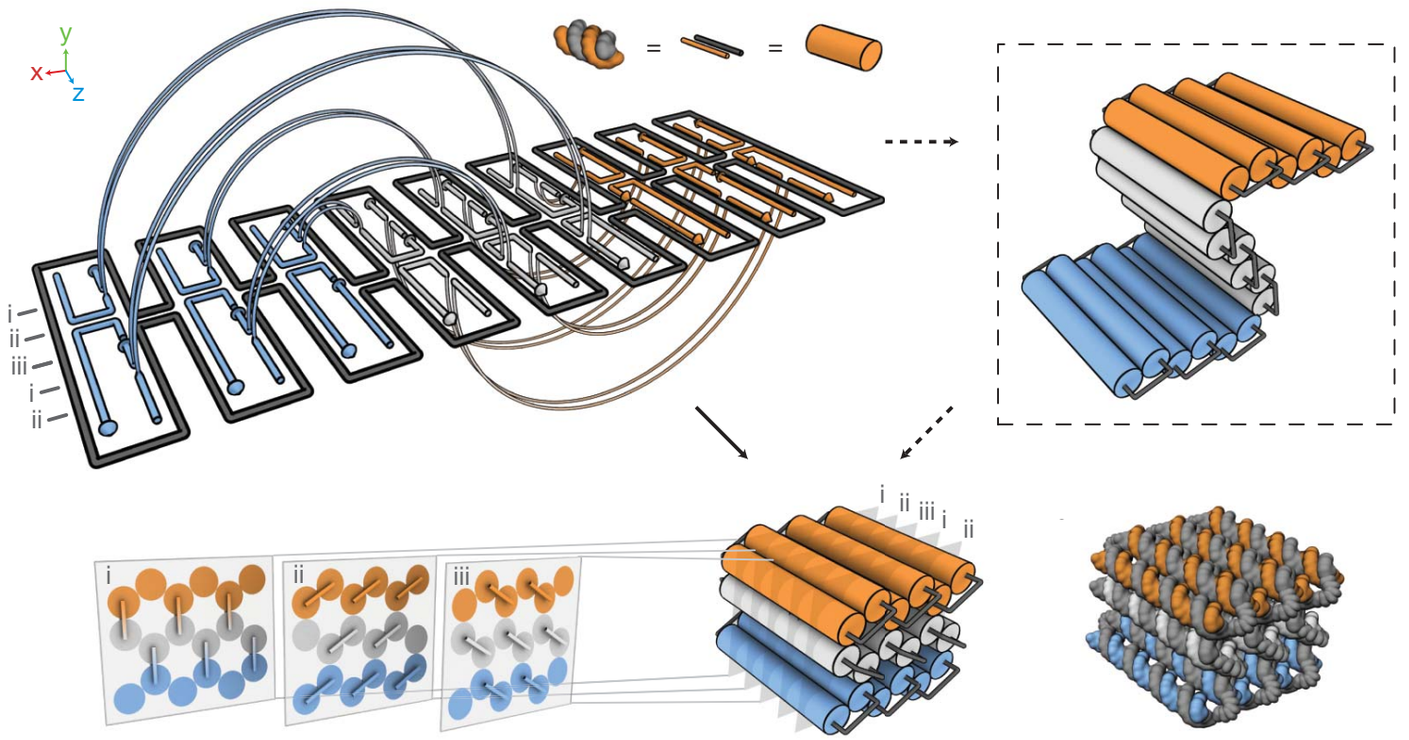


Computer-aided design of DNA origami with **caDNAno** (Shih group, Harvard U.)

Shawn Douglas
(UCSF)



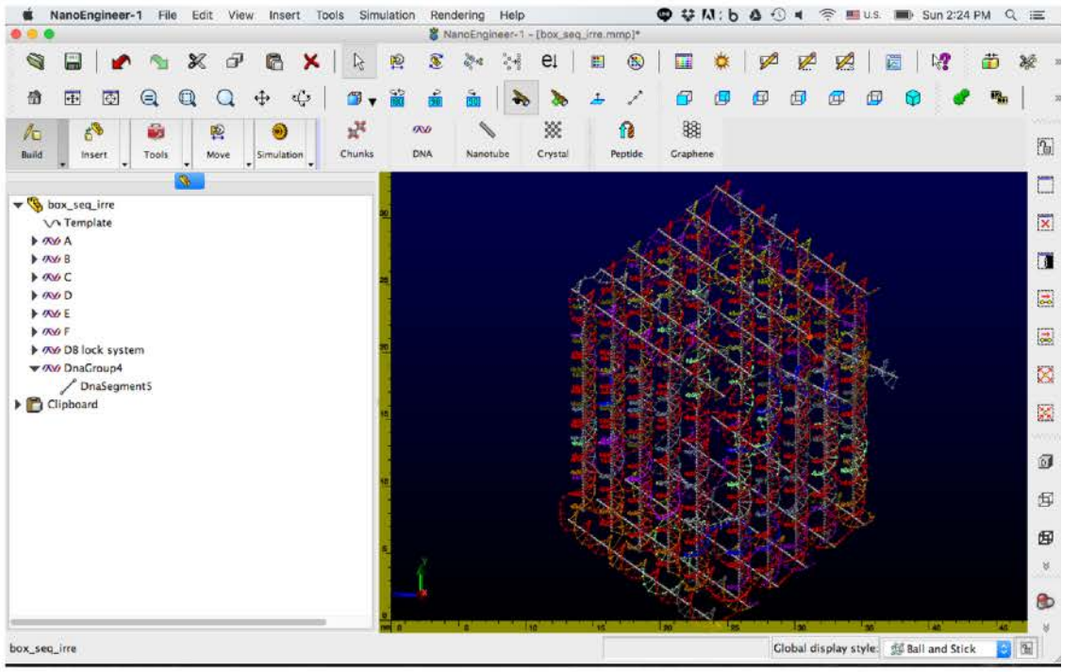




S.M. Douglas, at el. Nature (2009)

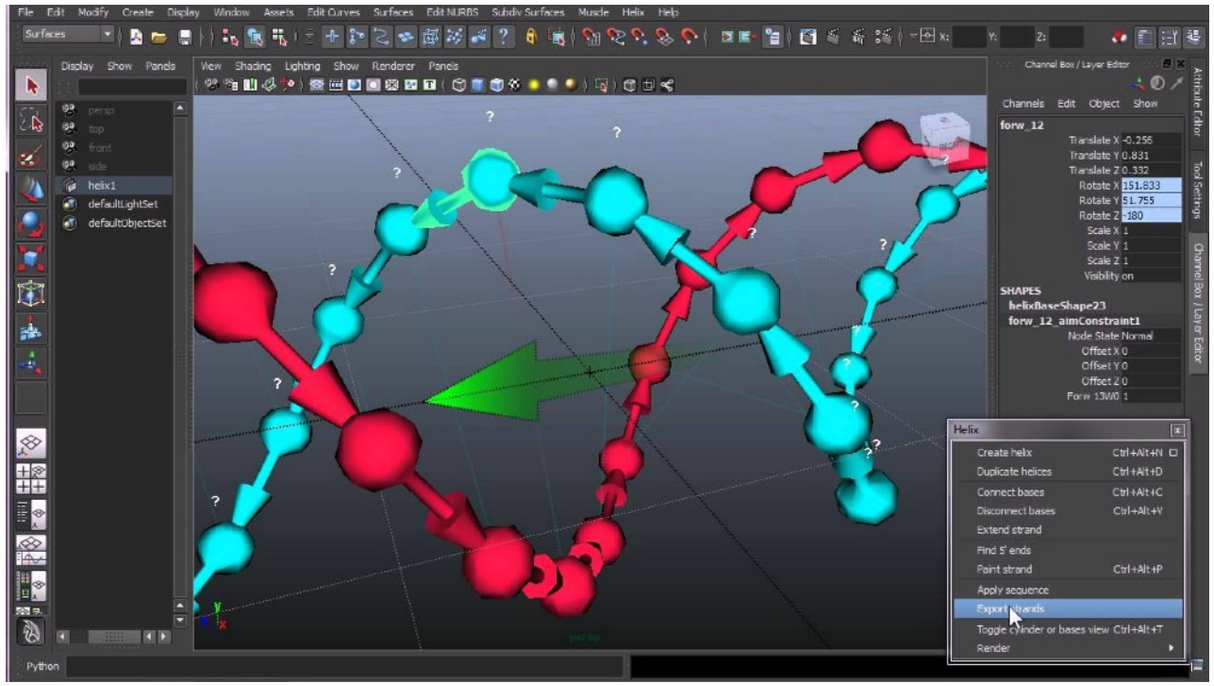
Other DNA structure design tools

NanoEngineer-1

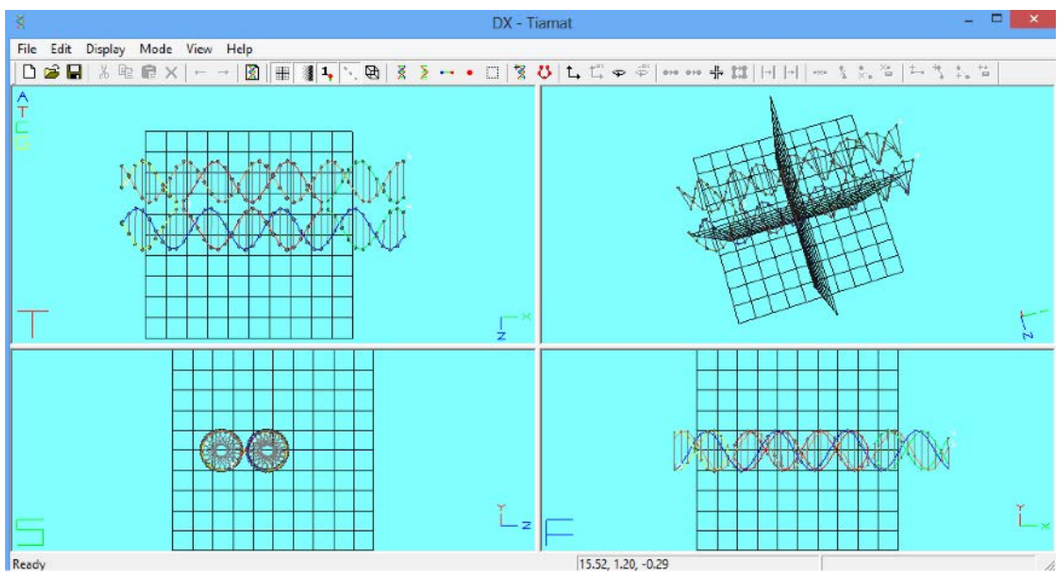


Nanorex, Inc

vHelix (Bjorn Hogberg, Karolinska)



Tiamat (Hao Yan, ASU)



DAEDALUS: DNA Origami Sequence Design Algorithm for User-defined Structures

(Mark Bathe, MIT)

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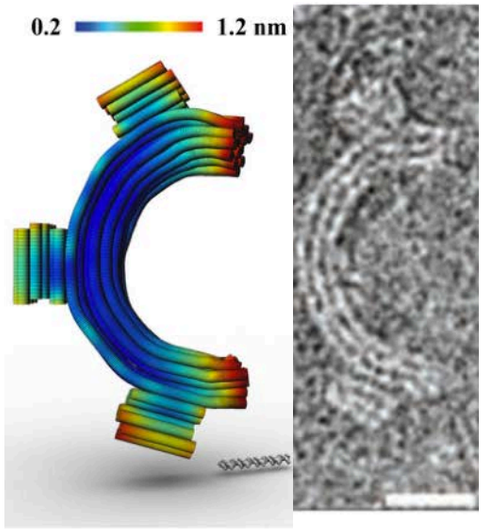
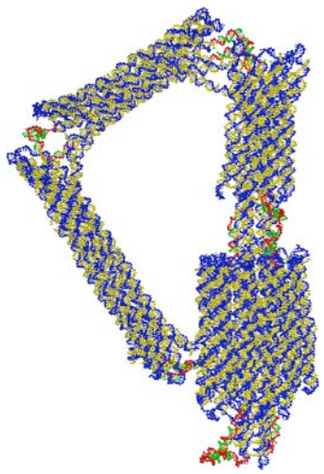
Physics-based computational model of DNA nanostructure

CanDo



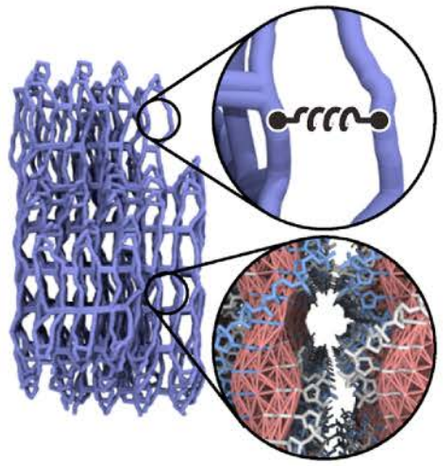
Nature Methods 8:221

oxDNA



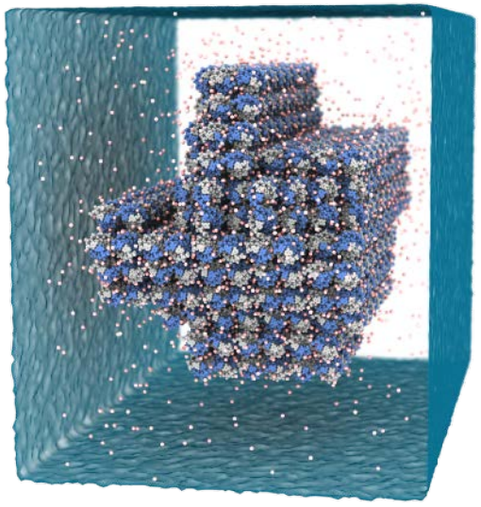
ACS Nano 11:12426

ENRG MD

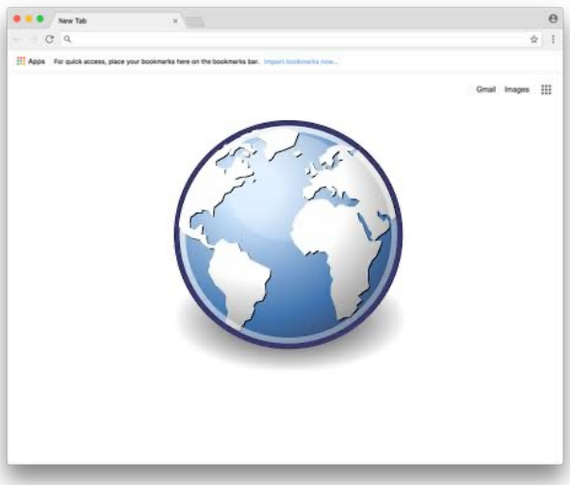


Nucleic Acids Research 44:3013

All-atom MD



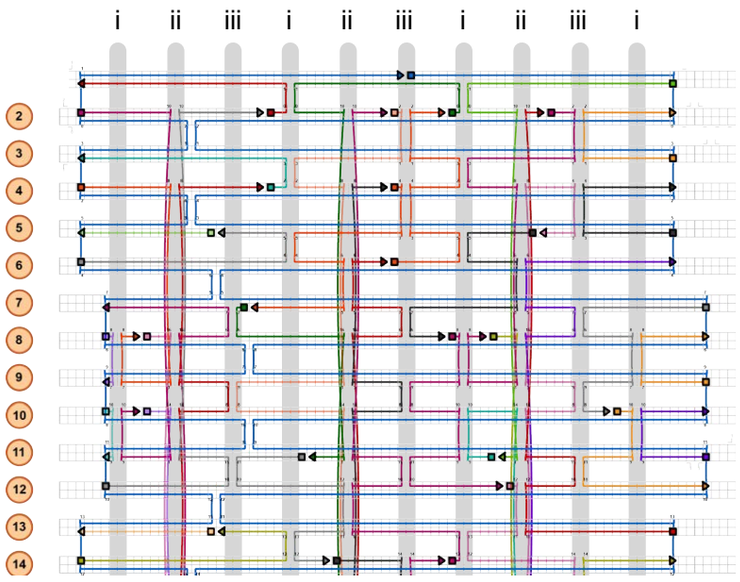
PNAS 110:20099 (2013)

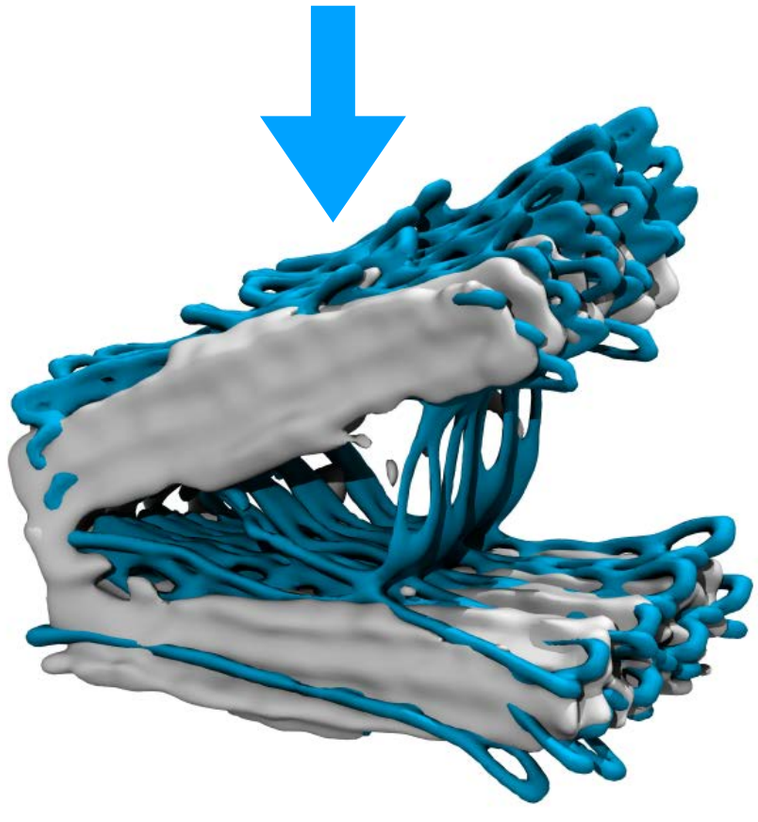


Faster dynamics, less computation

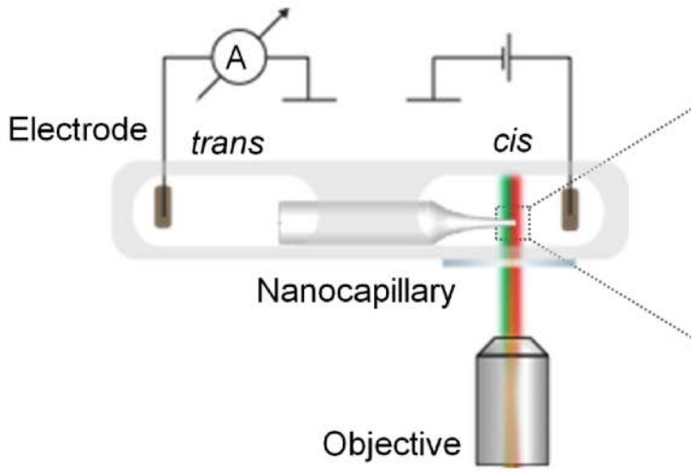
More detail

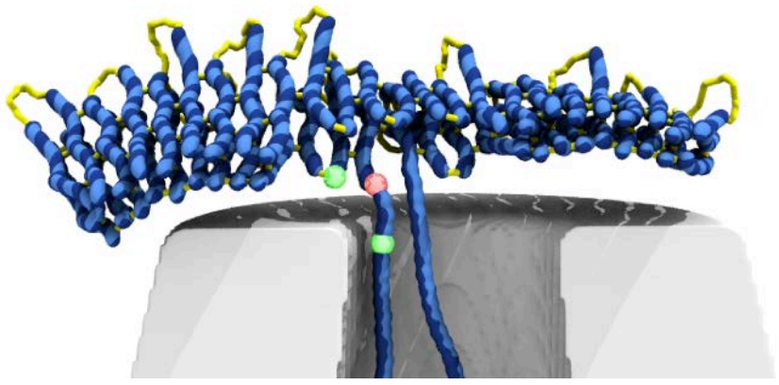
Typical problems

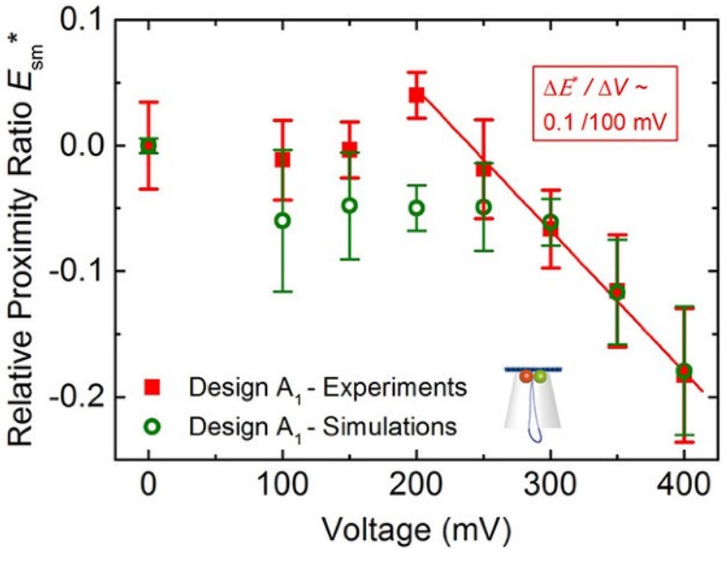




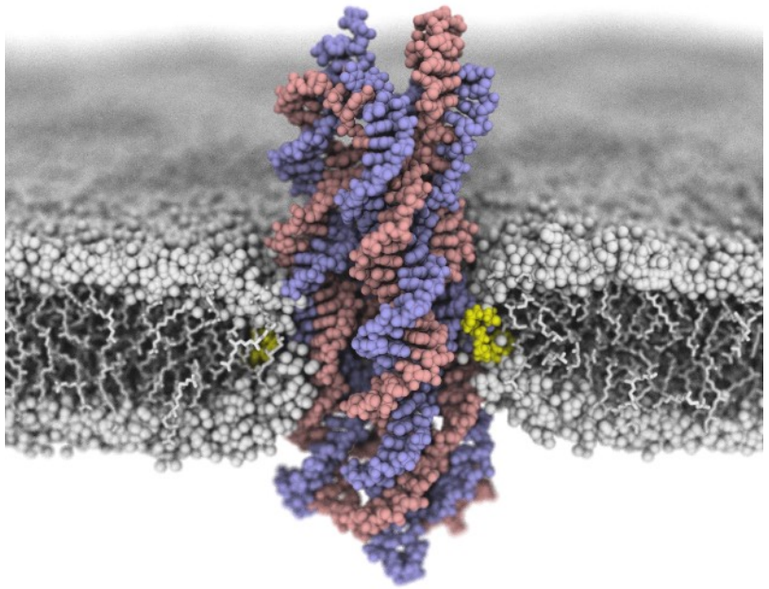
Structure prediction
doi: 10.1101/865733

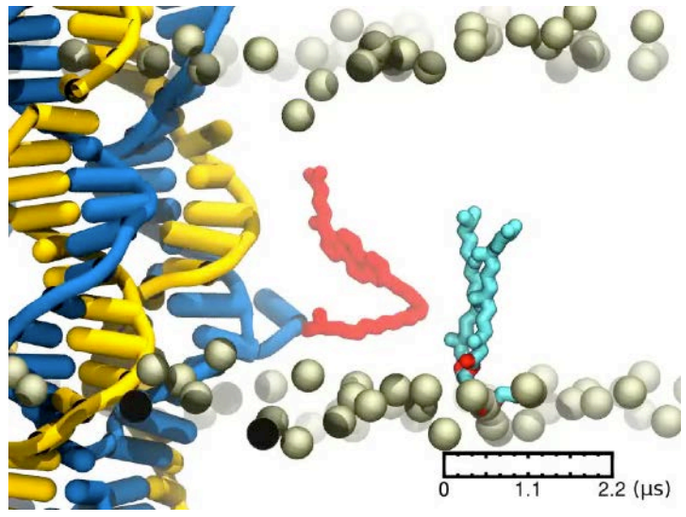


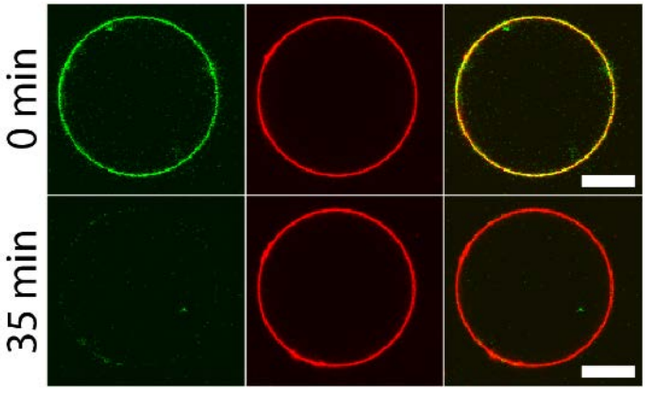




Interpretation of experiment
Nano Letters 18:1962 (2018)



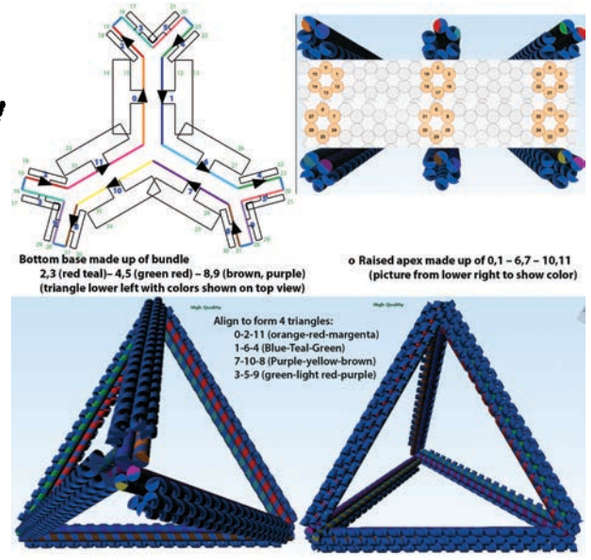




Design of new systems
Nature Comm. 9:2426 (2018)

The DNA origami design process

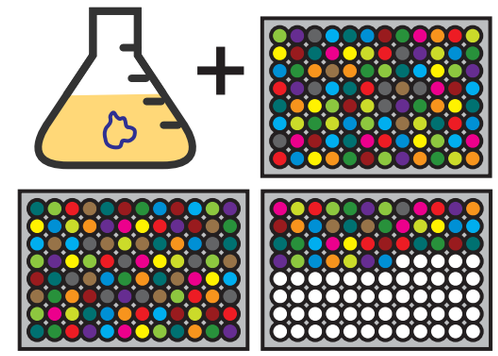
Schematic model



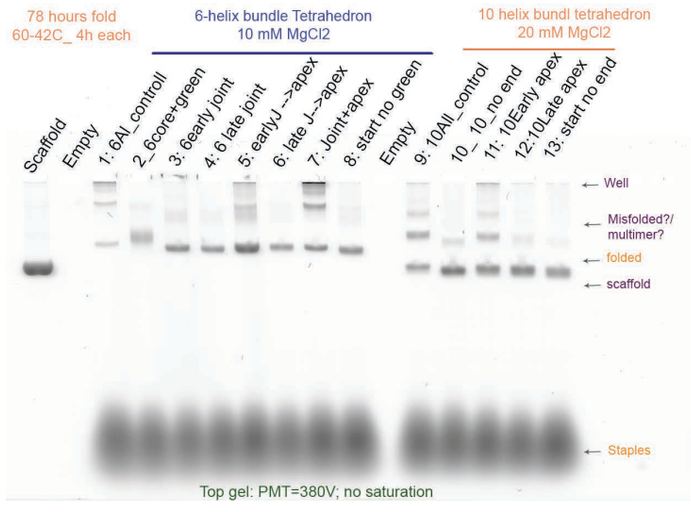
Sequence assignment

```
ATCTGACTGATCGTACGT
CATGCTAGCATGCCGCGGCG
CGTAGCTGCATGATCGTACG
GATGATCGGGTTACTGC
ACTGTCGTGACTGACTAG
TACTACTCTCGCGCGCGTAC
ATTATTGAGCGTCACTGCTG
TGATCGTACGTACGTA
TGATCGTACGTACGATCGTAG
ATCGTAGCTACGTACGTACG
GCTGGGCGCGTATCGATTTAT
....
```

DNA synthesis

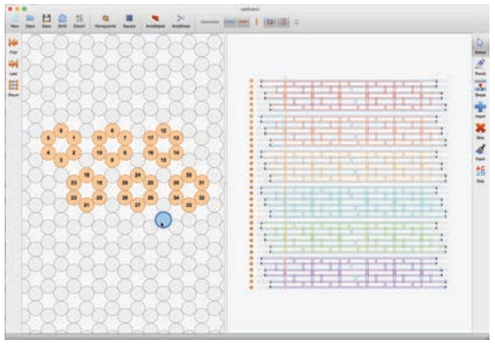


Assembly

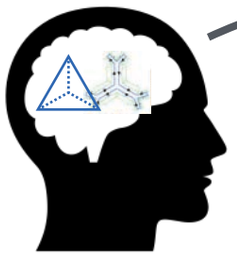


Characterization

DNA origami



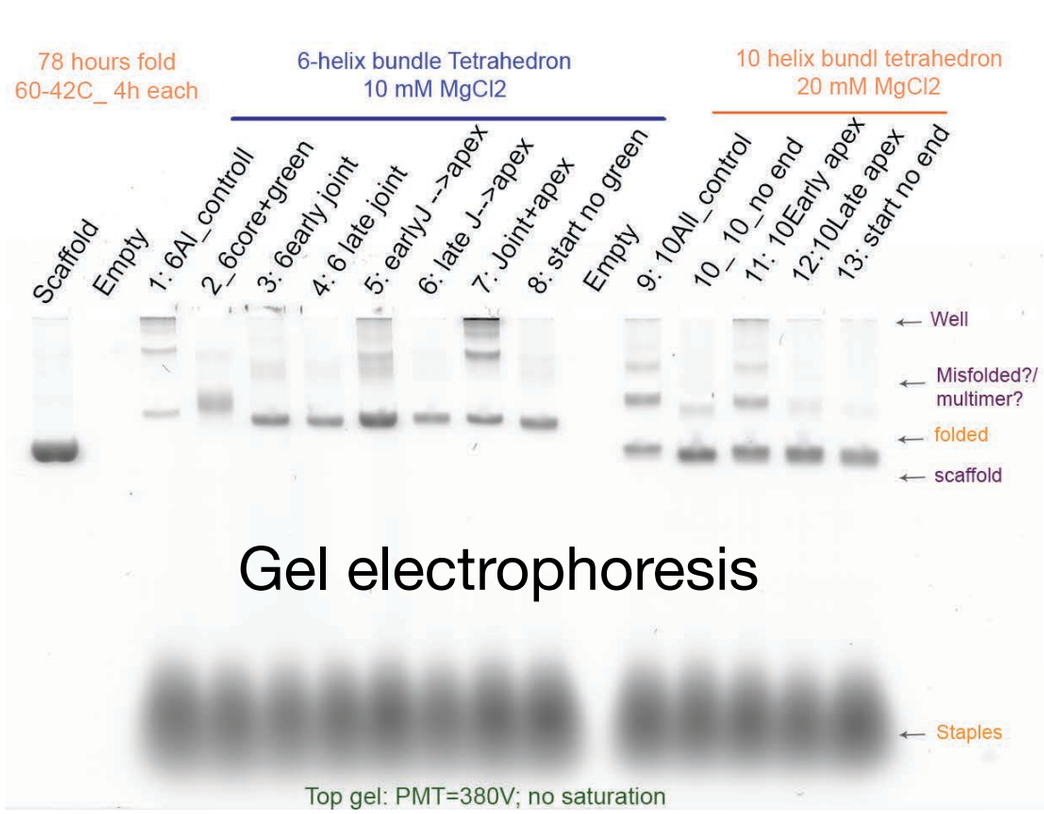
Cadnano



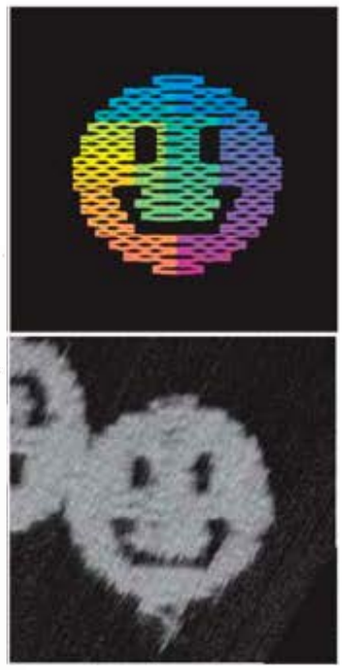
3–4 weeks for

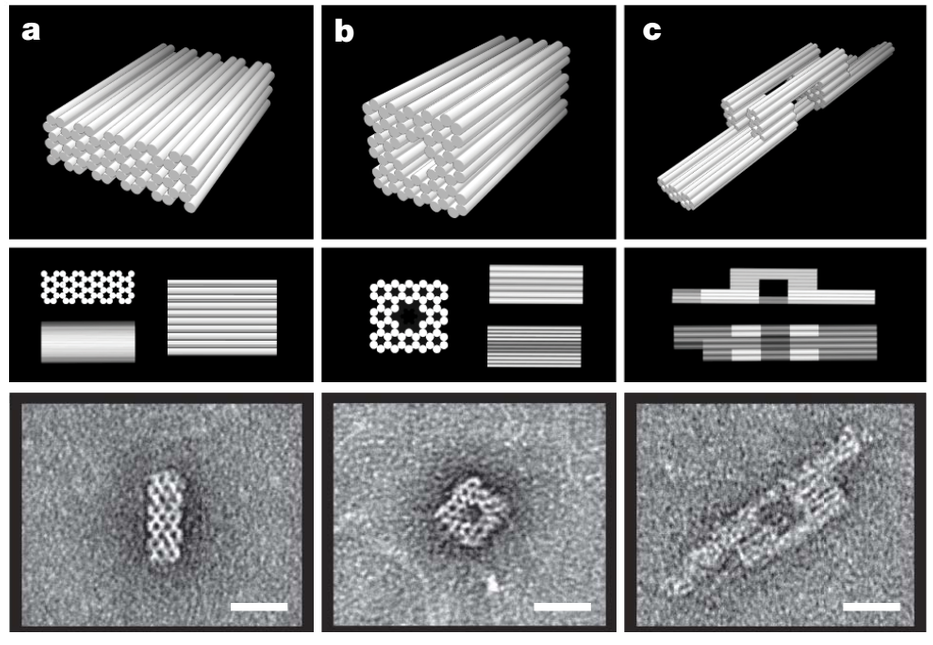
< 24 hours through simulation feedback (*Nucleic Acids Research* 48: 5135)

Experimental characterization of DNA nanostructures



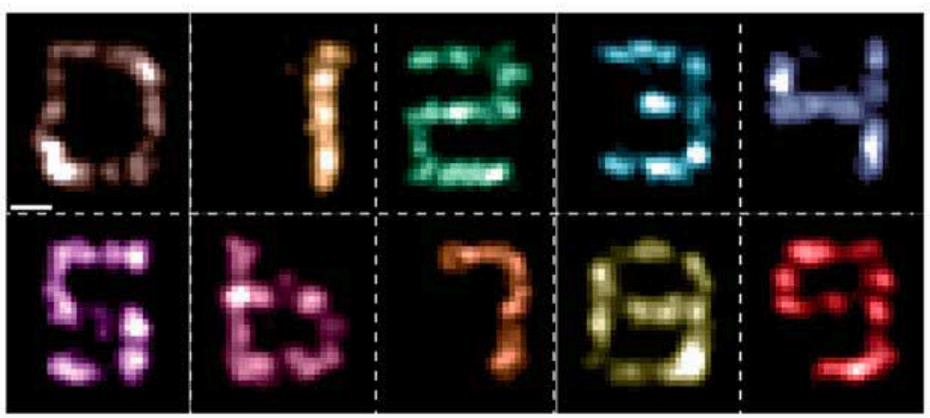
Gel electrophoresis



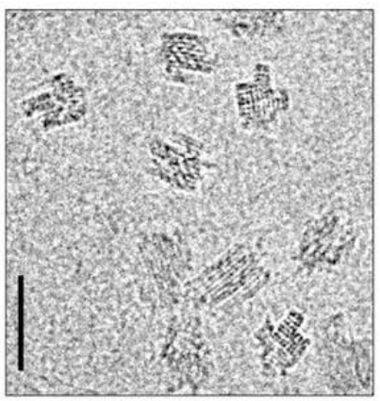


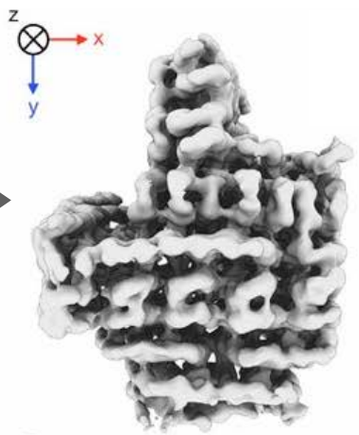
Atomic force / Transmission electron microscopy

Fluorescence



DNA-PAINT super-resolution imaging
DOI: 10.1007/978-1-4939-6454-3_13





Cryo-EM reconstruction, the only experimentally derived structural model

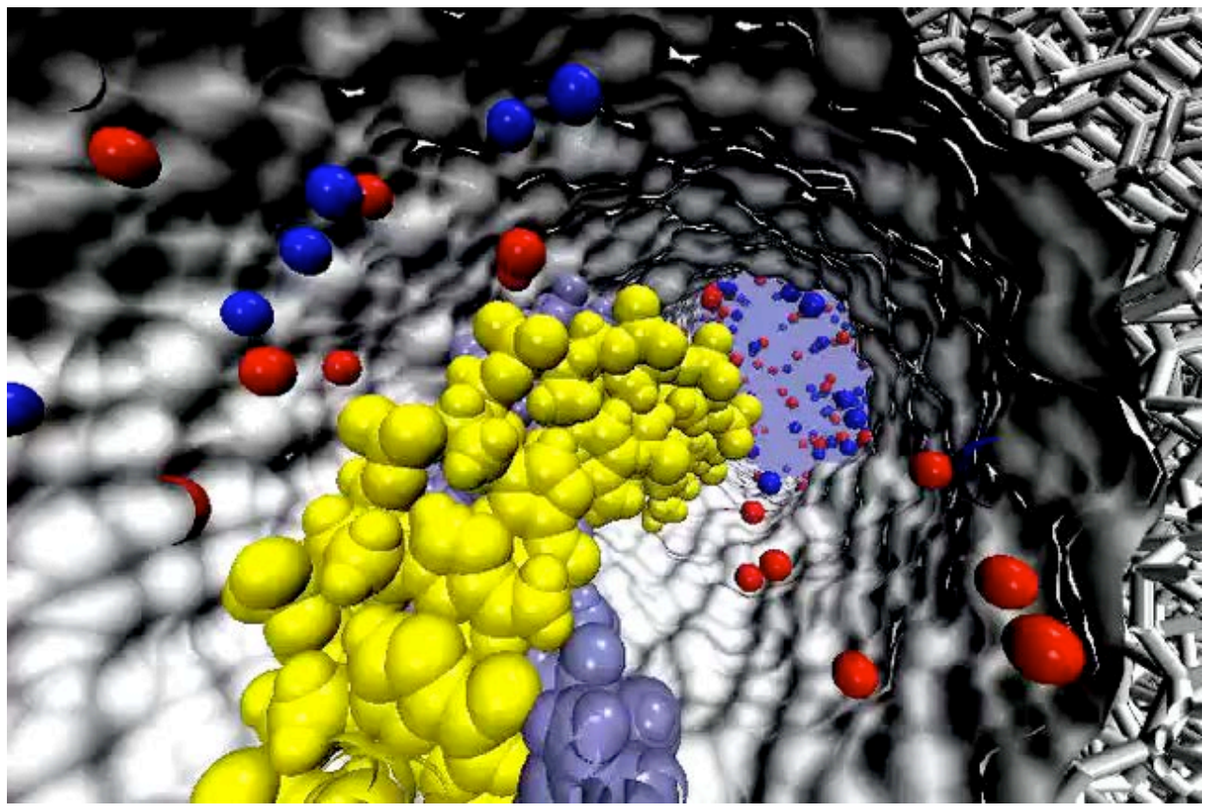
Bai, … , Dietz, PNAS (2012)

All-atom molecular dynamics simulations: the computational microscope

Klaus Schulten

Massive parallel computer
Blue Waters (UIUC): ~200,000 CPUs

Atoms move according to
classical mechanics (F= ma)



Time scale: ~ 0.1-100 μs

Length scale: 10K - 1000M atoms or (< 70 nm)^3

Time resolution: 2 fs

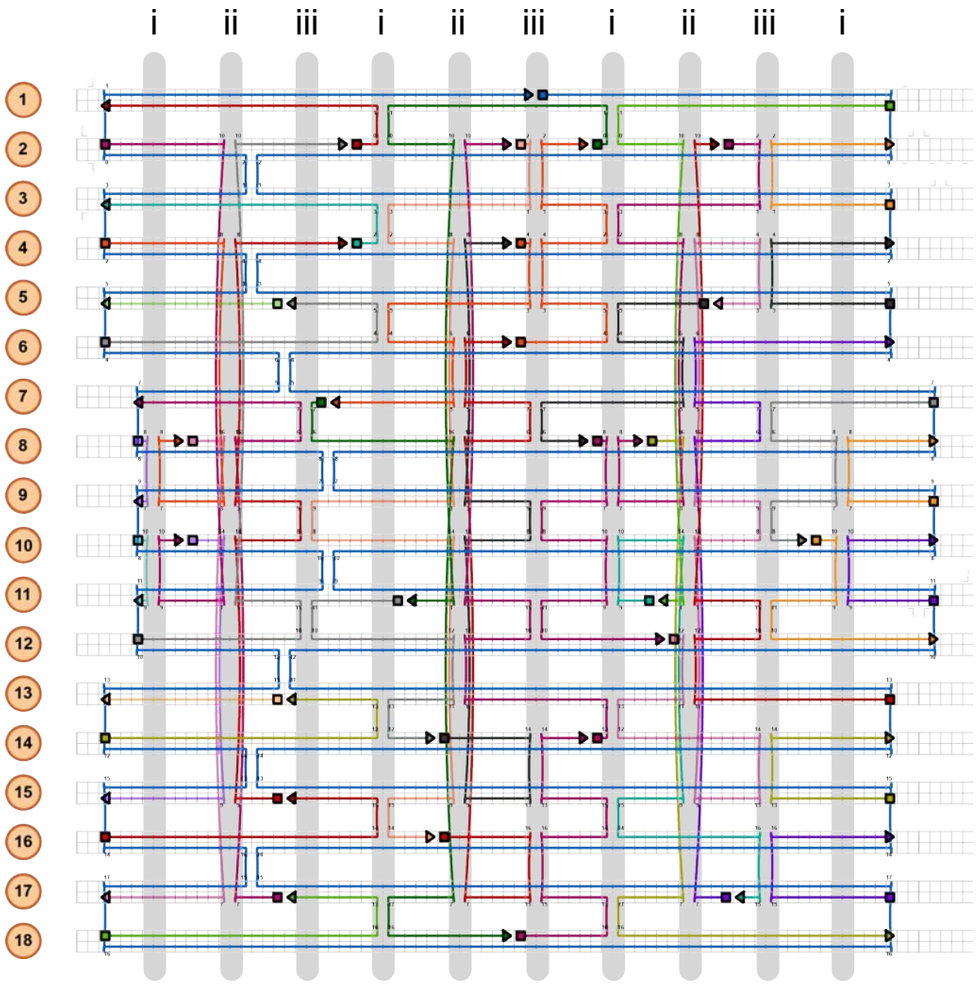
Spacial resolution: 0.1 A

Interaction between atoms is
defined by molecular force field

From caDNAno to all-atom

Jejoong Yoo

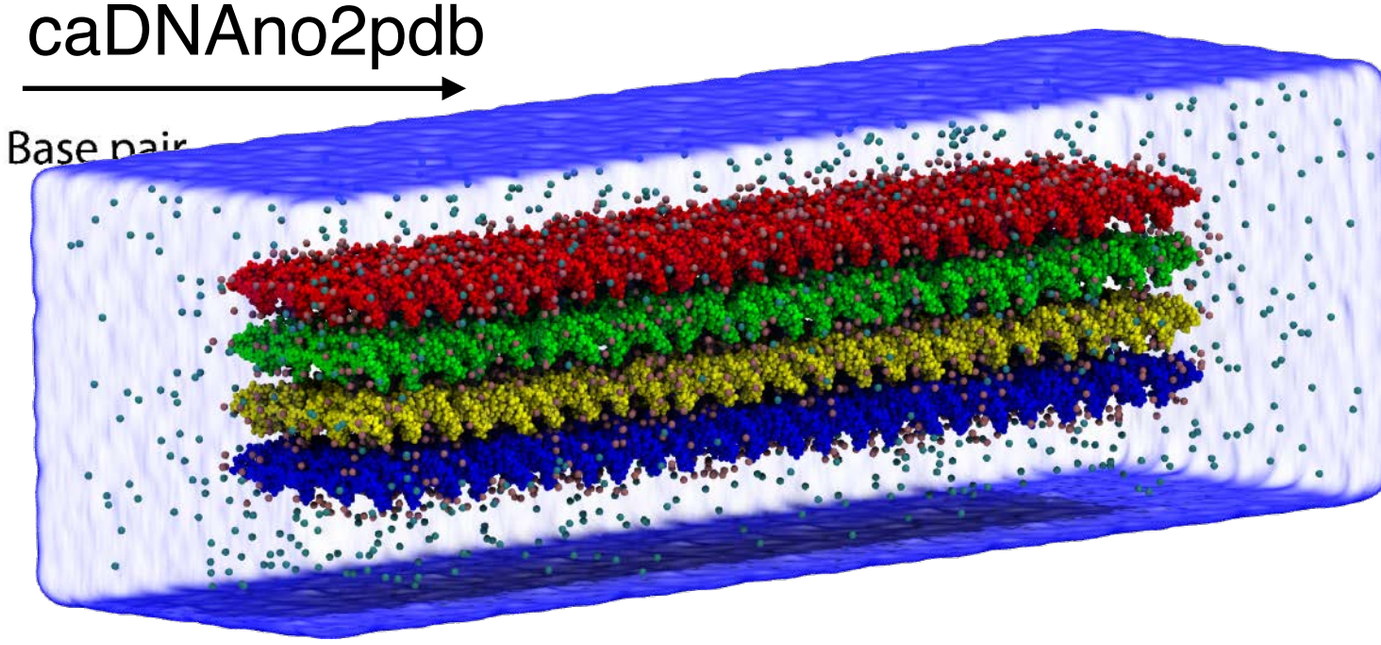
Cadnano design



SM. Douglas,…, WM. Shih,
Nucleic Acids Res., 2009

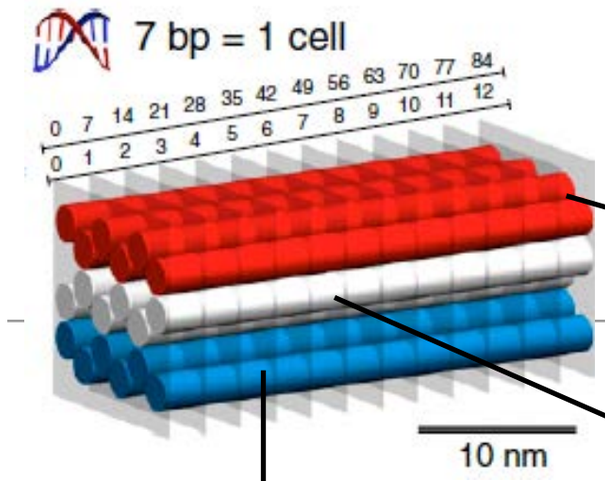
Solvated structure

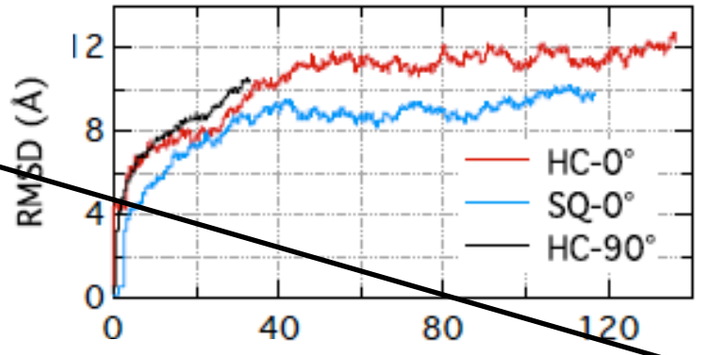
caDNAno2pdb

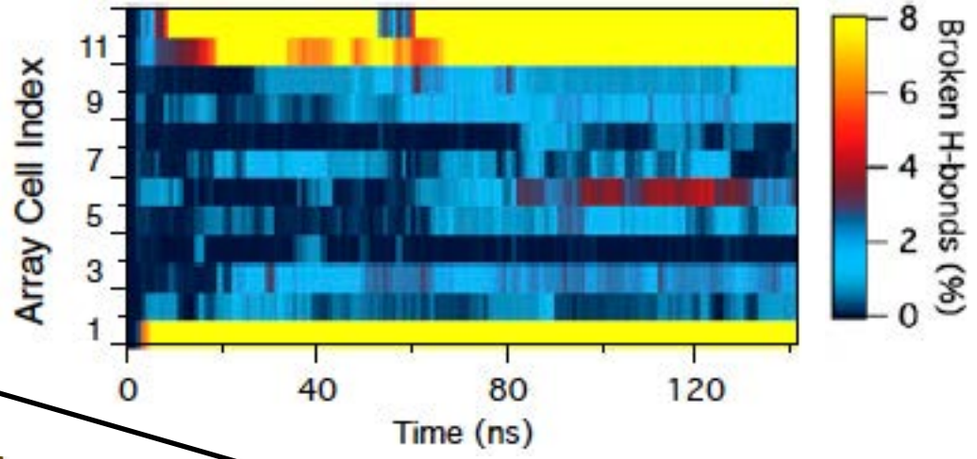


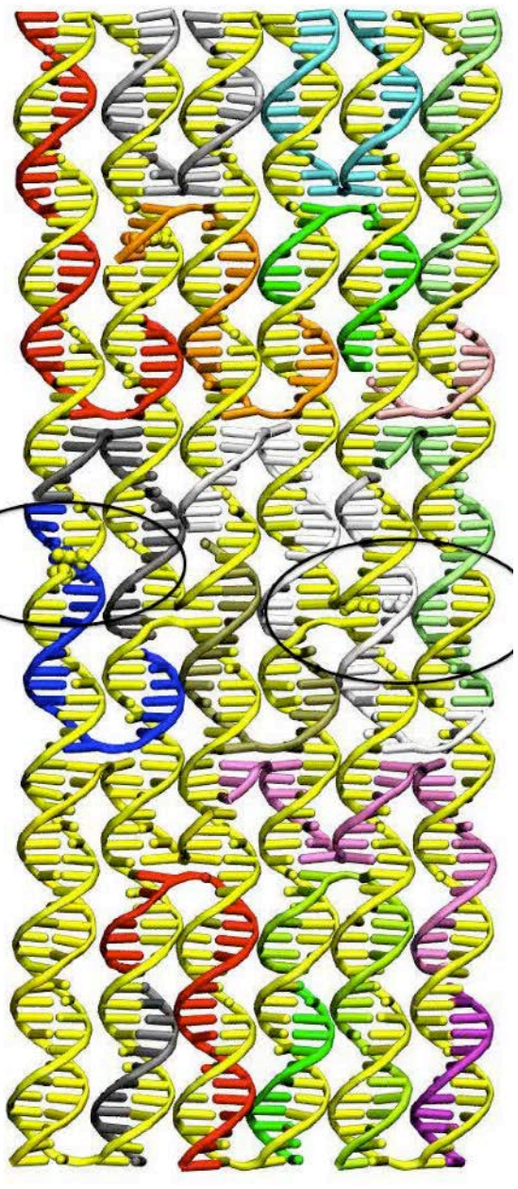
- CHARMM36 force field
- Explicit water
- [$MgCl_2$] ~ 10 mM
- NAMD
- 1 to 3M atoms
- 500 to 1,000 CPUs

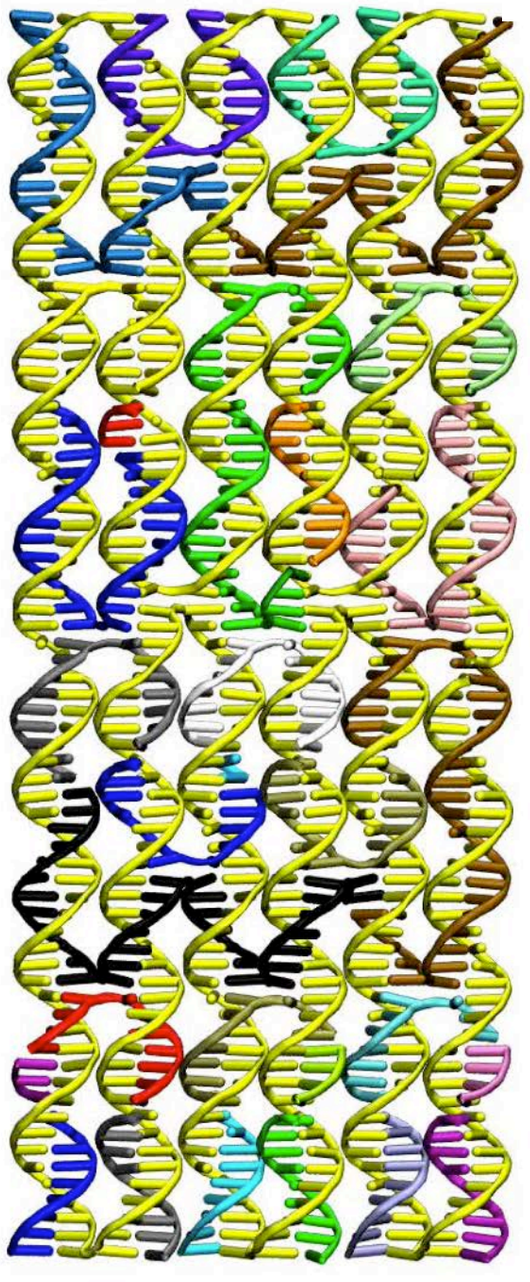
Structural dynamics

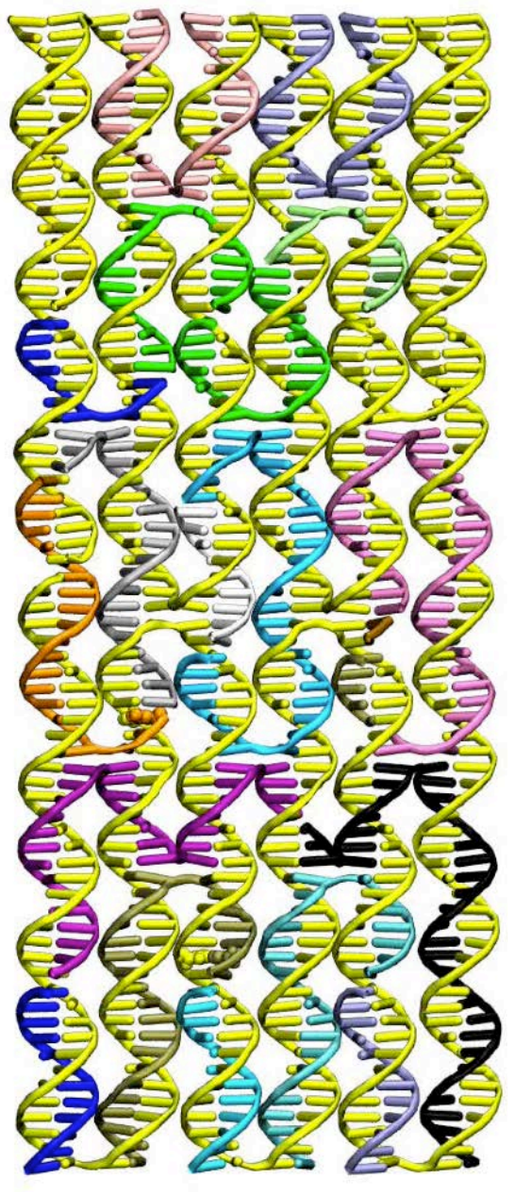






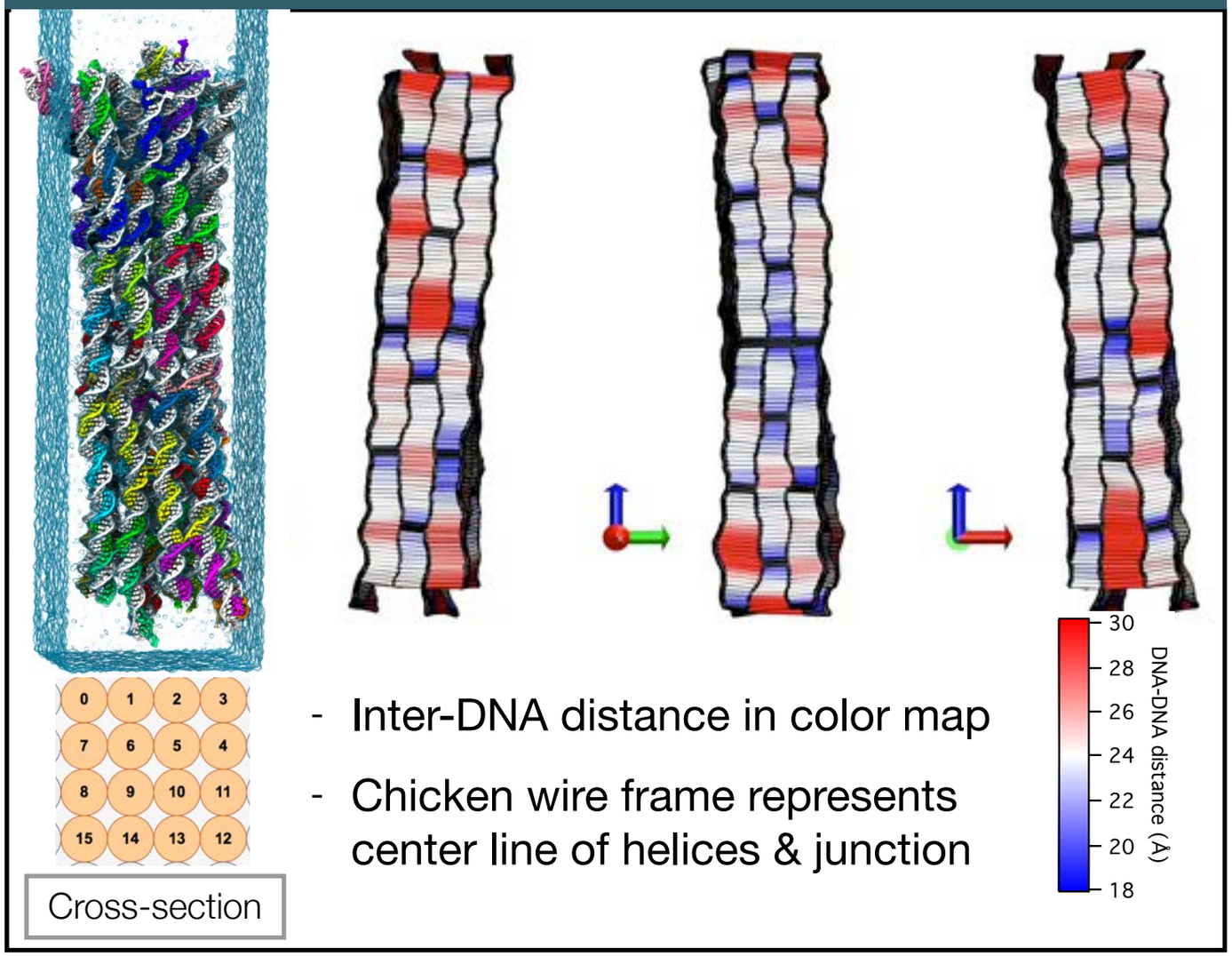




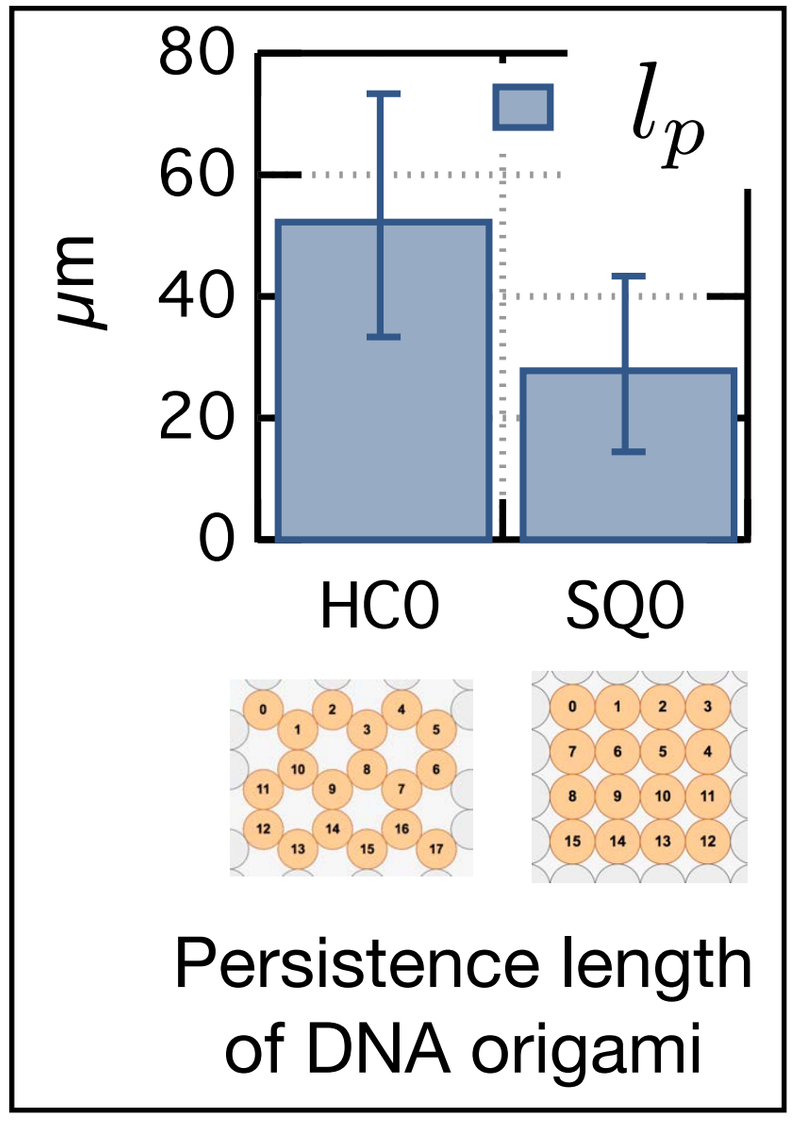


Structural fluctuations reveal local mechanical properties

Structural fluctuation of DNA origami nanorod



Yoo and Aksimentiev, PNAS 110:20099 (2013)



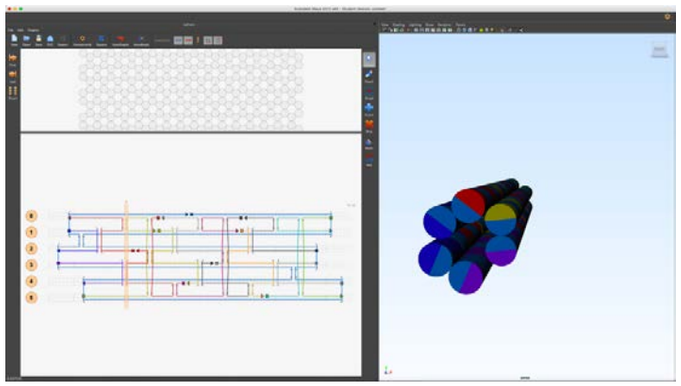
Frenet analysis of MD trajectories characterizes local elasticity

Tutorial: A Practical Guide to DNA Origami Simulations Using NAMD

Methods in Molecular Biology (Springer Nature) 1811: 209-229 (2018)

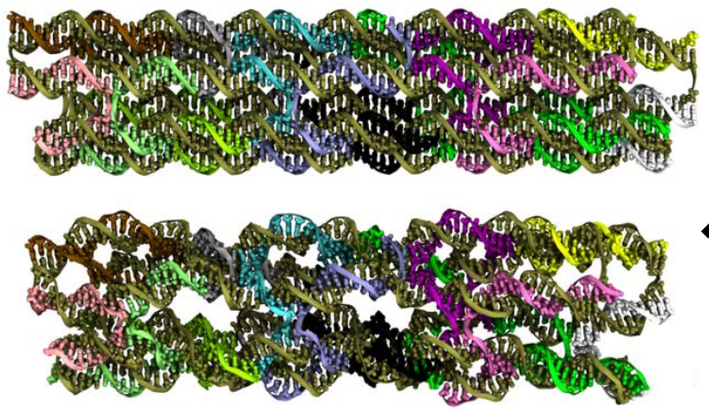
http://bionano.physics.illinois.edu/tutorials/practical-guide-dna-origami-simulations-using-namd

Cadnano design



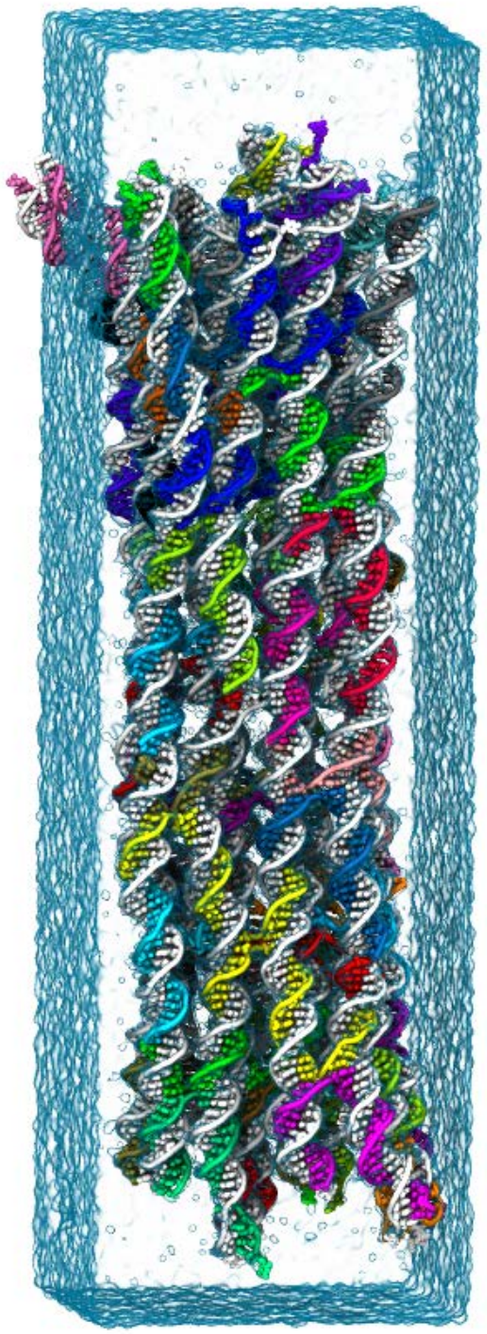
cadnano2pdb web server

All-atom model

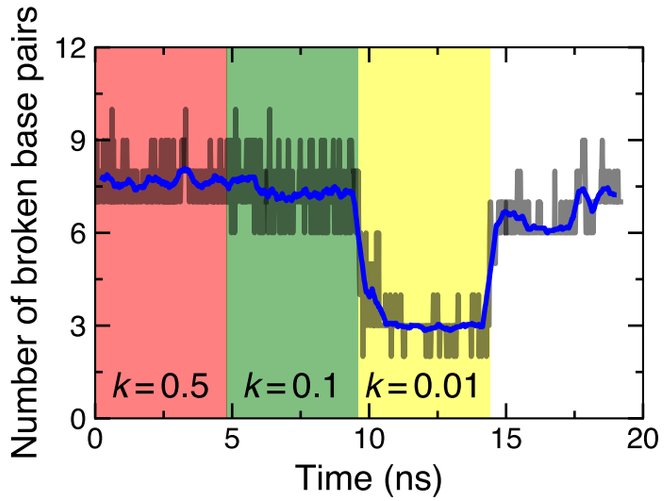


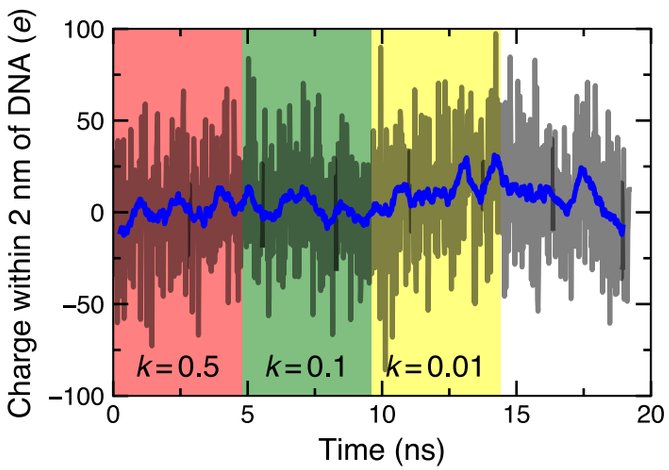
After optimization

All-atom structure in a $MgCl_2$ solution



Perform MD simulation and analysis

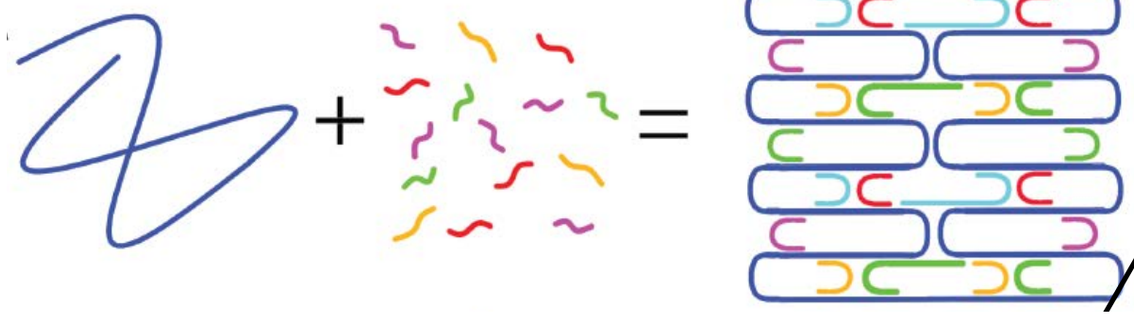


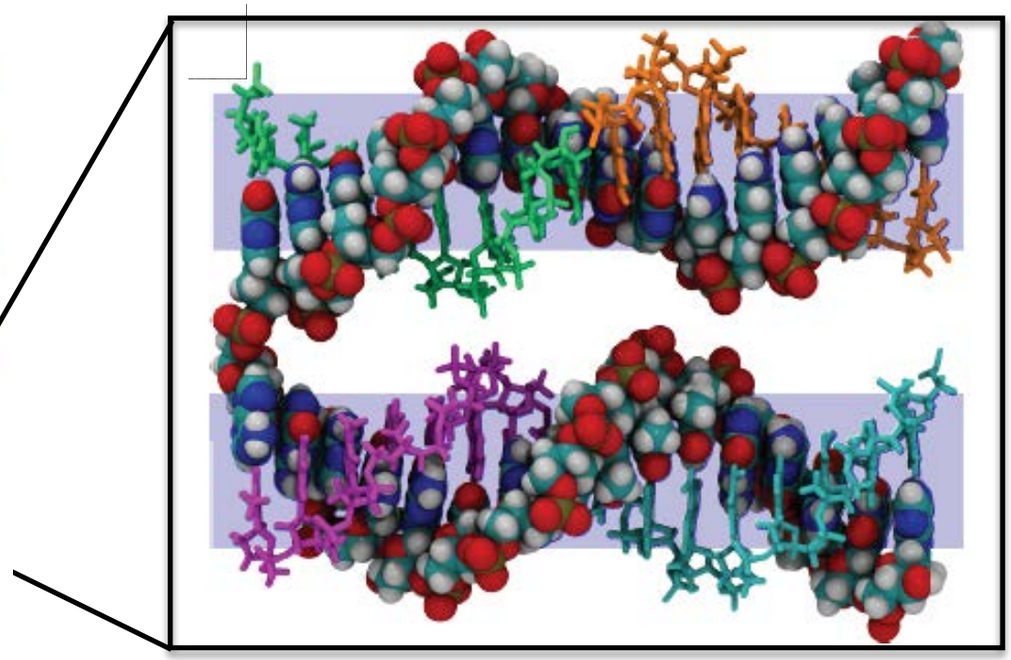


— Walk through the protocol for all-atom simulations of DNA origami using the NAMD package

Tiled DNA nanostructures

DNA Origami

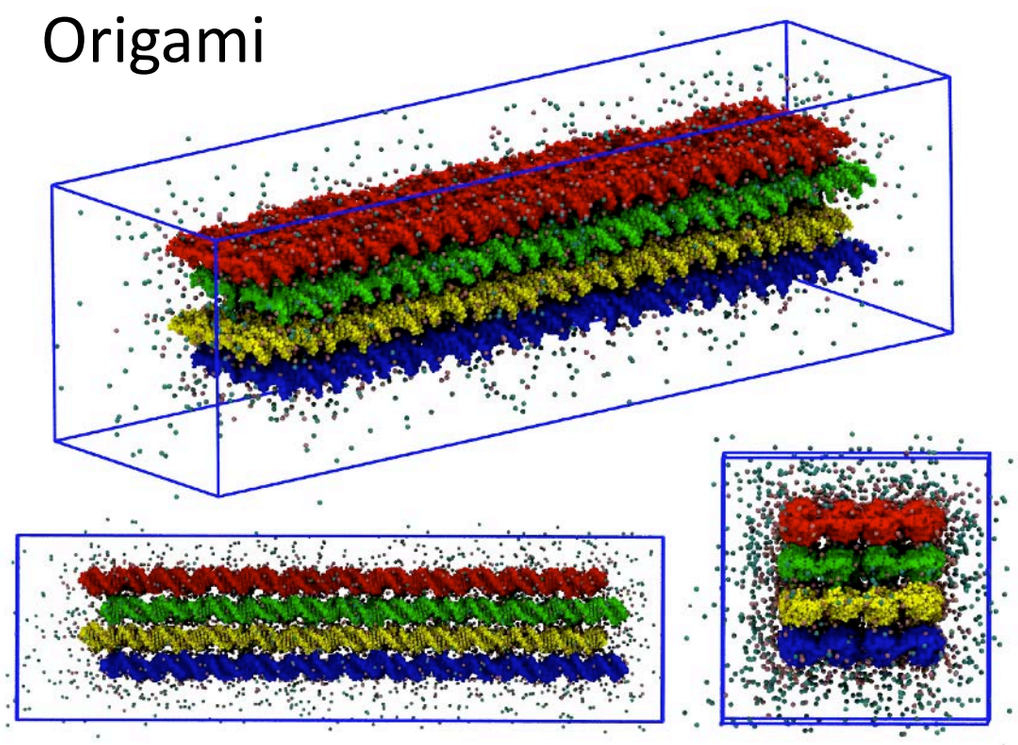




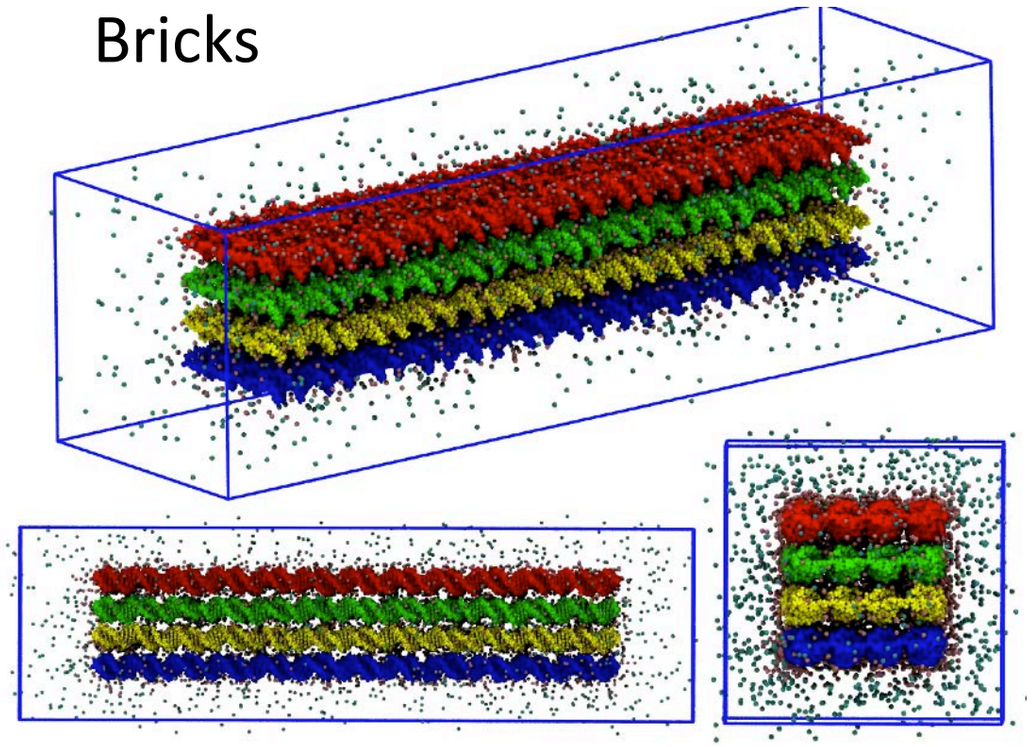
Identical nucleotide sequence in both structures

All-atom MD 165 ns each

Origami



Bricks



Slone et al., New J. Phys. 18:055012 (2016)

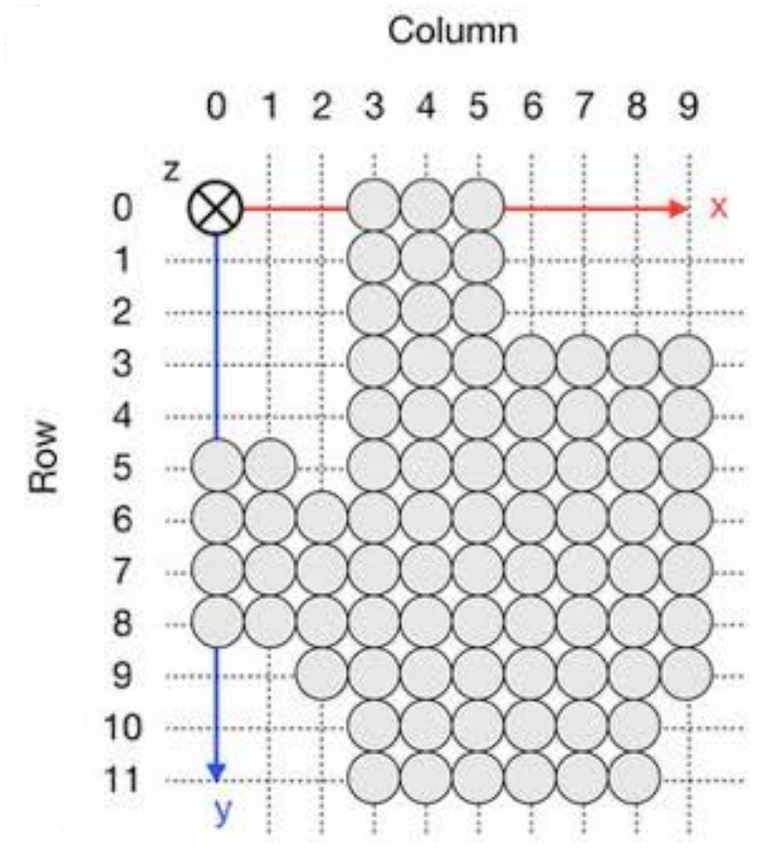
nanohub.org/resources/legogen

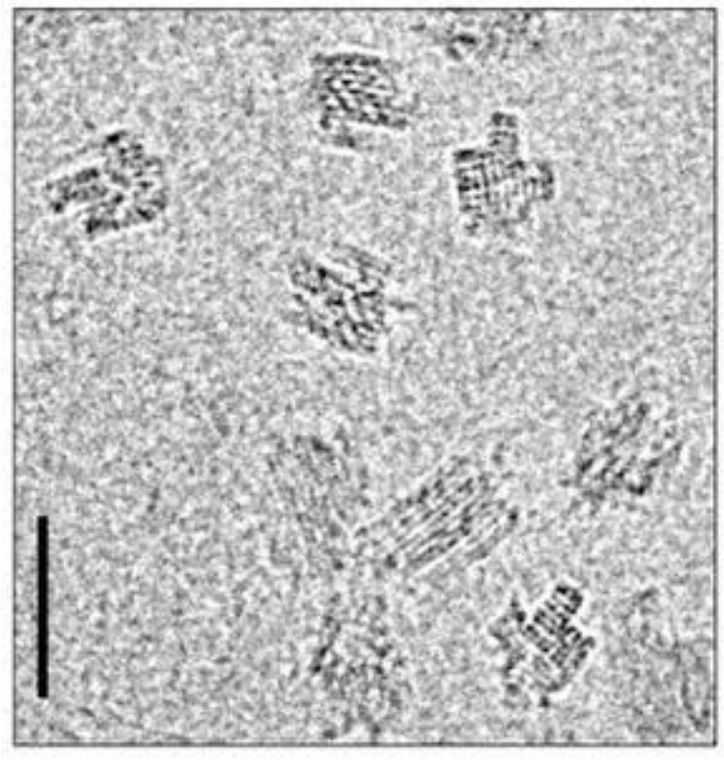
Cryo-EM reconstruction versus all-atom simulation

High-resolution cryo-electron microscop

Petascale computer system

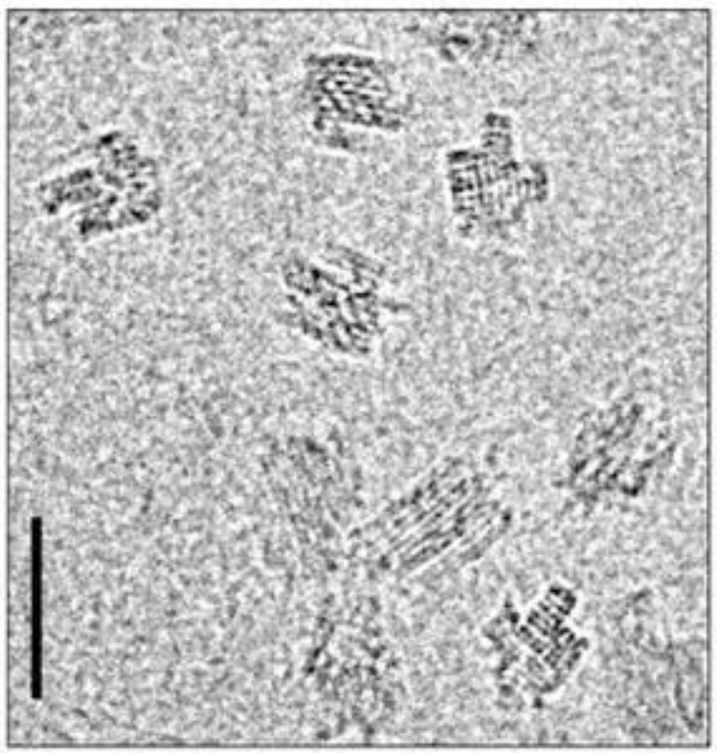
Cryo-EM reconstruction versus all-atom simulation

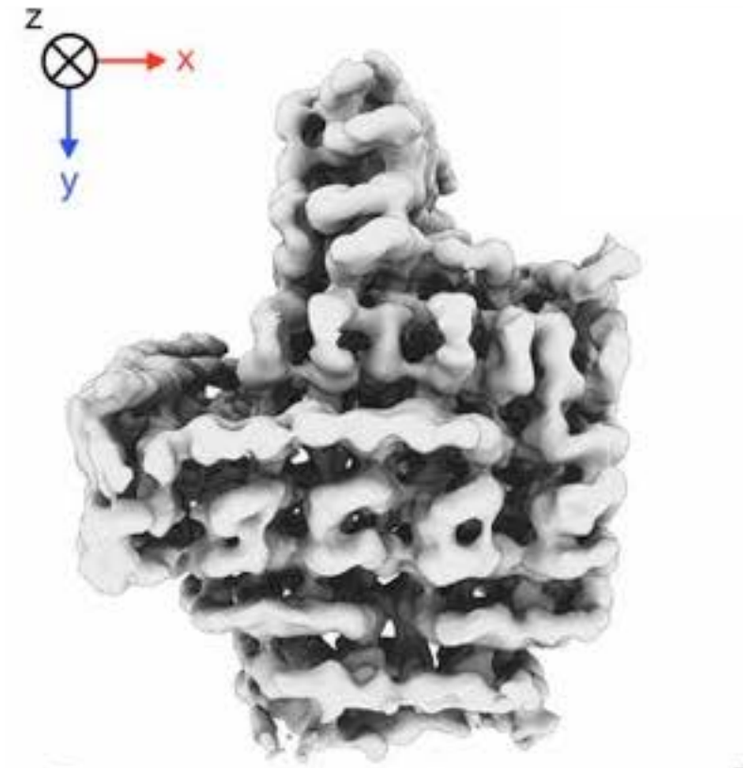




Bai *et al*, PNAS 109:20012 (2012)

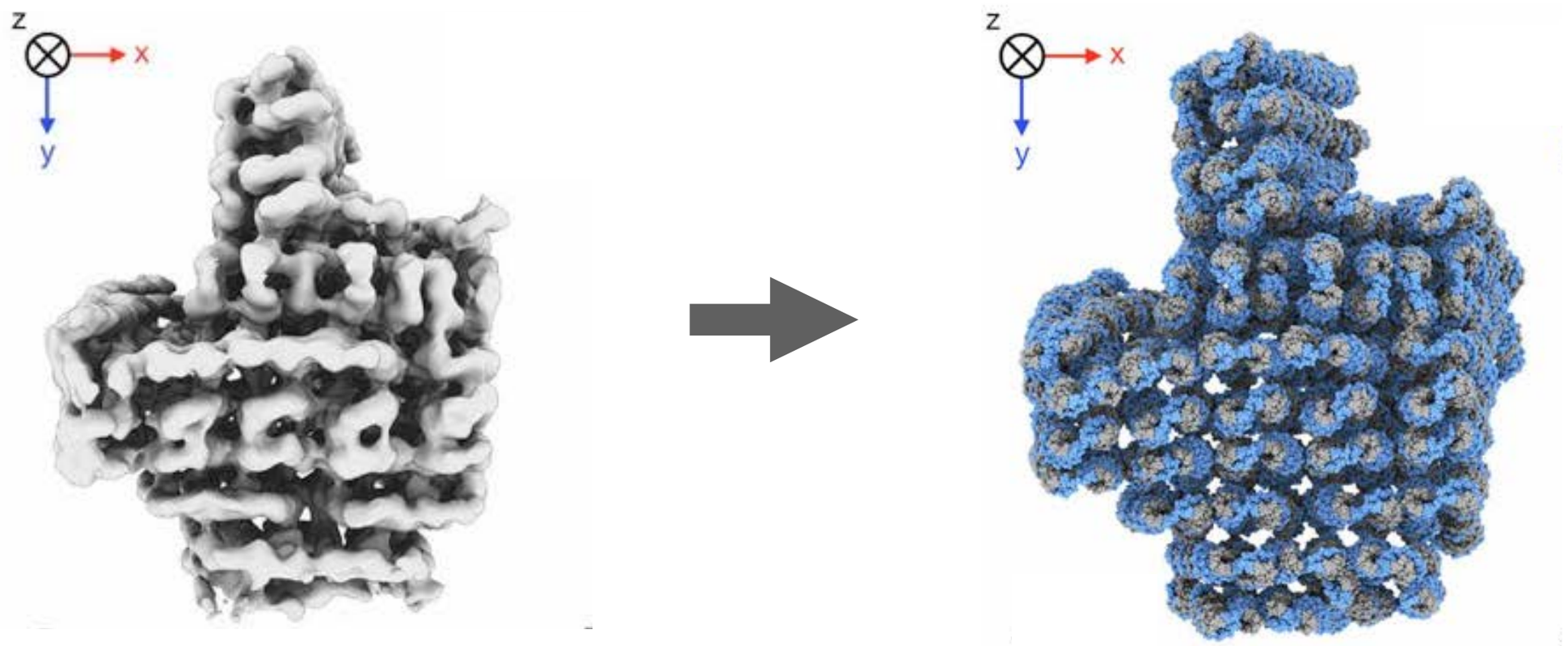
Cryo-EM reconstruction versus all-atom simulation





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

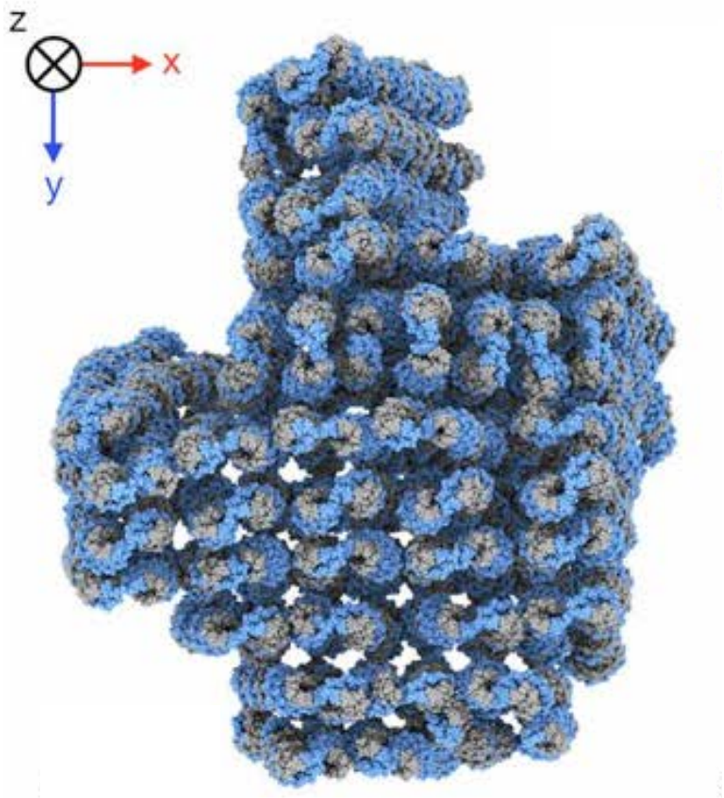
Cryo-EM reconstruction versus all-atom simulation



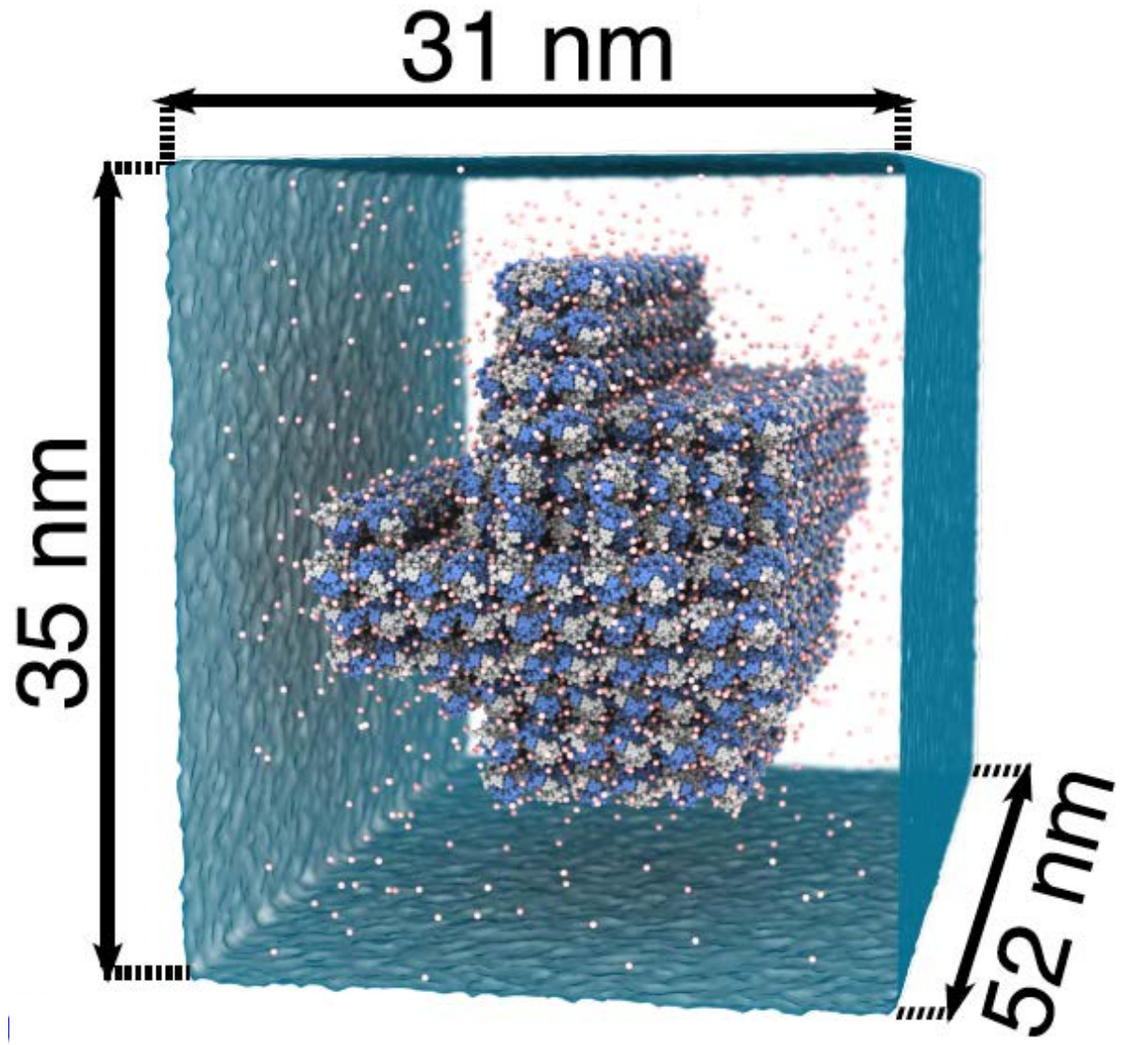
Pseudo-atomic model

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

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

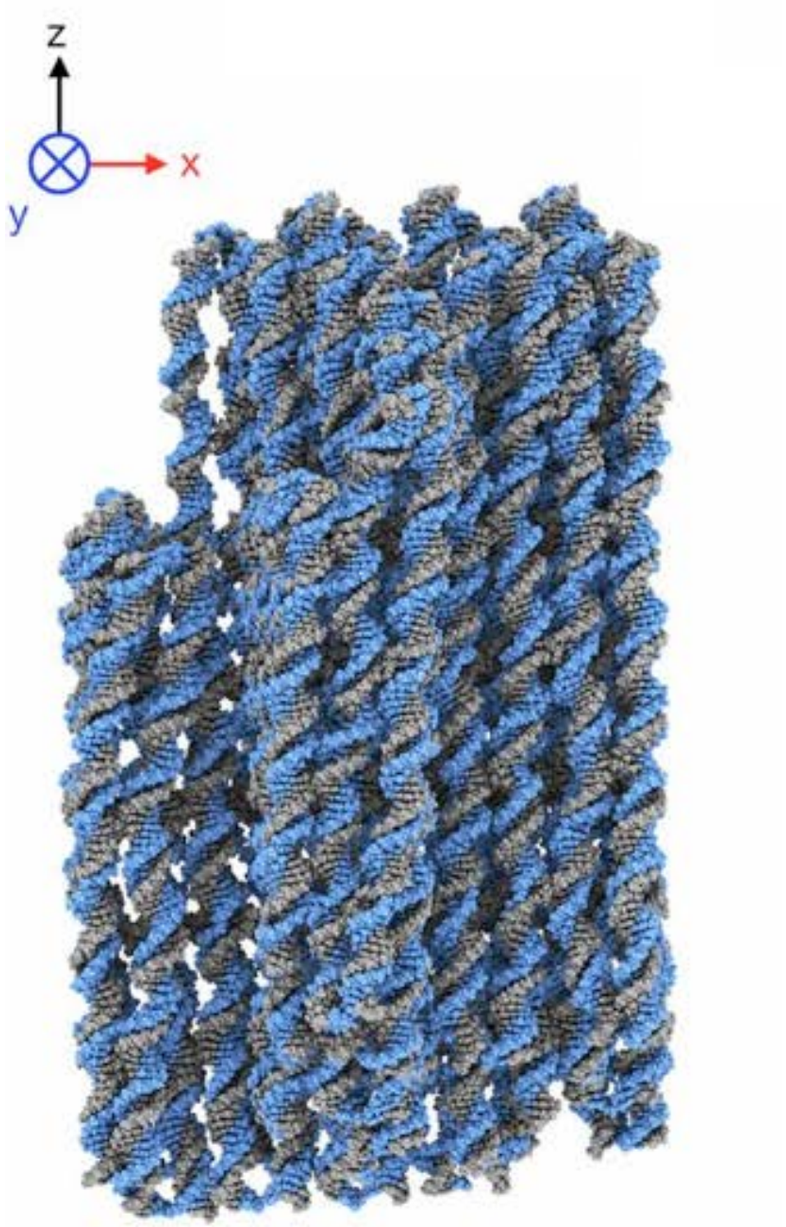


Bai *et al*, PNAS 109:20012 (2012)

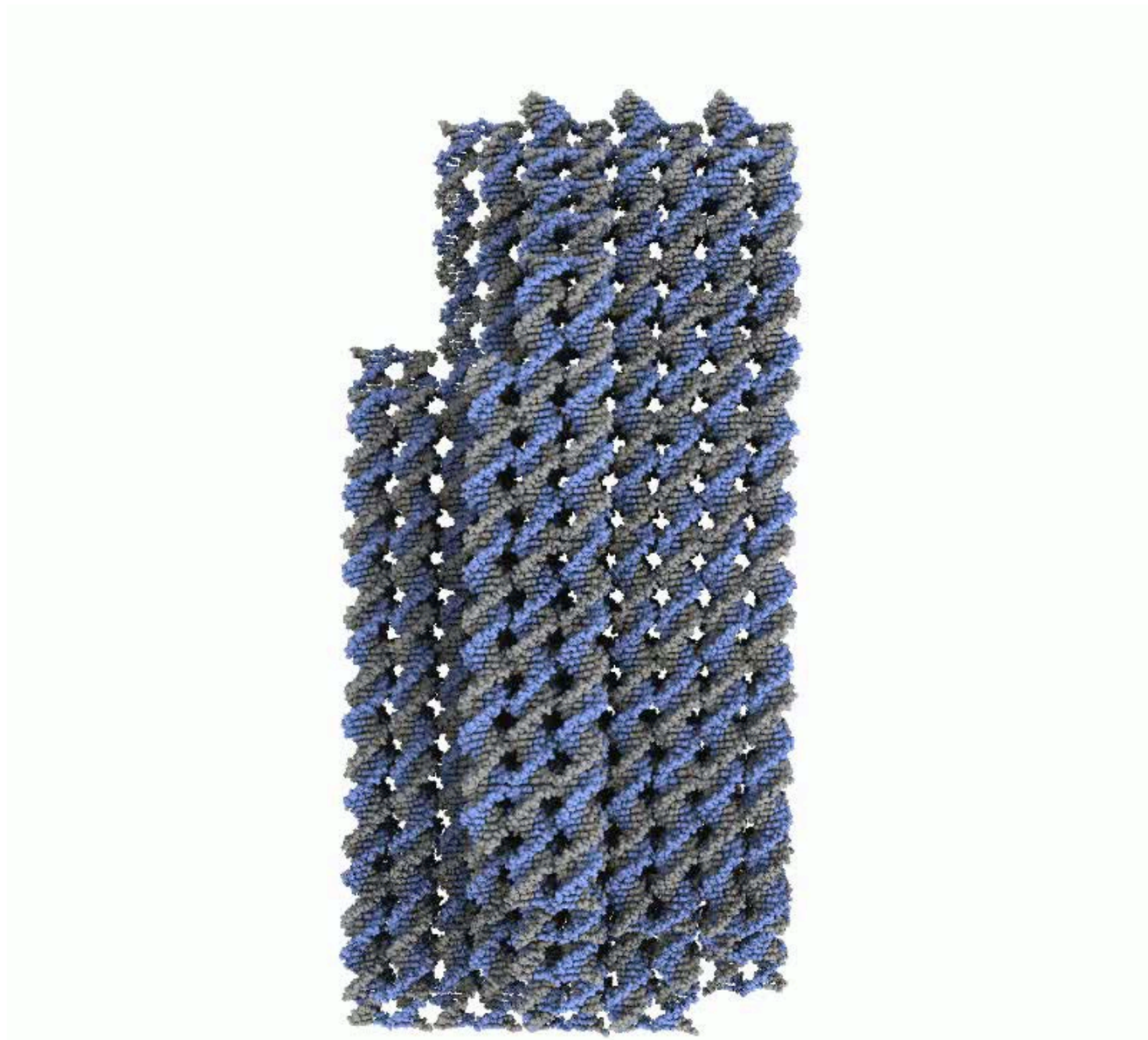


7M atom solvated model
~200 ns MD trajectory

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



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



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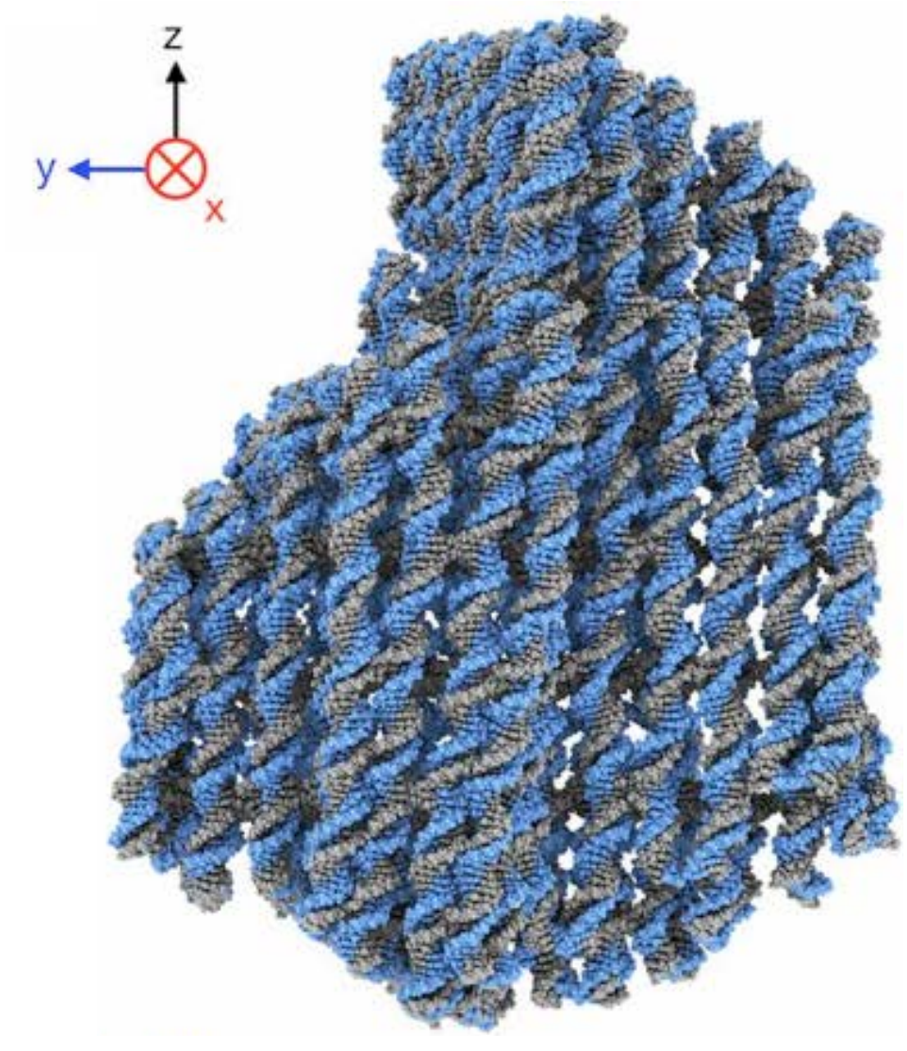
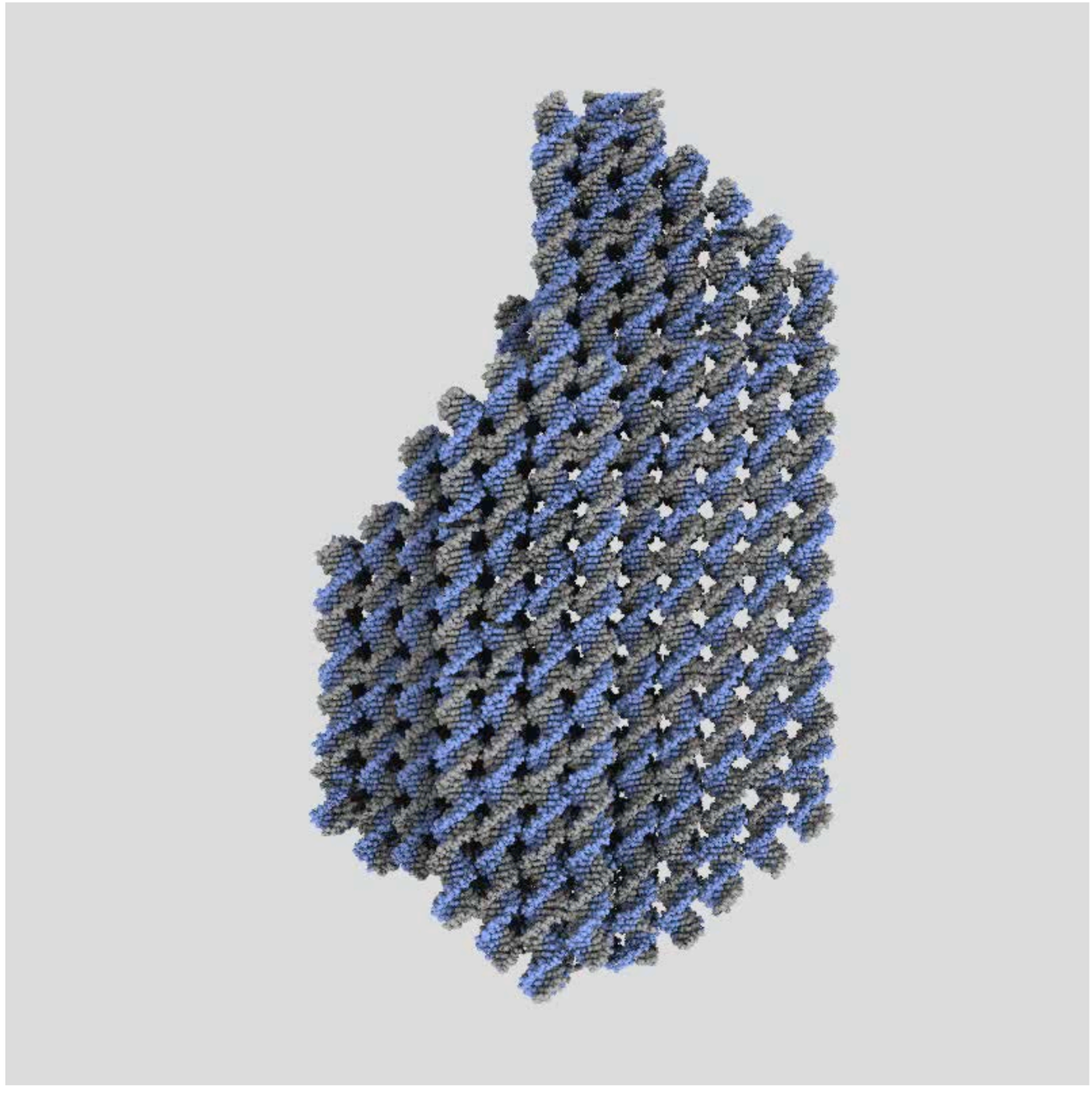
MD simulation of the cryo-EM object starting from a caDNAno design

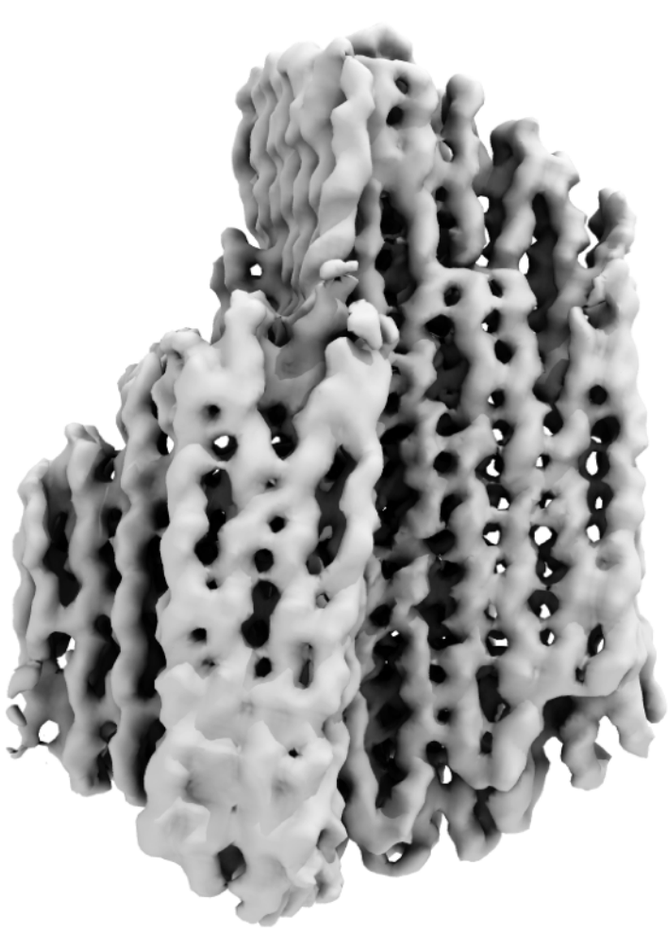


Bai *et al*, PNAS 109:20012 (2012)

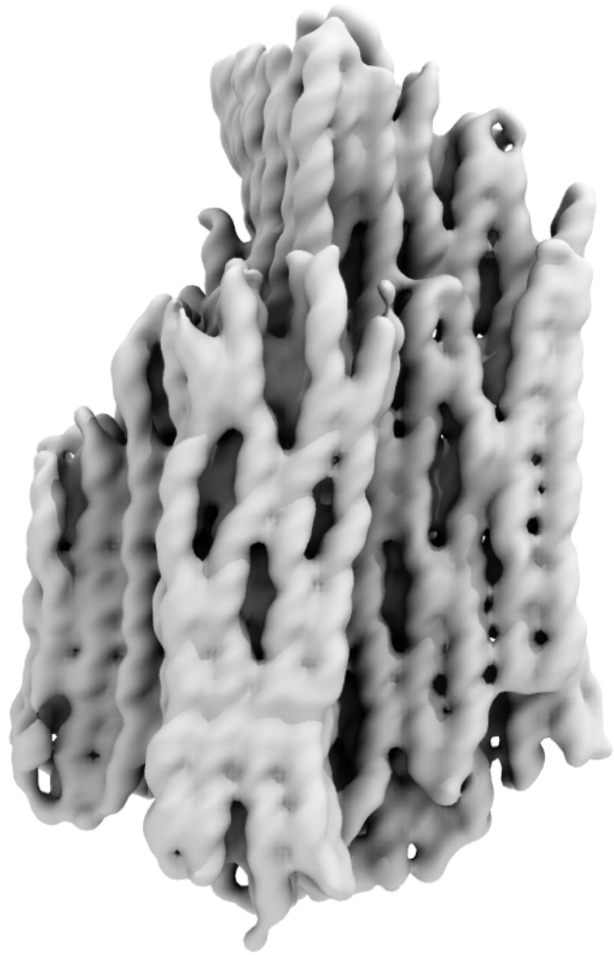


7M atom solvated model
~200 ns MD trajectory

Electron density maps



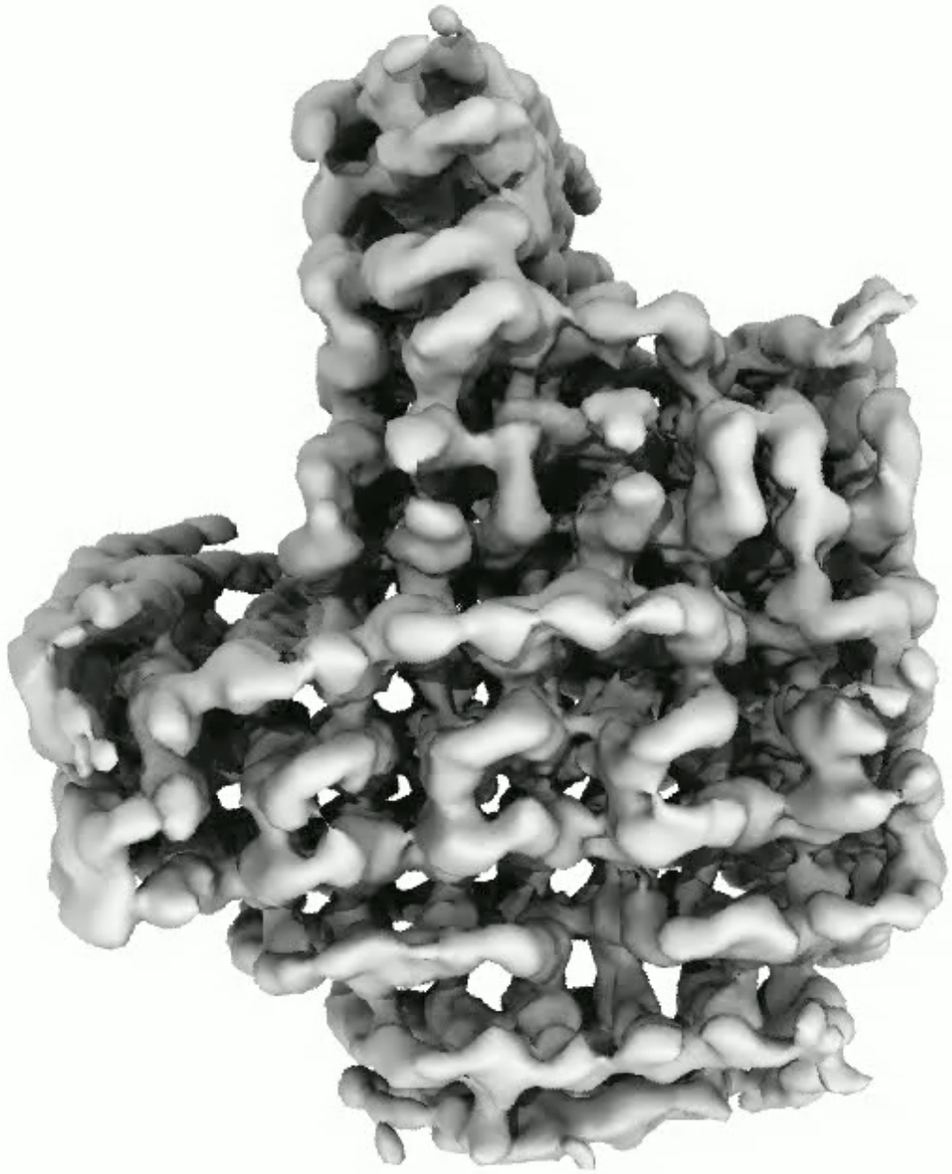
Cryo-EM reconstruction



All-atom MD simulation

Comparison with experiment

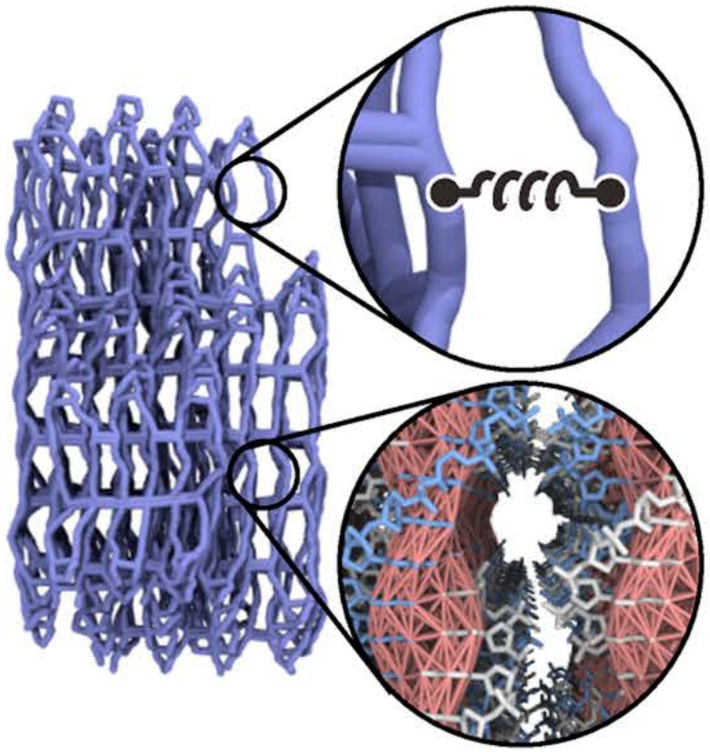
Maffeo, Yoo & Aksimentiev, *NAR 44: 3013 (2016)*



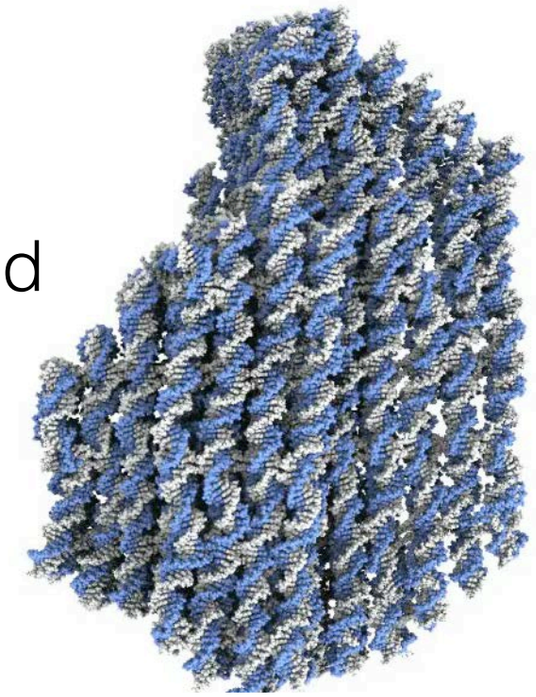
EM density psuedo-atomic model simulation

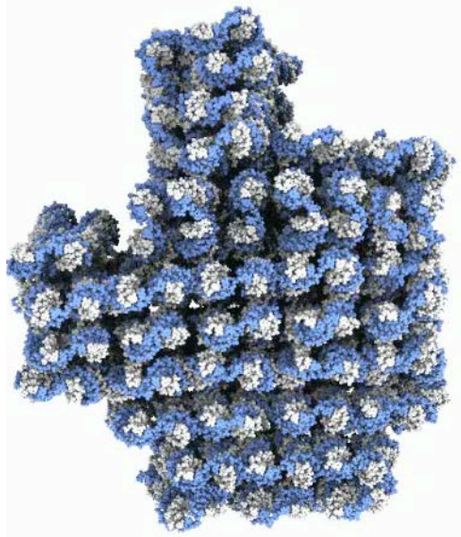
Elastic network of restraints guided MD (ENRG MD)

~10,000 times more efficient

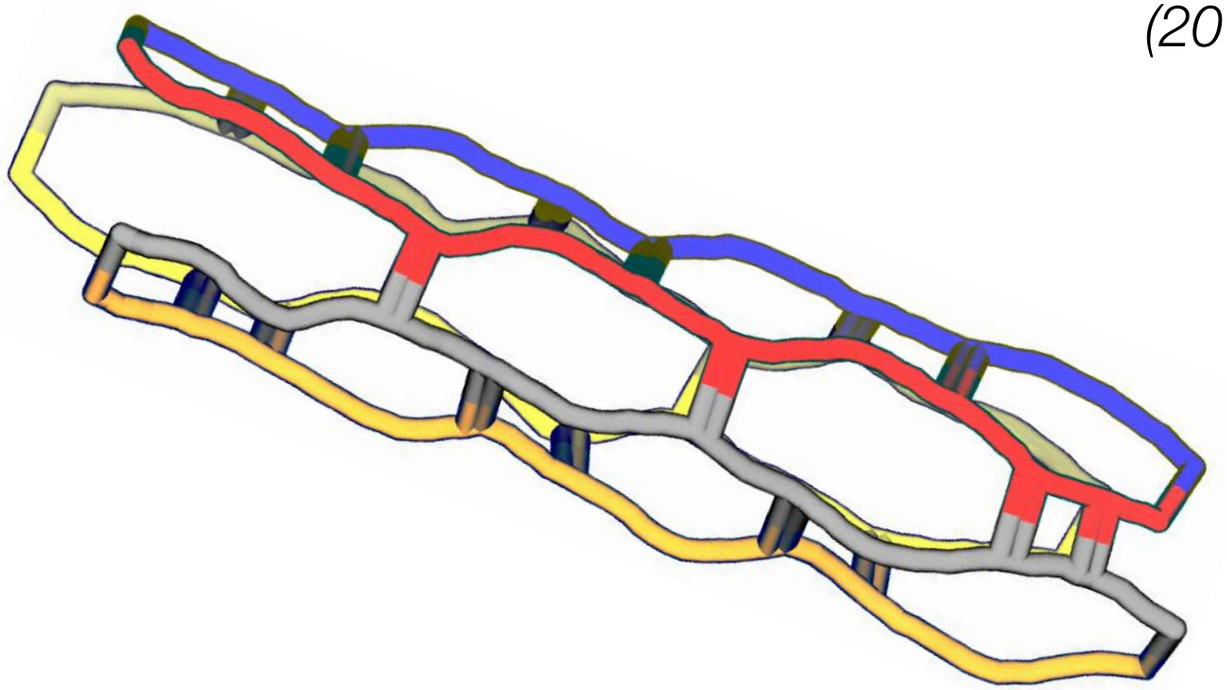


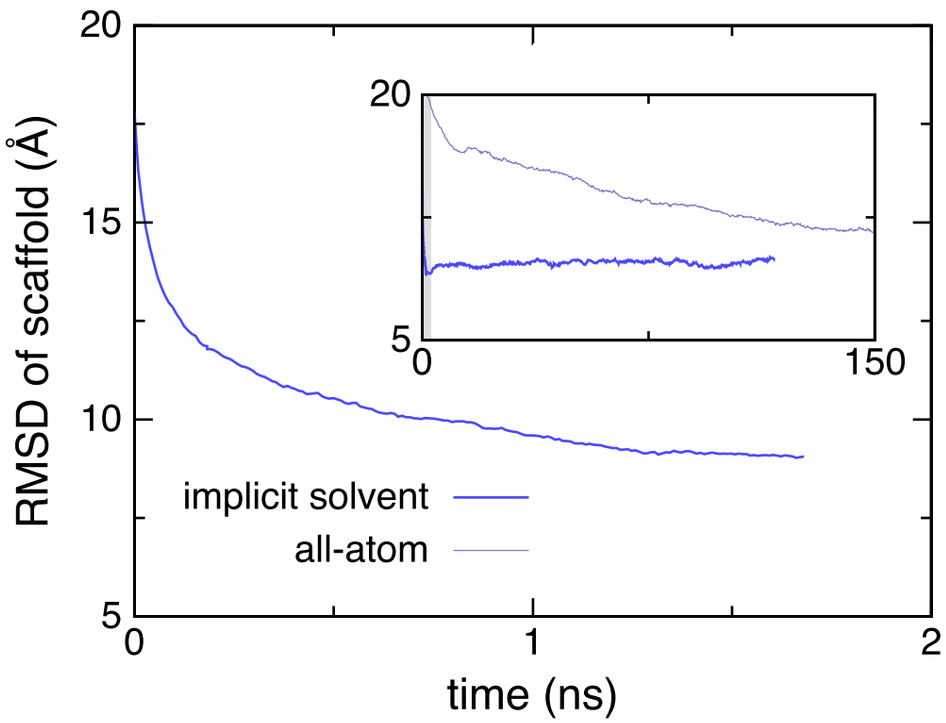
Solvent replaced with elastic network





Maffeo, Yoo & Aksimentiev, *NAR 44: 3013 (2016)*





ENRG MD For Origami Structure Prediction

Upload a DNA origami design .json file

Choose File No file chosen

Select the origami lattice. *

- (•) Square
- ( ) Honeycomb

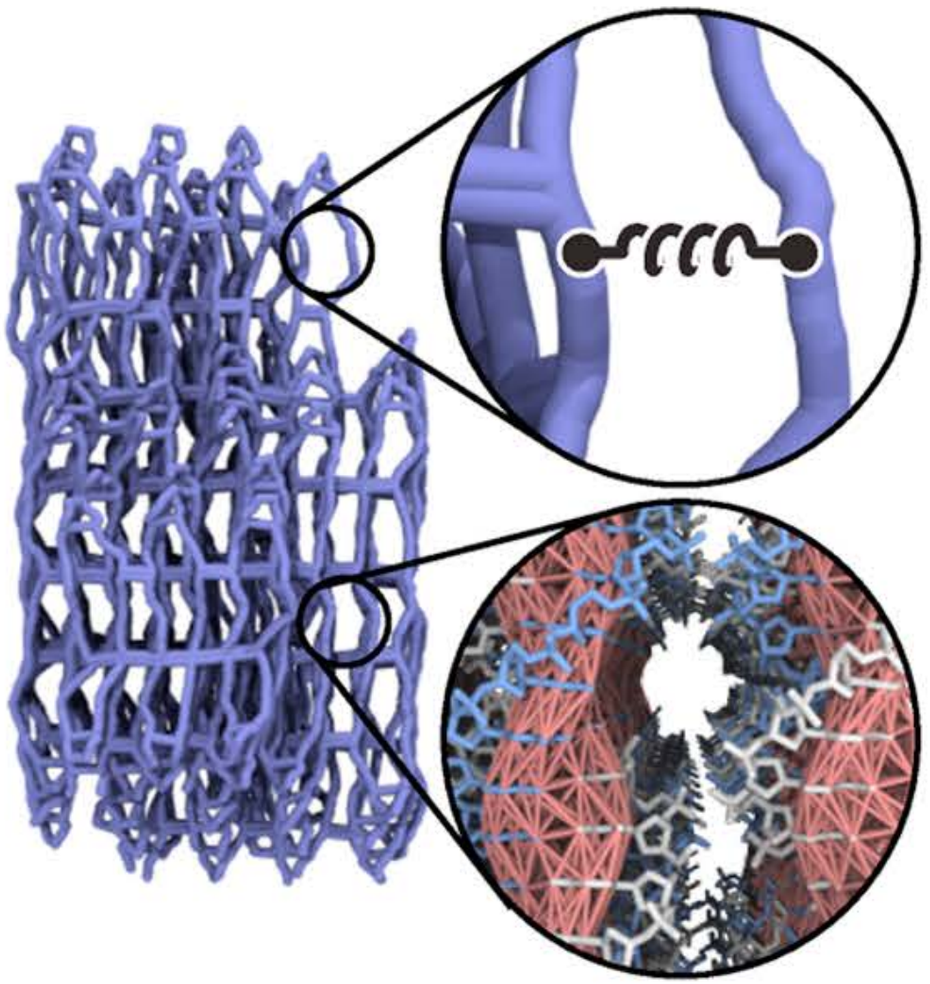
Select the scaffold sequence. *

- (•) m13mp18 (up to 7,249 bases)
- ( ) Custom

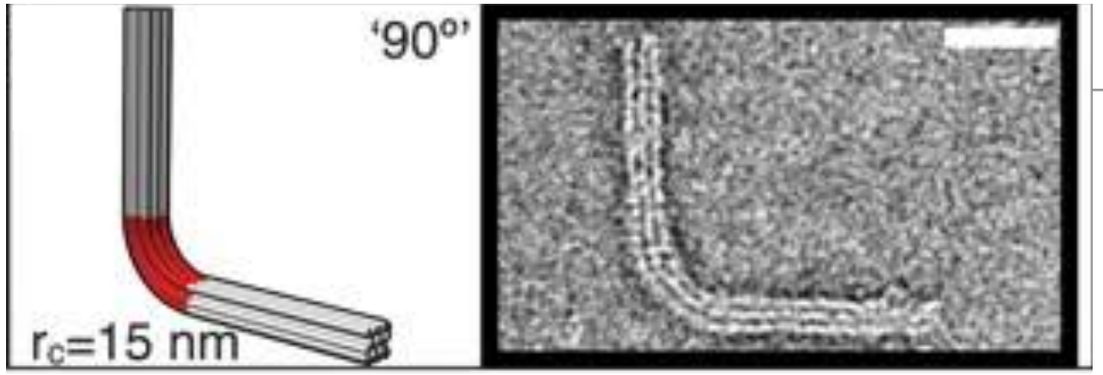
Simulation package *

- (•) NAMD (CHARMM FF)
- ( ) Gromacs (AMBER FF; beta coming soon)

Create simulation files

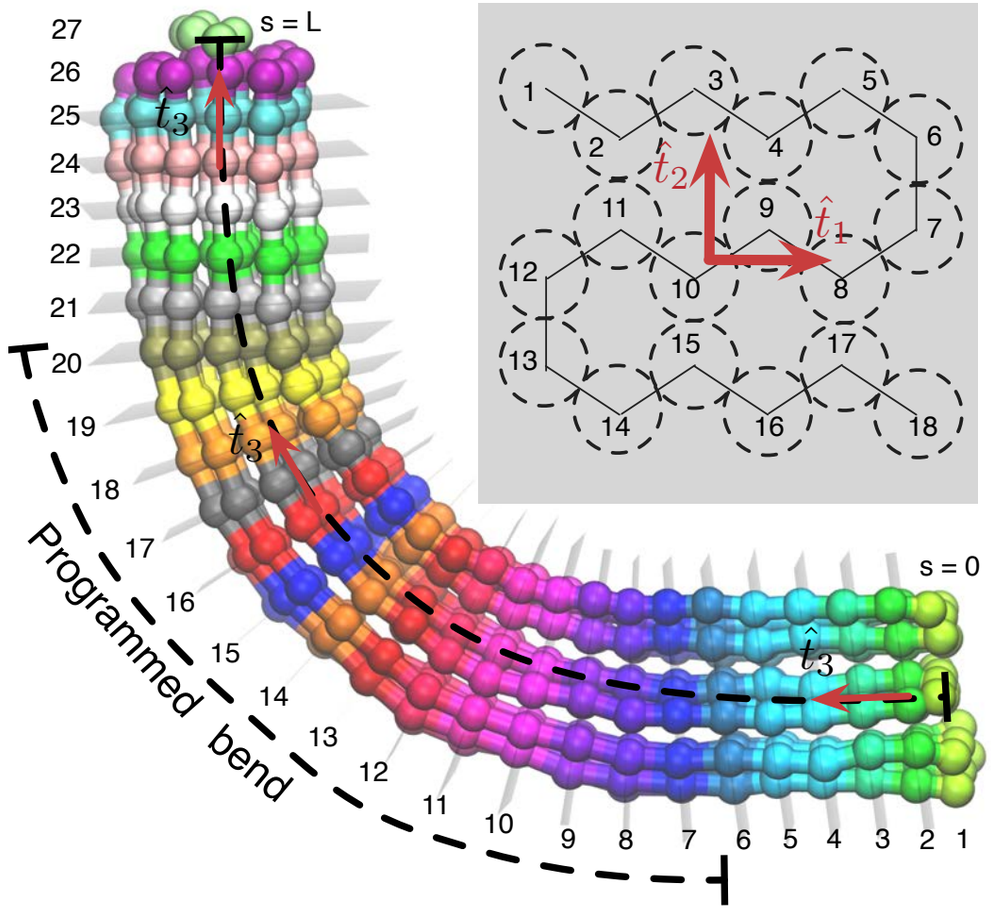


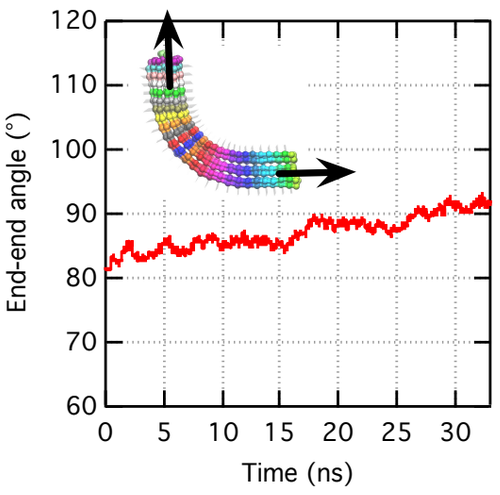
Curved DNA origami systems

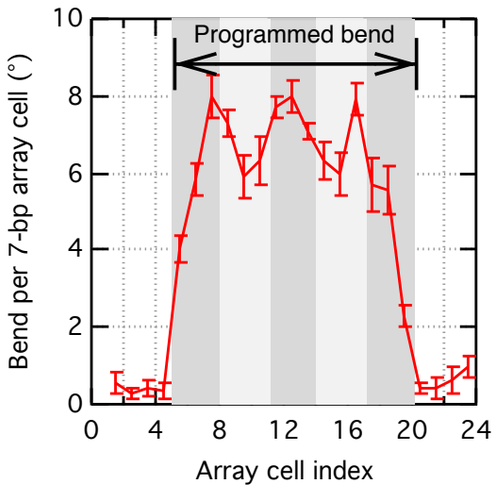


Experiment

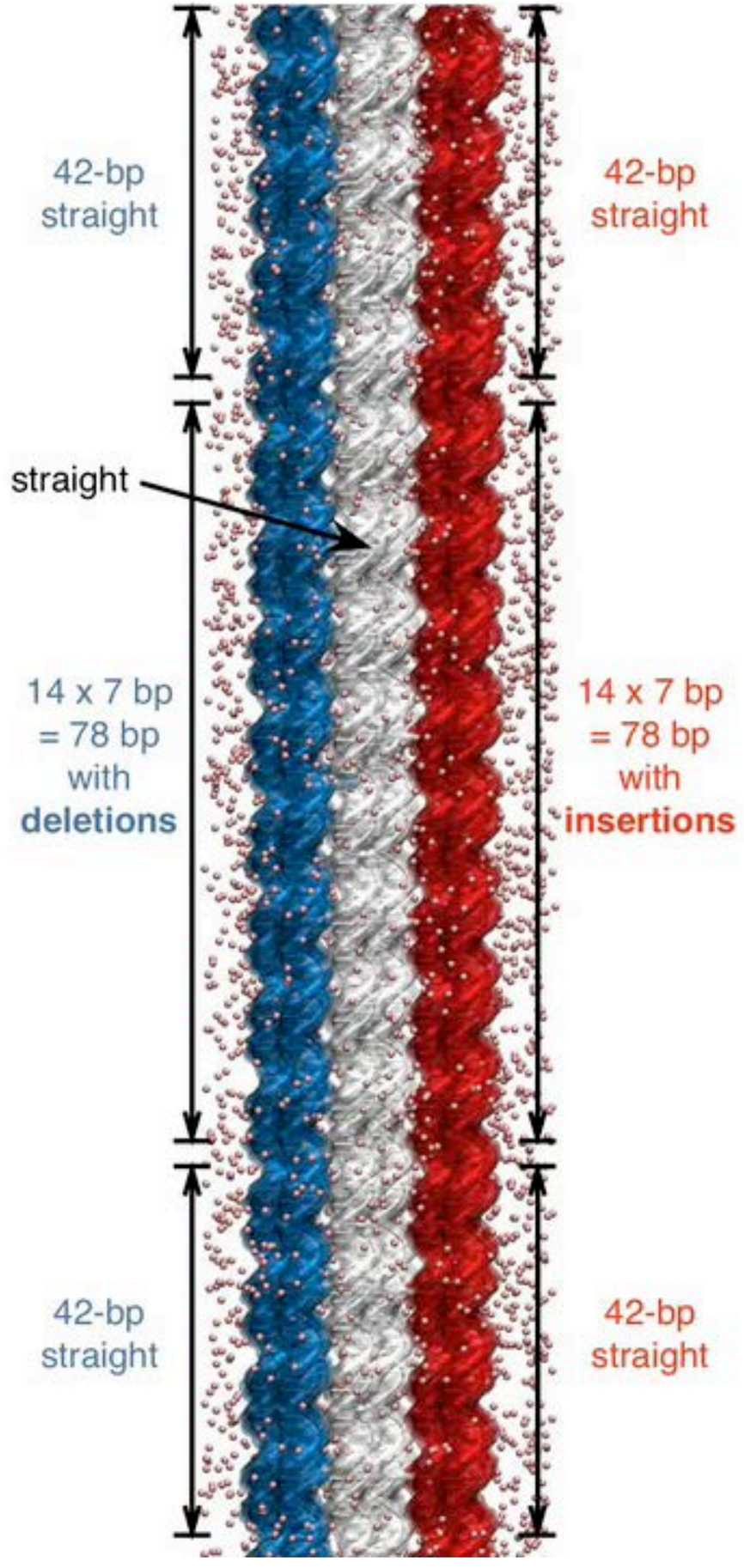
Dietz, H. et al, *Science*, 325



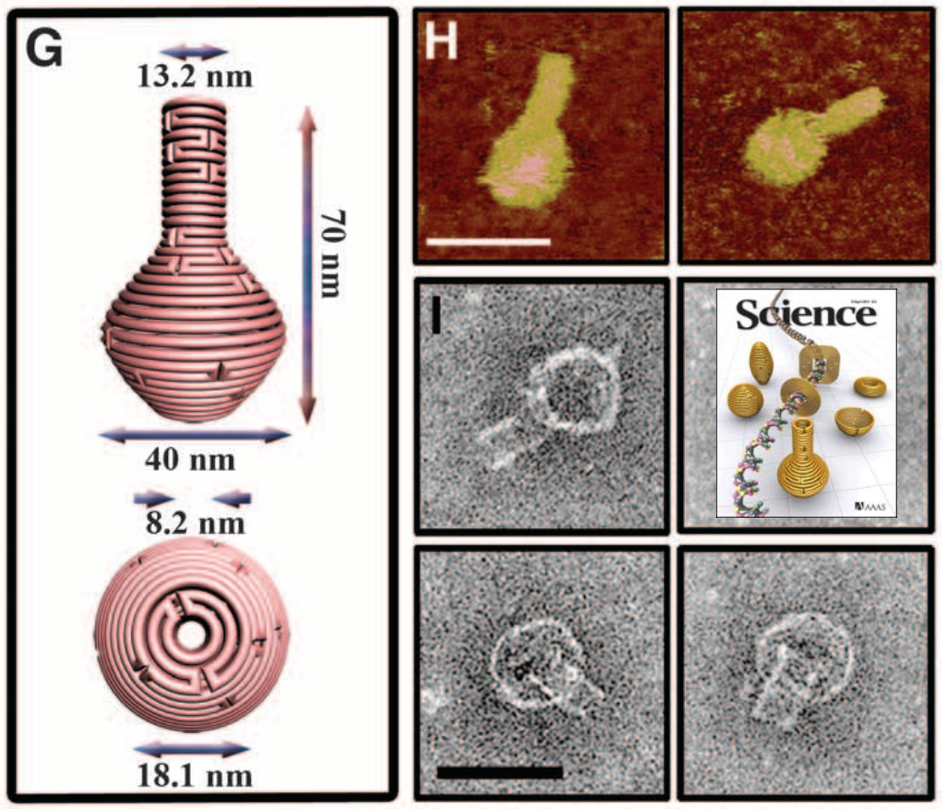




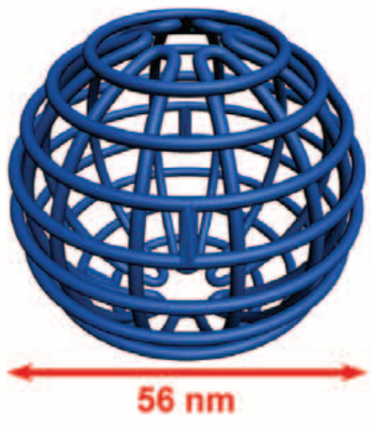
Yoo and Aksimentiev, PNAS 110:20099 (2013)



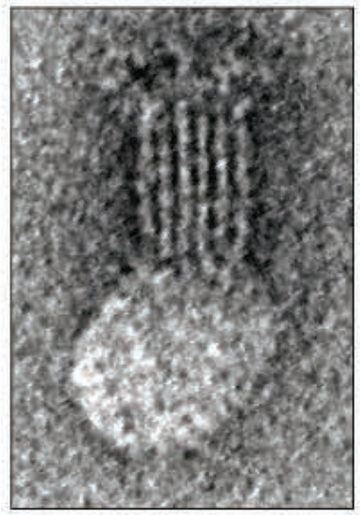
DNA origami structures

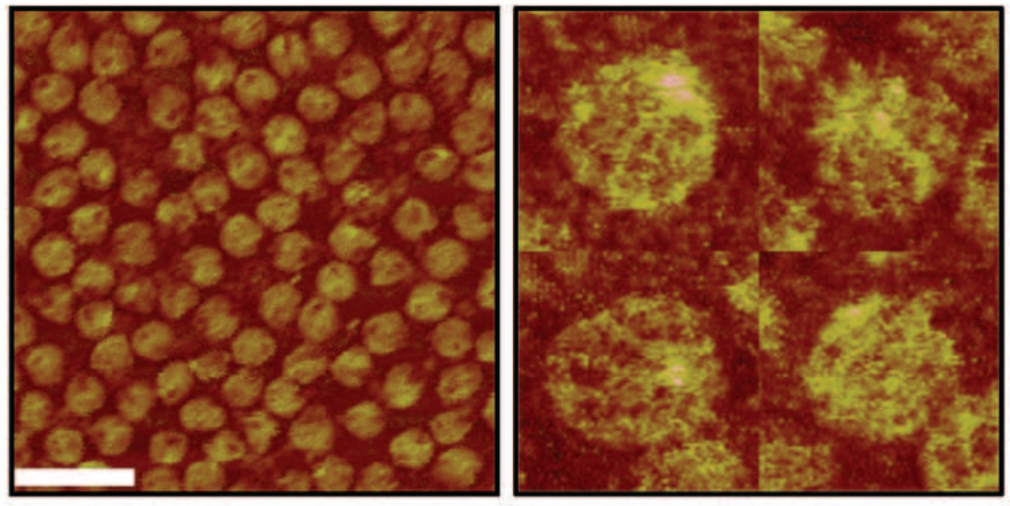


Yan and coworkers, Science (2011)

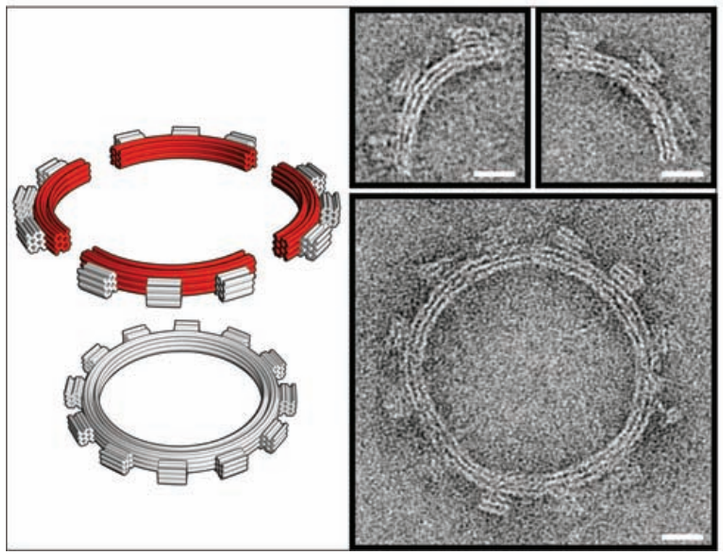


Dietz and coworkers, Science (2012)

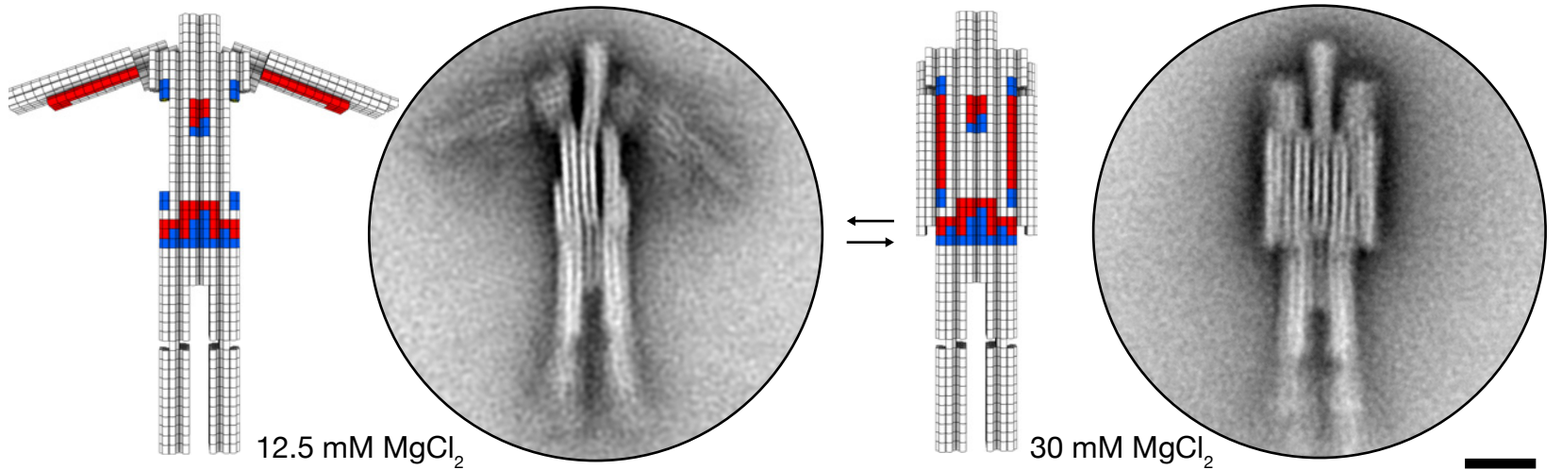




Yan and coworkers, Science (2013)



Shih and coworkers, Science (2009)



Dietz and coworkers, Science (2015)

Multi-Resolution DNA (mrDNA) model

Nucleic Acids Research 48: 5135 (2020)

Chris Maffeo

Strategy: change resolution for speed and detail

Multi-Resolution DNA (mrDNA) model

Nucleic Acids Research 48: 5135 (2020)

Chris Maffeo

Strategy: change resolution for speed and detail

Multi-Resolution DNA (mrDNA) model

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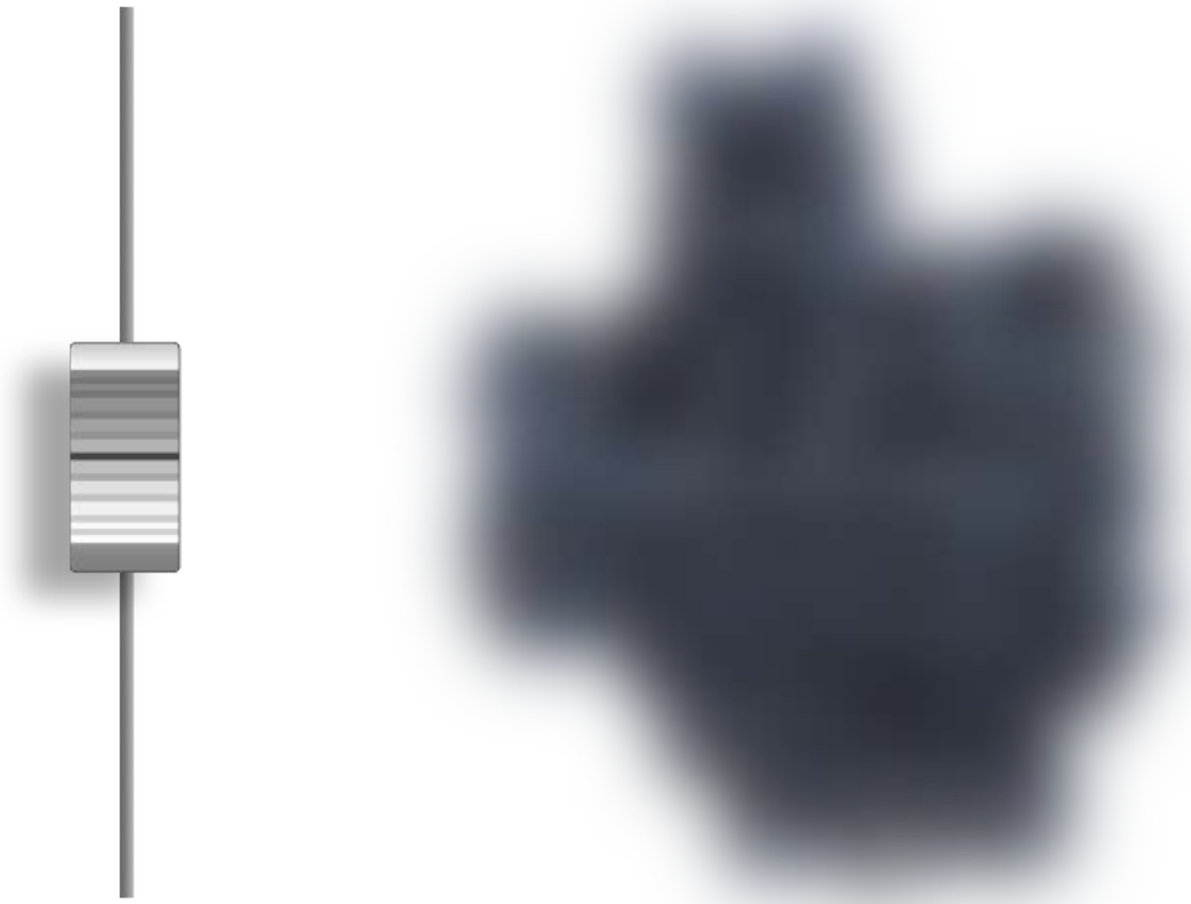
Chris Maffeo

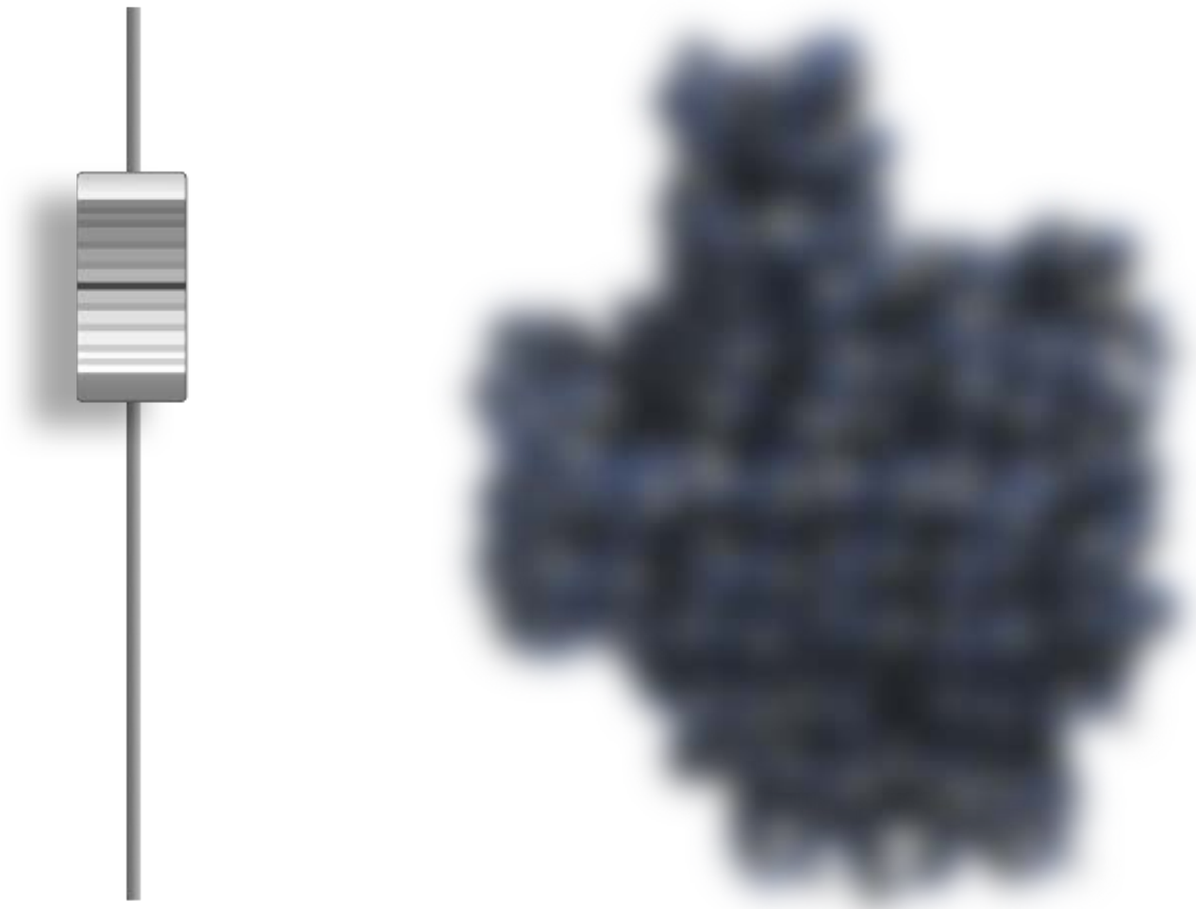


Strategy: change resolution for speed and detail

Multi-Resolution DNA (mrDNA) model

Nucleic Acids Research 48: 5135 (2020)

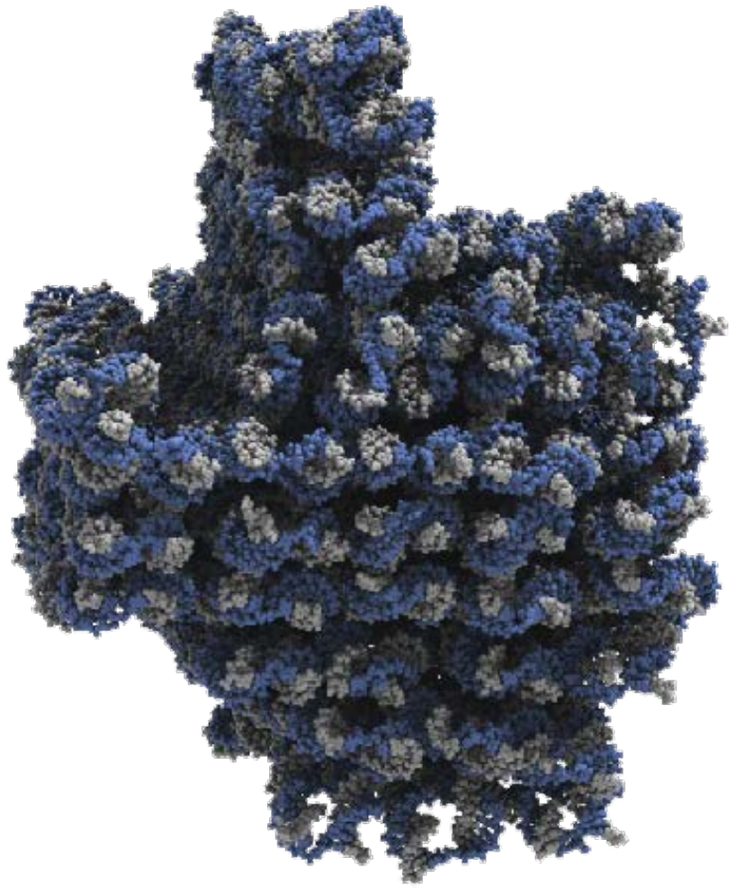
Chris Maffeo



Strategy: change resolution for speed and detail

Multi-Resolution DNA (mrDNA) model

Nucleic Acids Research 48: 5135 (2020)

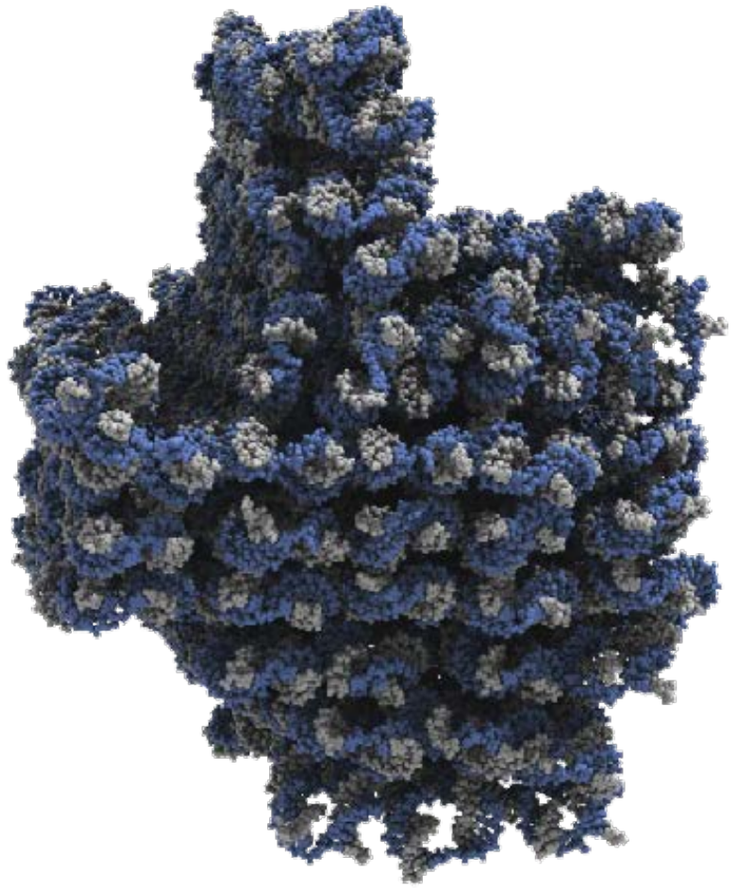


Chris Maffeo

Strategy: change resolution for speed and detail

Multi-Resolution DNA (mrDNA) model

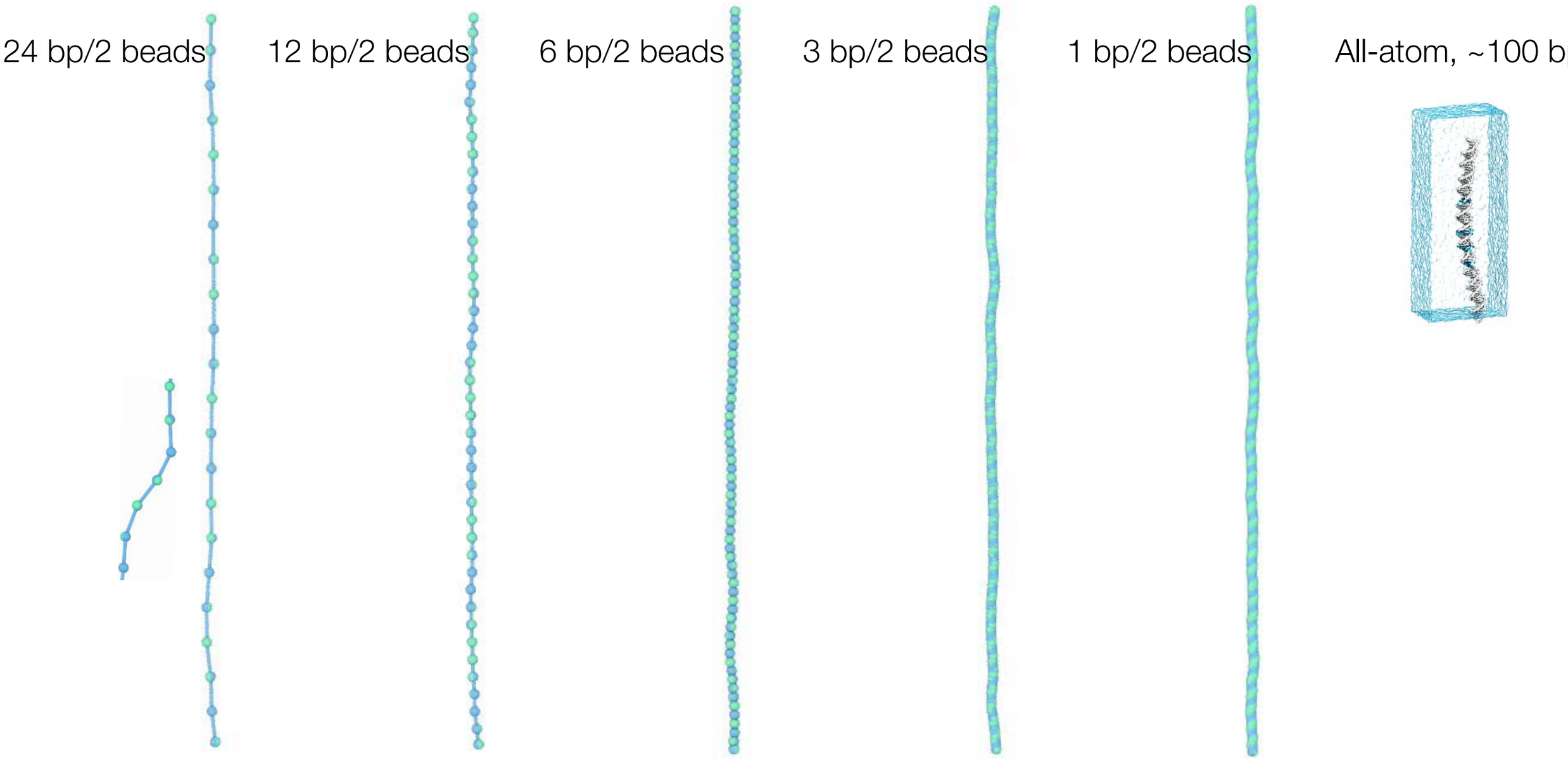
Nucleic Acids Research 48: 5135 (2020)



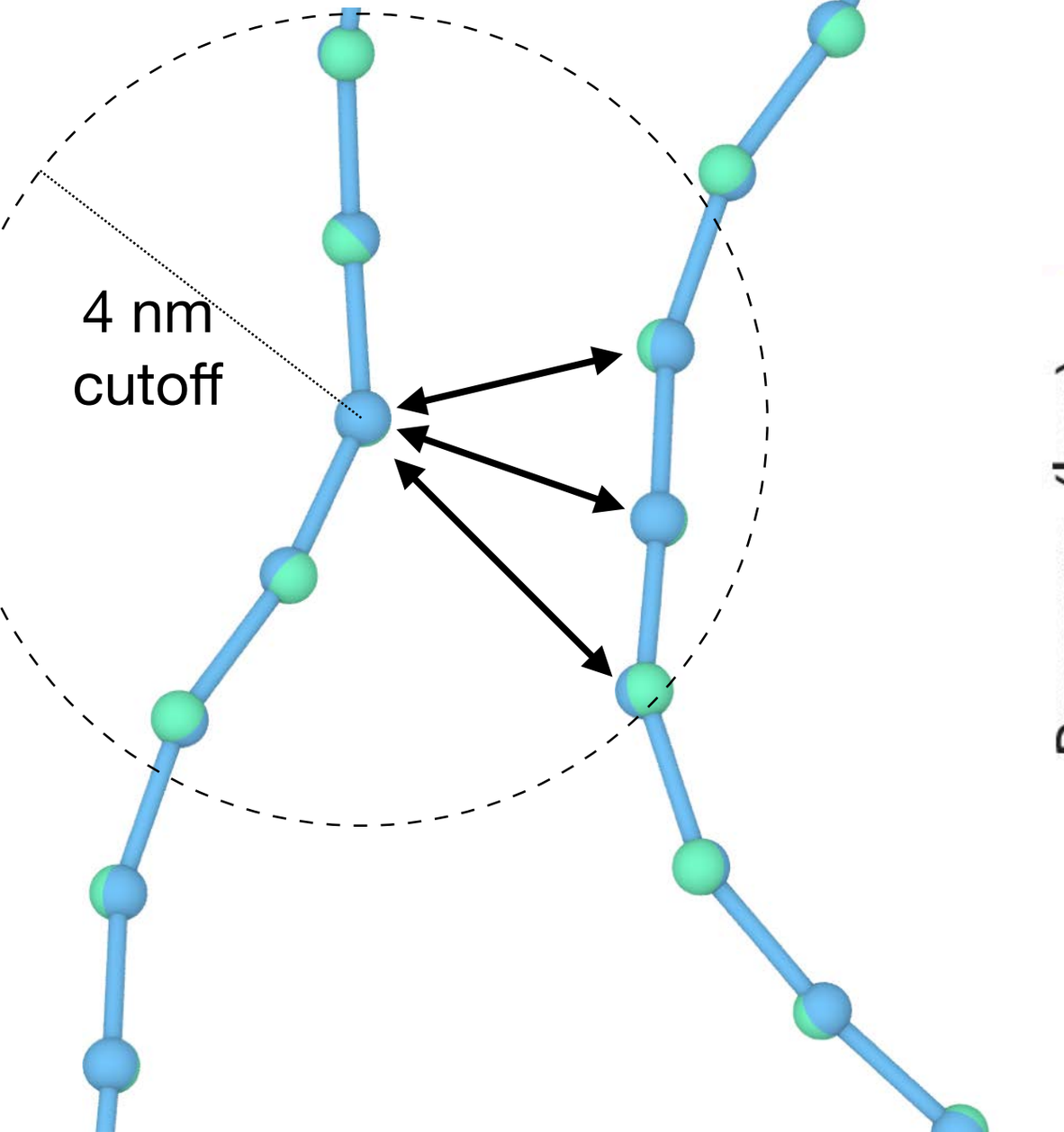
Chris Maffeo

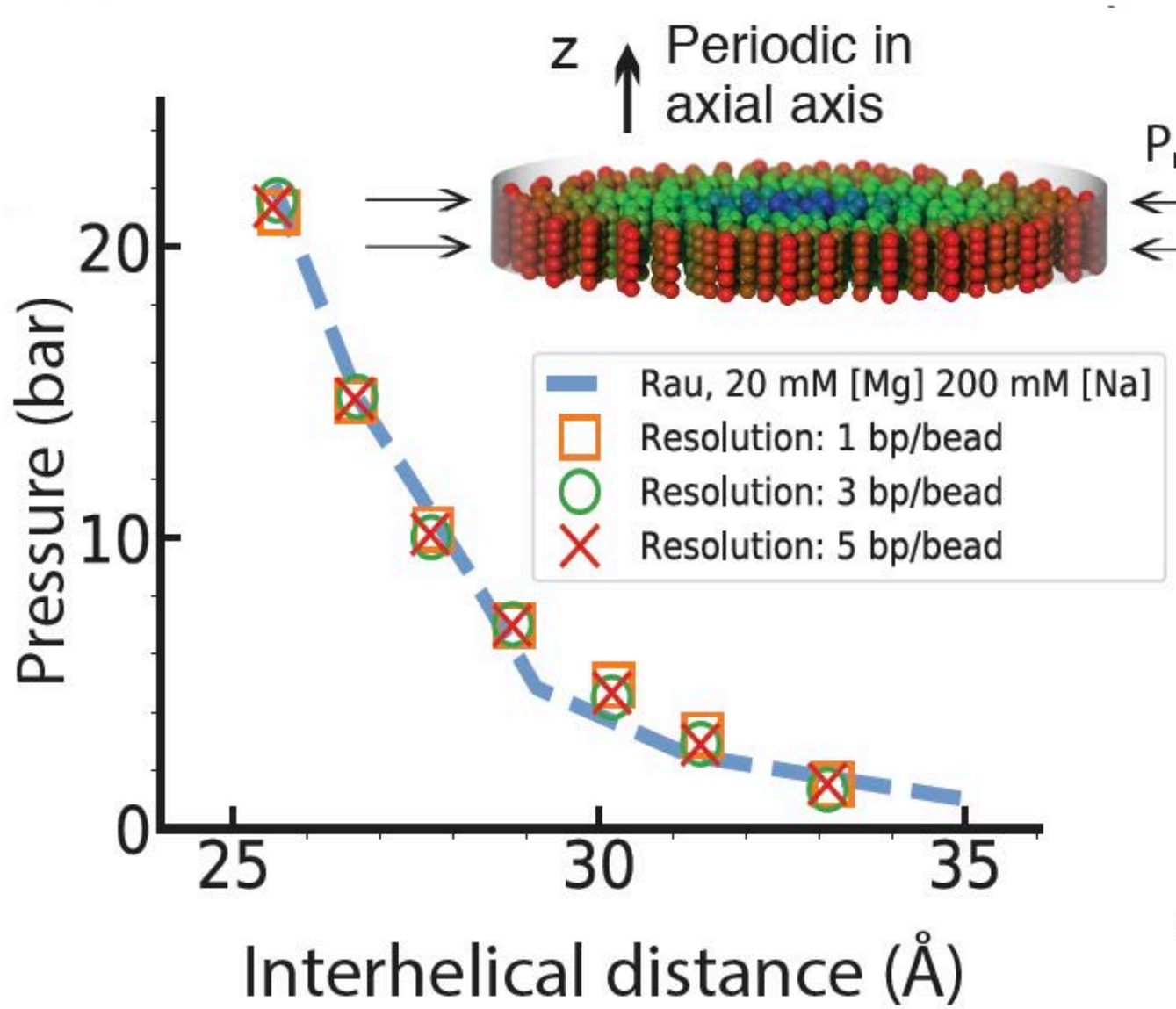
Strategy: change resolution for speed and detail

500 bp dsDNA fragment modeled at different resolutions

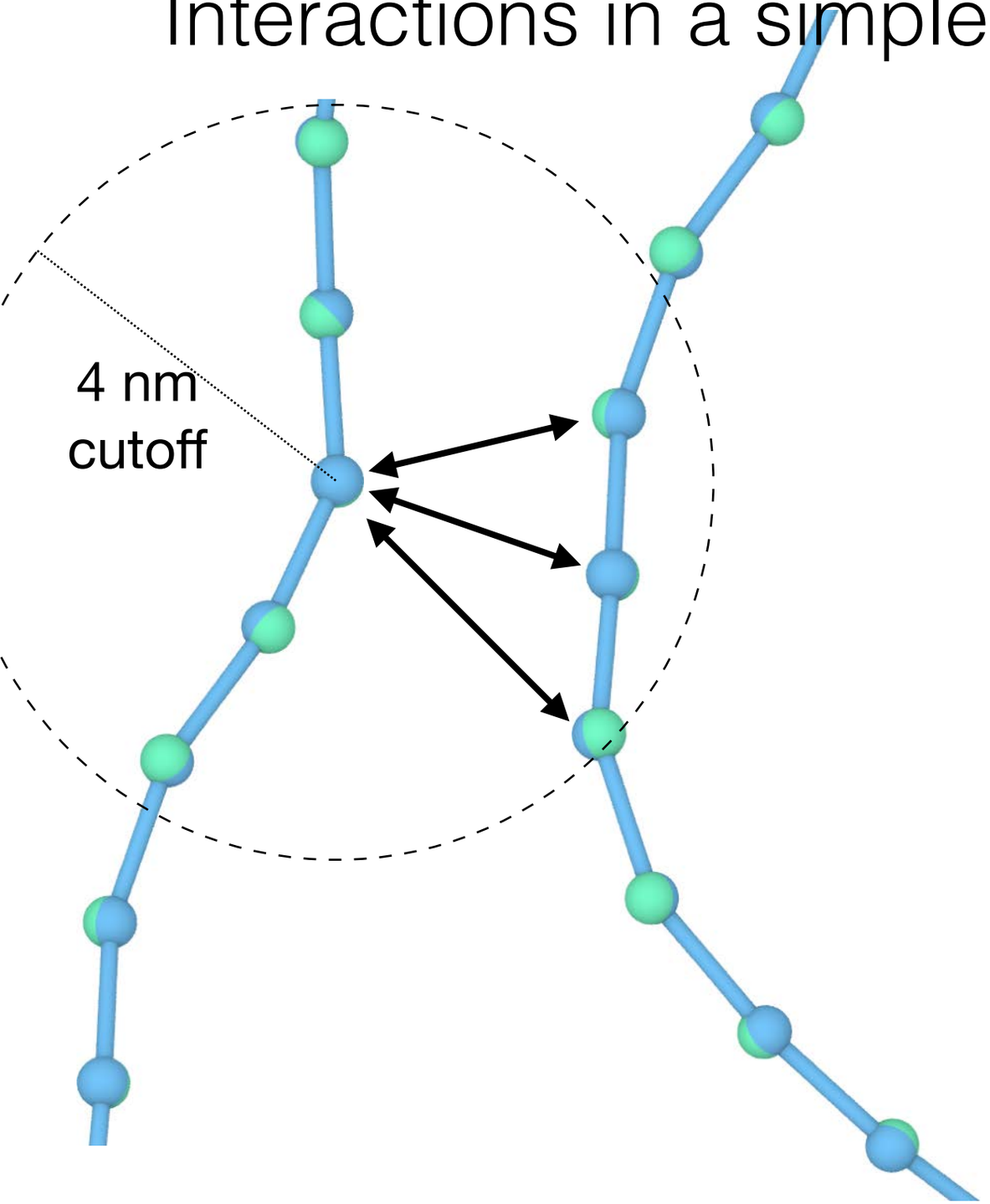


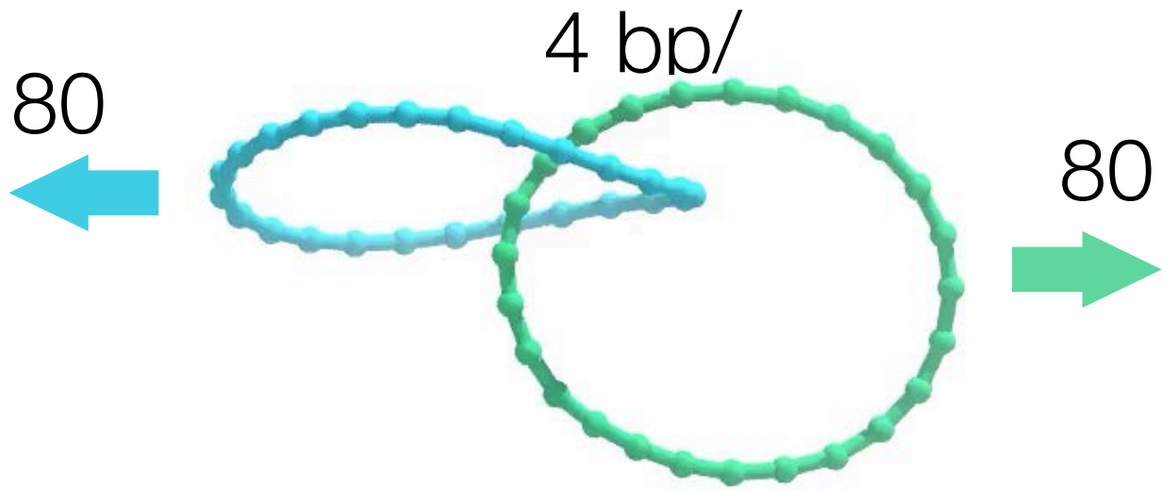
Interactions in a simple coarse-grained DNA model

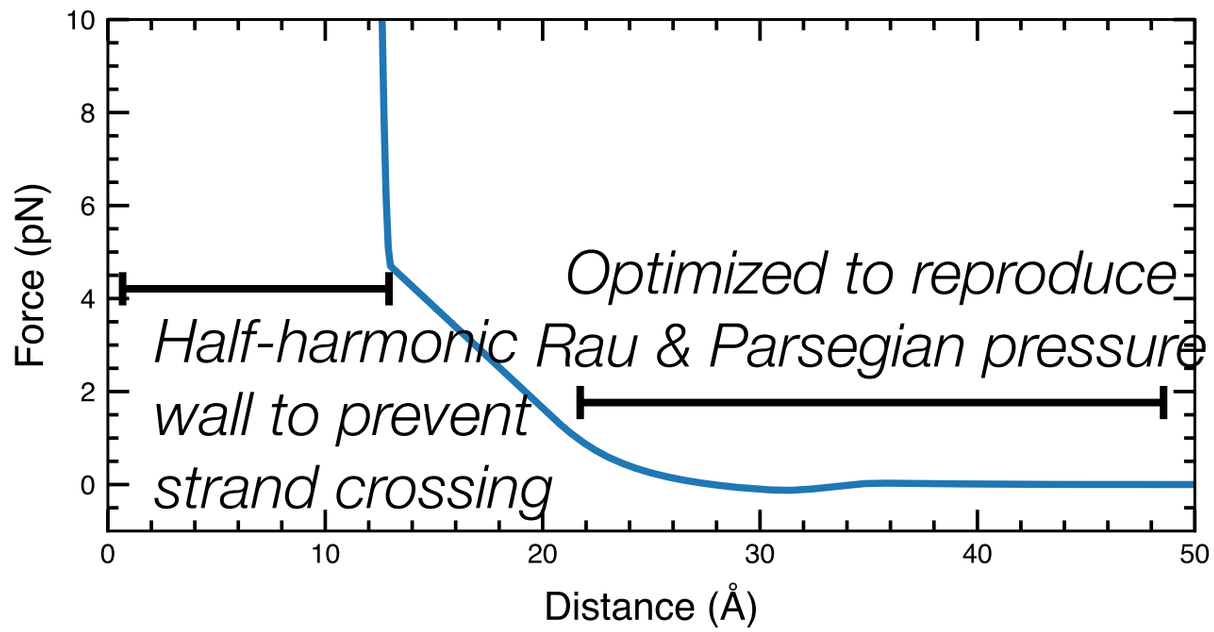




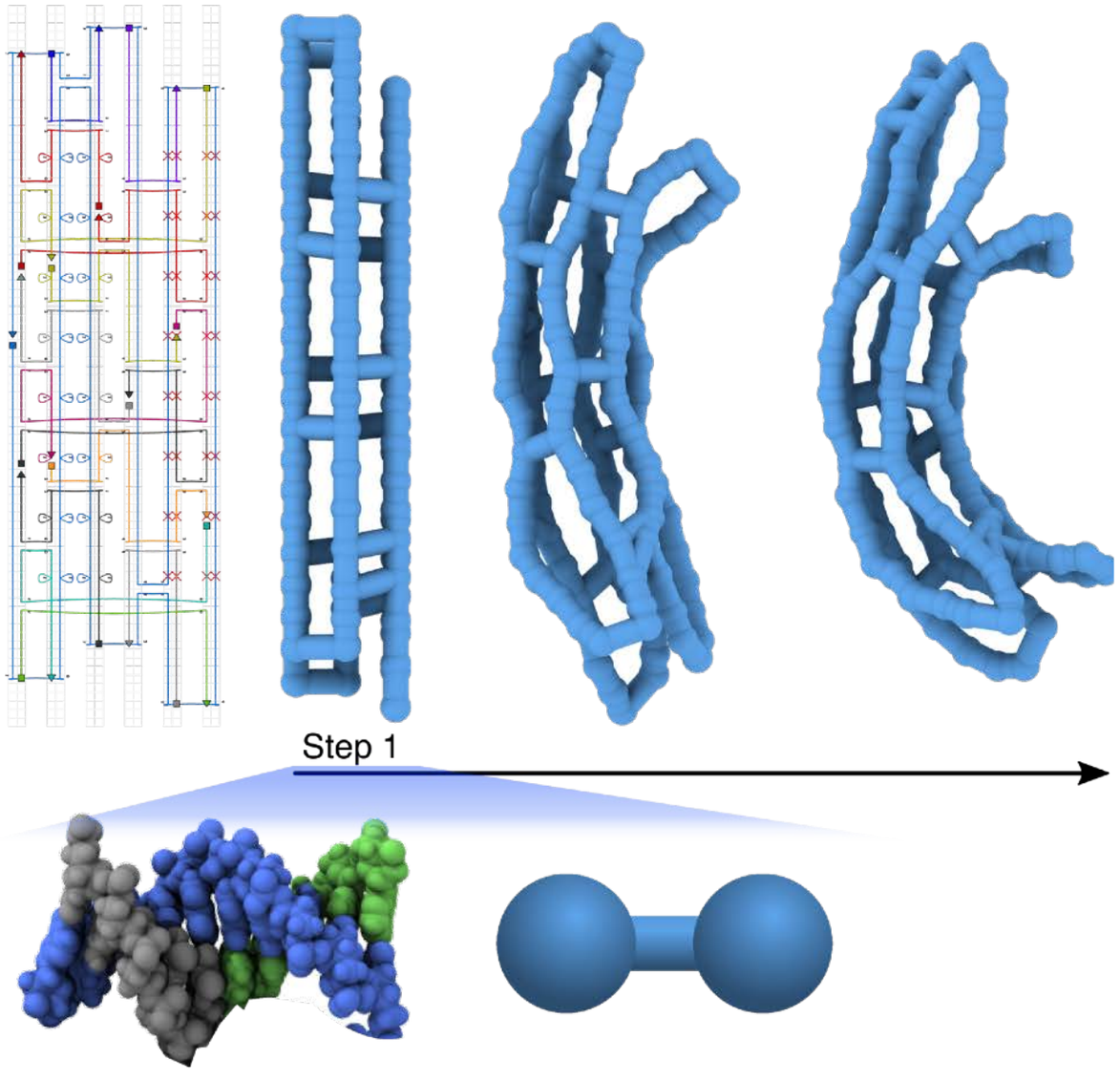
Interactions in a simple coarse-grained DNA model





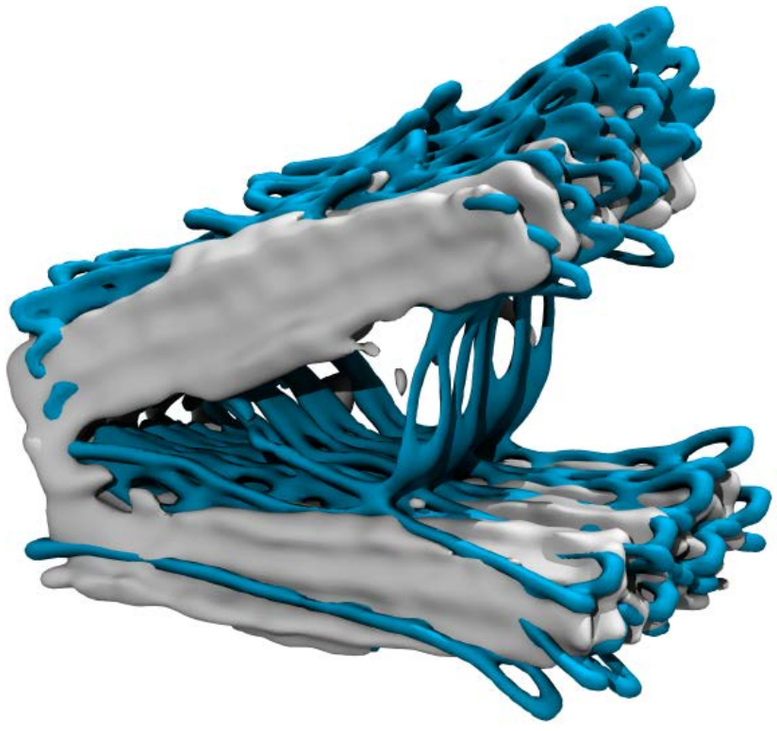


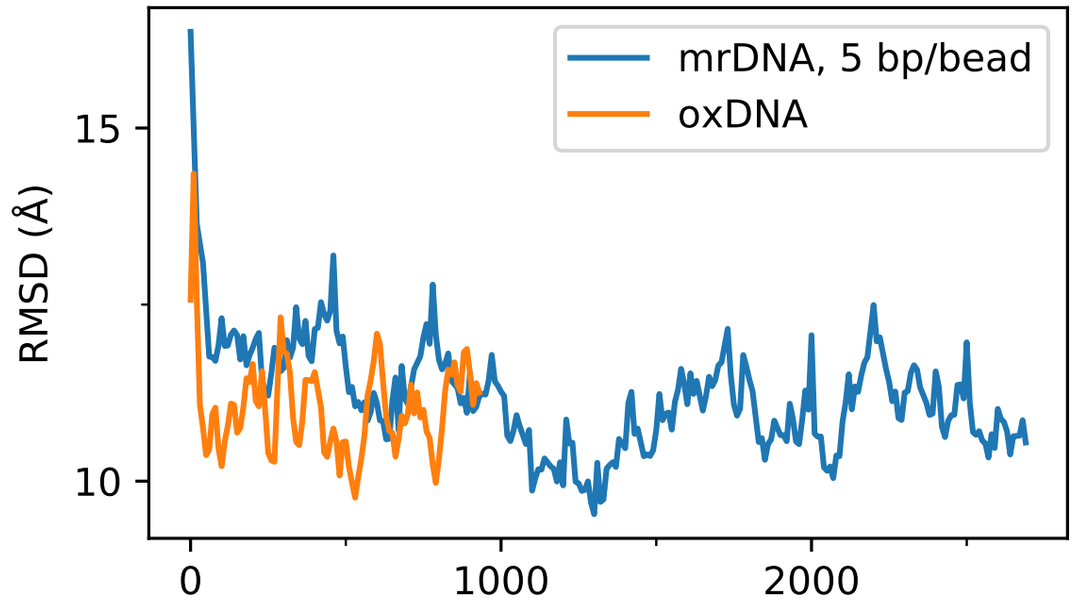
Typical structural relaxation procedure



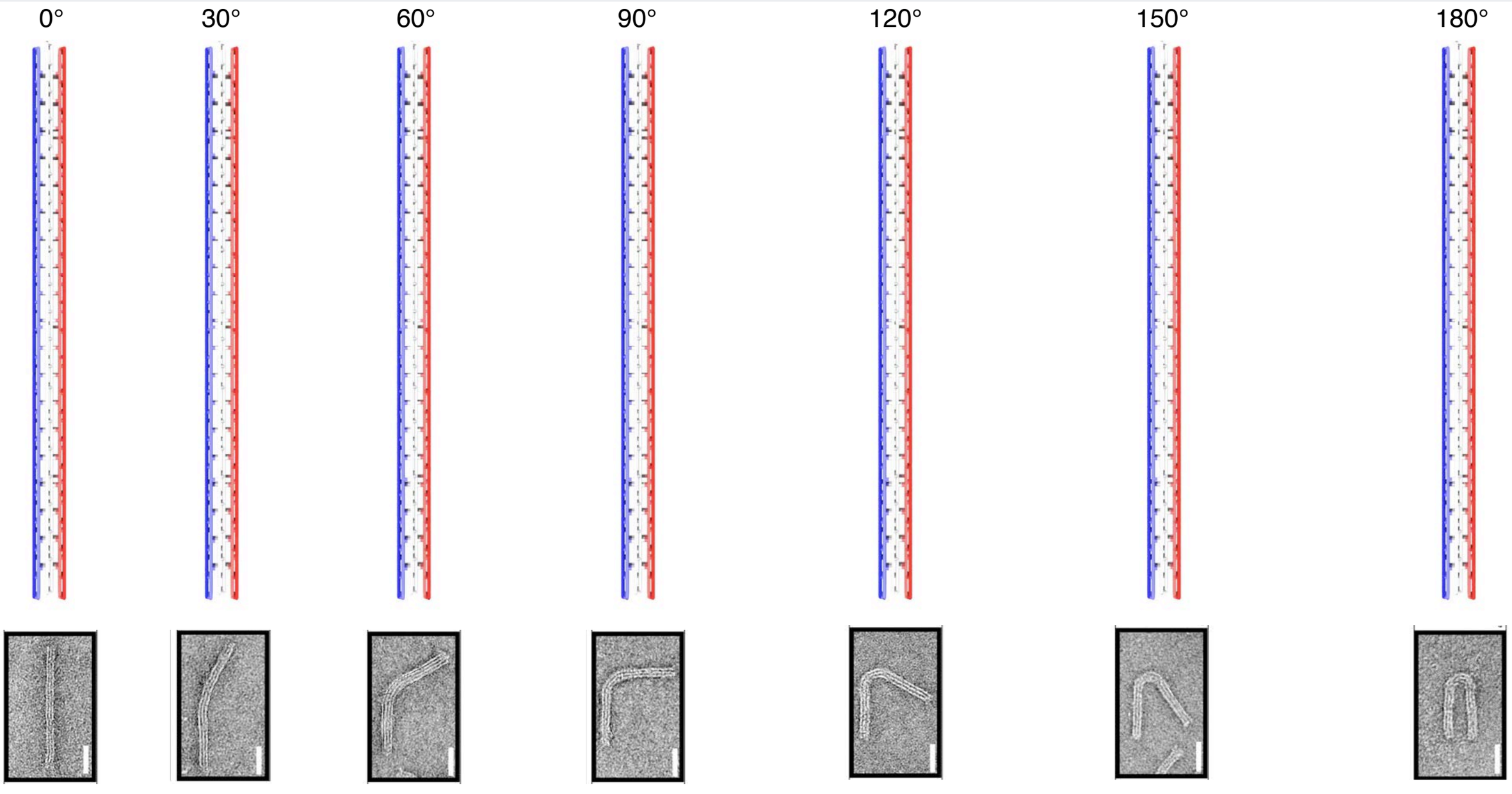
Nucleic Acids Research 48: 5135 (2020)

Multi-resolution simulations provide highly detailed structures quickly





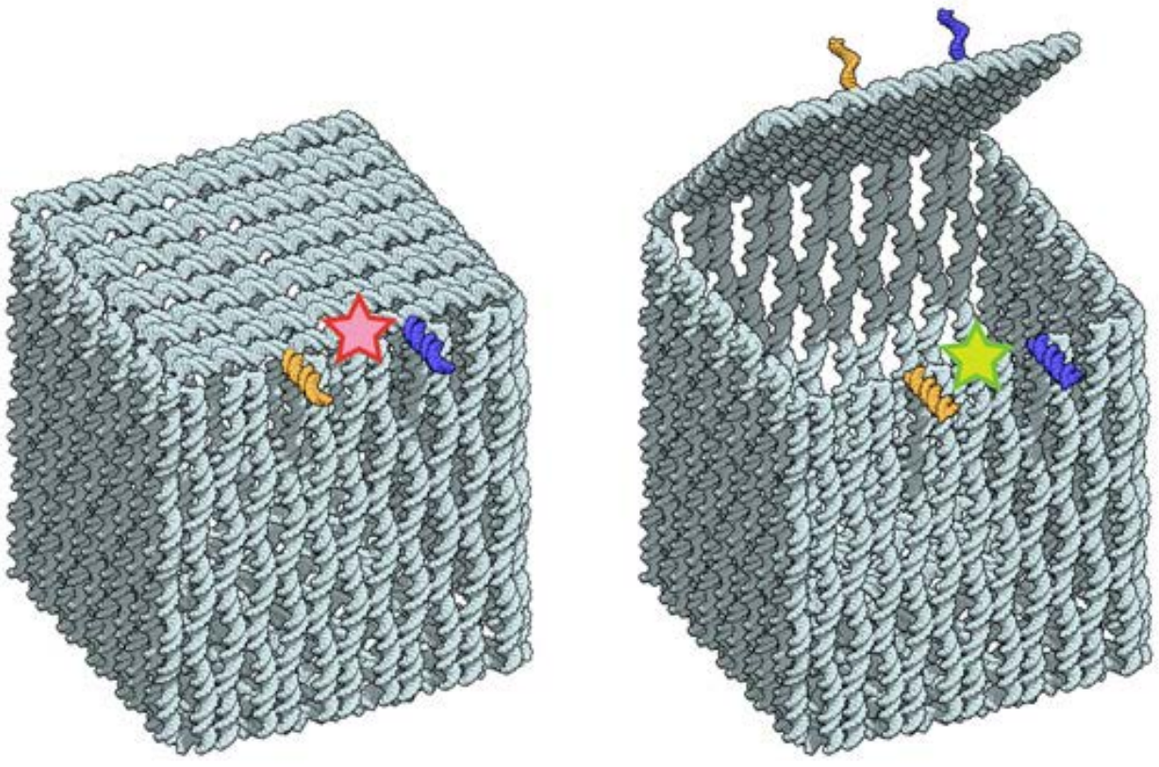
Coarse-grained model captures programmed curvature



Experiment from : Science 325:725

Adaptive resolution simulation of DNA origami systems

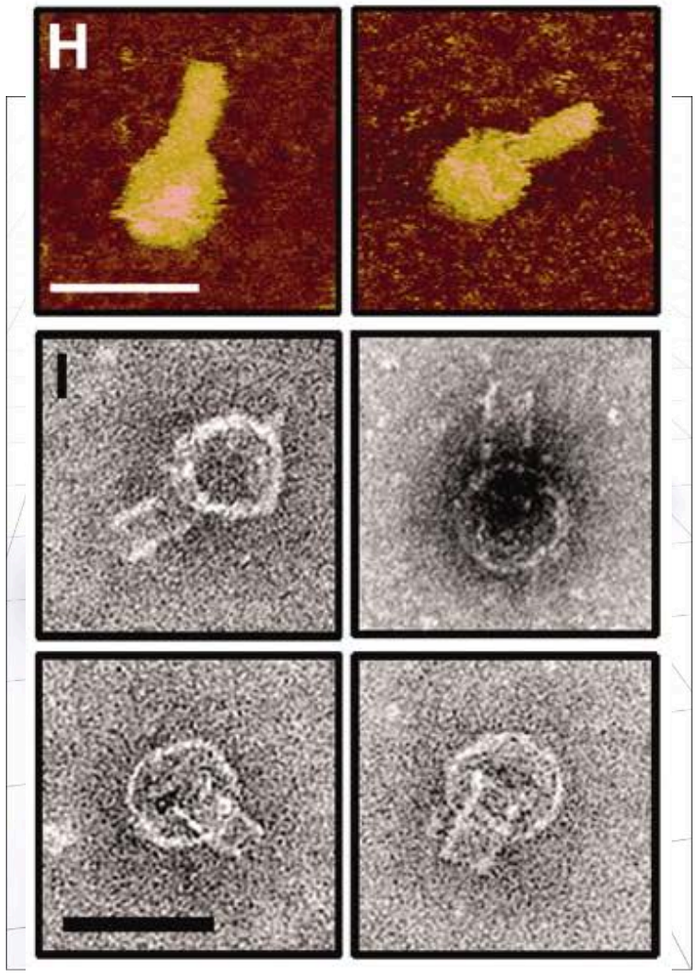
Victoria Birkedal Group



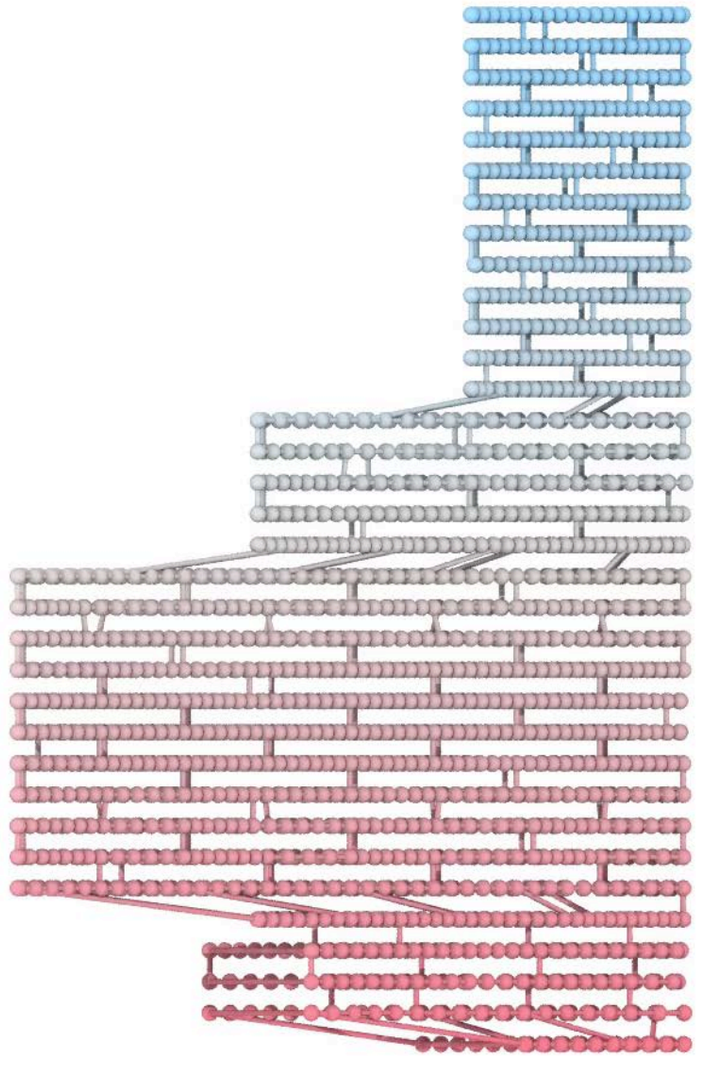
Andersen et al., Nature 2009

Used to interpret FRET characterization of DNA box variants: *Nanoscale* 11:18475 (2019)

Multi-resolution modeling of self-assembled DNA nanostructures

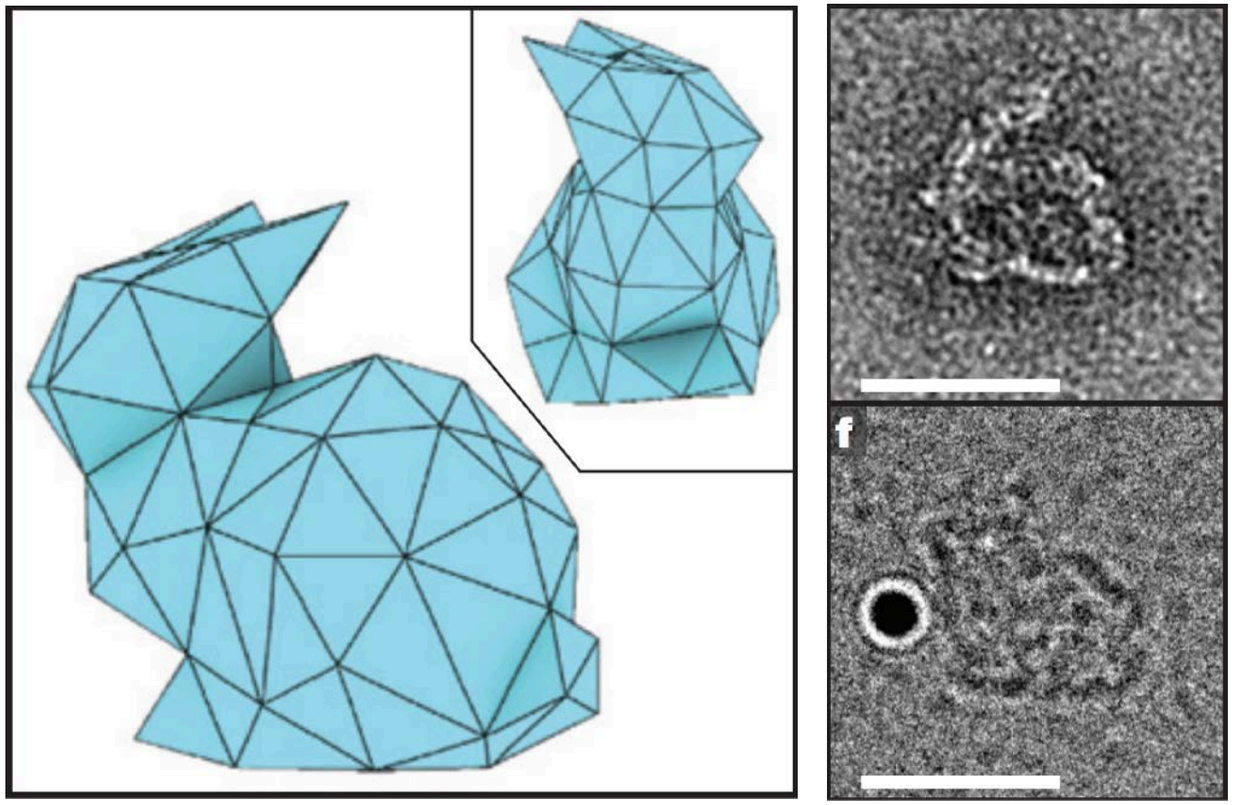


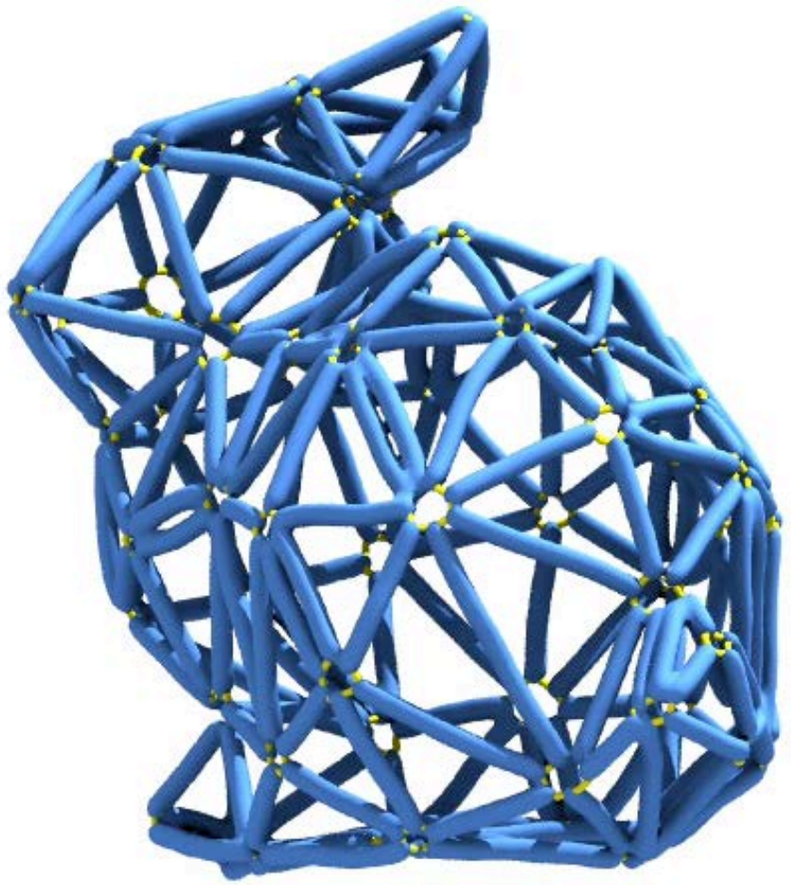
Dongran Han, Suchetan Pal, Jeanette Nangreave, Zhengtao Deng, Yan Liu, Hao Yan
Science 332:342



Nucleic Acids Research 48: 5135 (2020)

Multi-resolution workflow extended to DNA polyhedral meshes

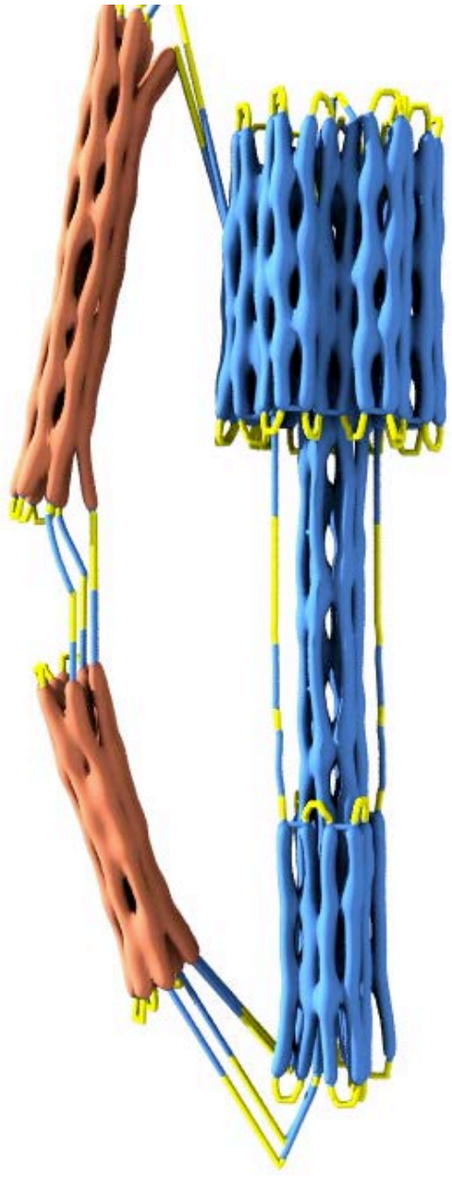




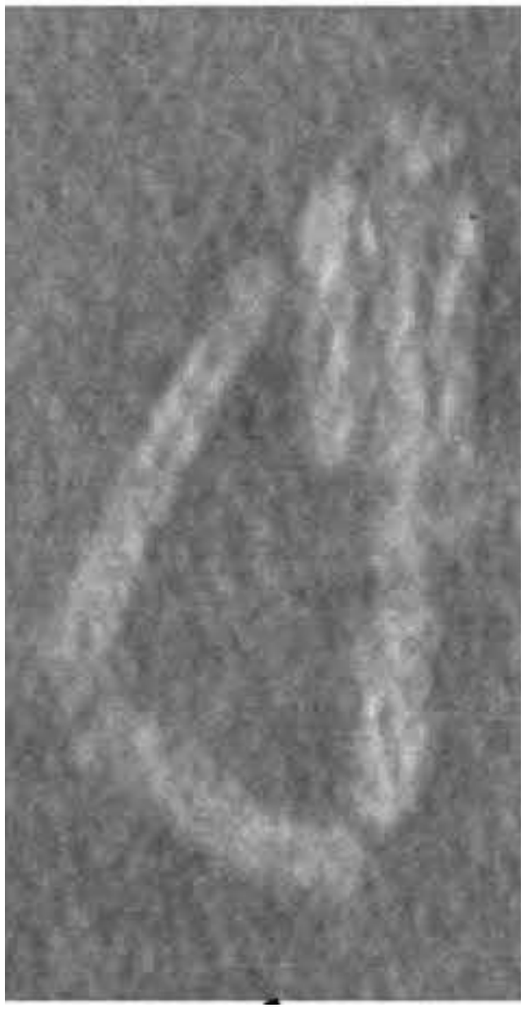
E Benson, A Mohammed, J Gardell, S Masich, E Czeizler, P Orponen & B Högberg
Nature 523:441

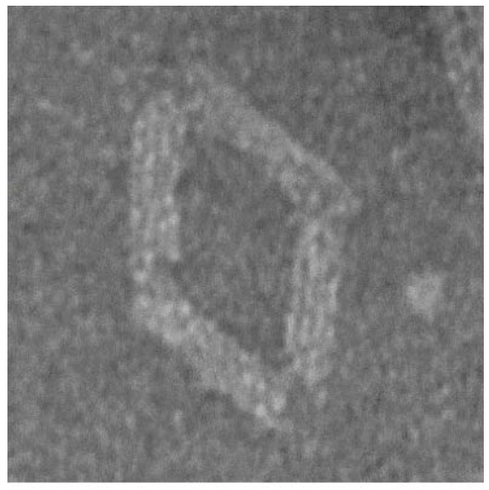
Nucleic Acids Research 48: 5135 (2020)

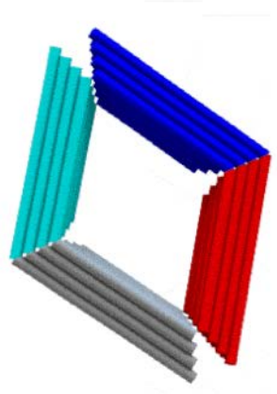
Coarse-grained simulations for sampling structural fluctuations



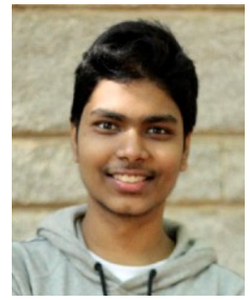
Alexander E. Marras, Lifeng Zhou, Hai-Jun Su and Carlos E. Castro PNAS 112:713

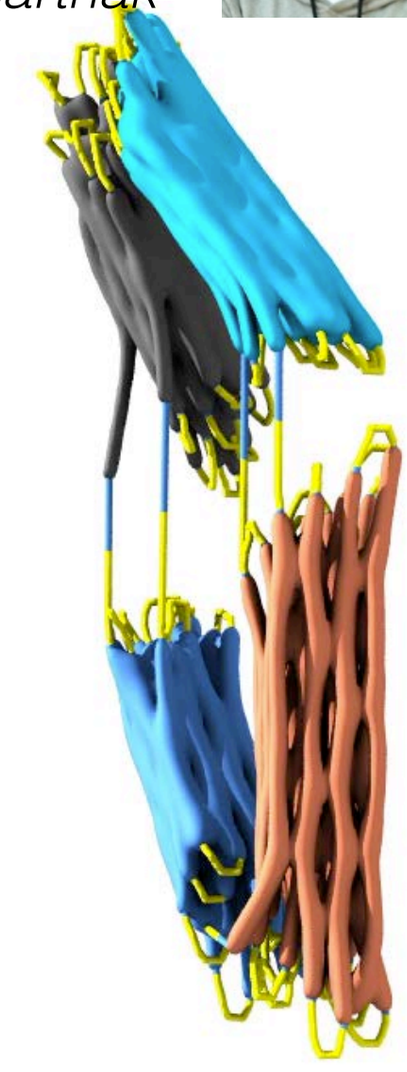






Kumar Sarthak



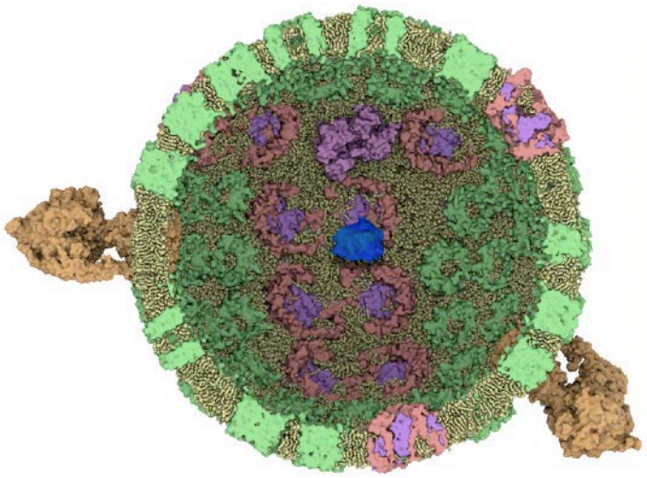


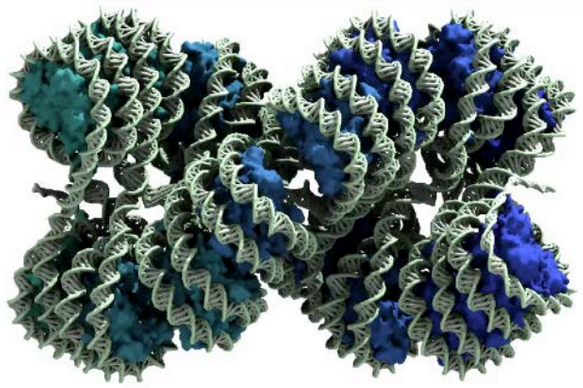
Nucleic Acids Research 48: 5135 (2020)

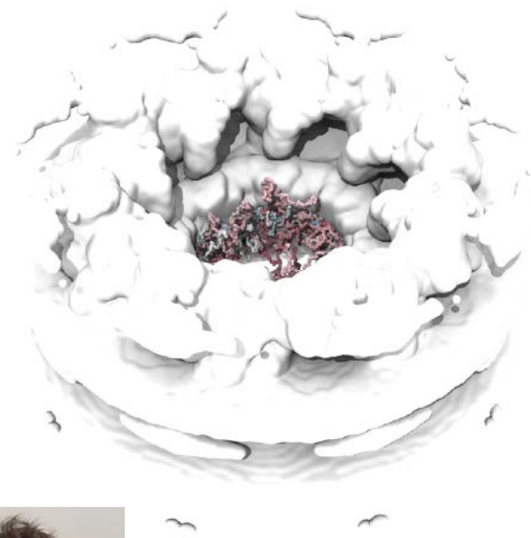
mrDNA simulations are powered by ARBD simulation package

http://www.ks.uiuc.edu/Development/Download/download.cgi?PackageName=ARBD

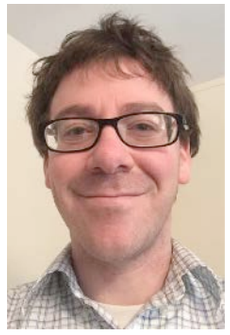
Cell 179:1098 (2019)







Abhi Singharoy

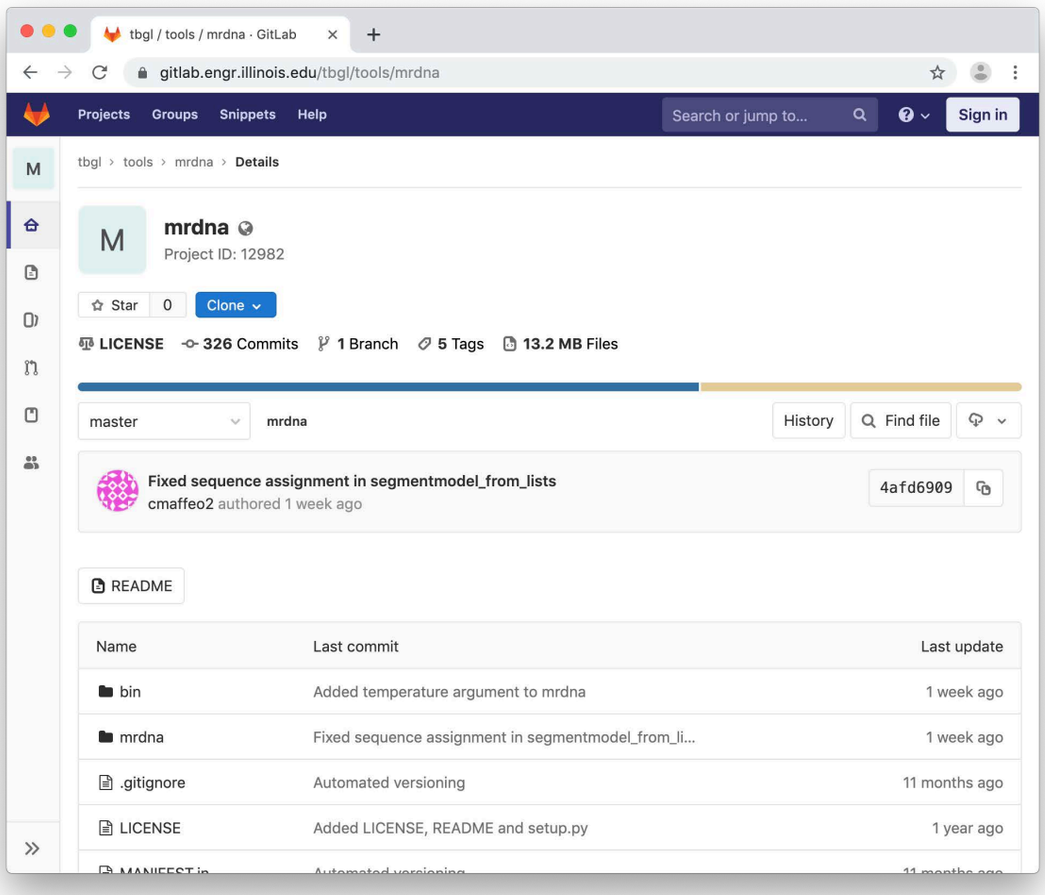


David Winogradoff

Where to find software, how to use it?

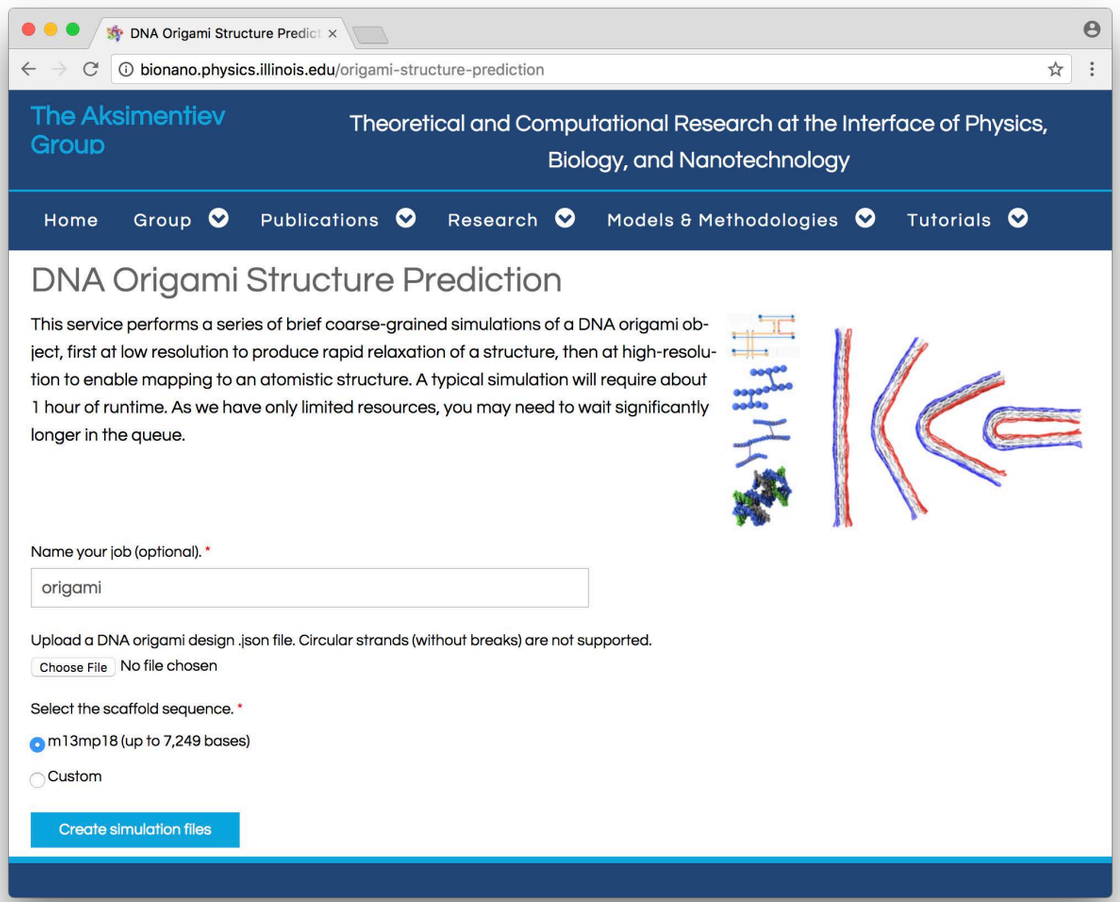
Gitlab

gitlab.engr.illinois.edu/tbgl/tools/mrdna



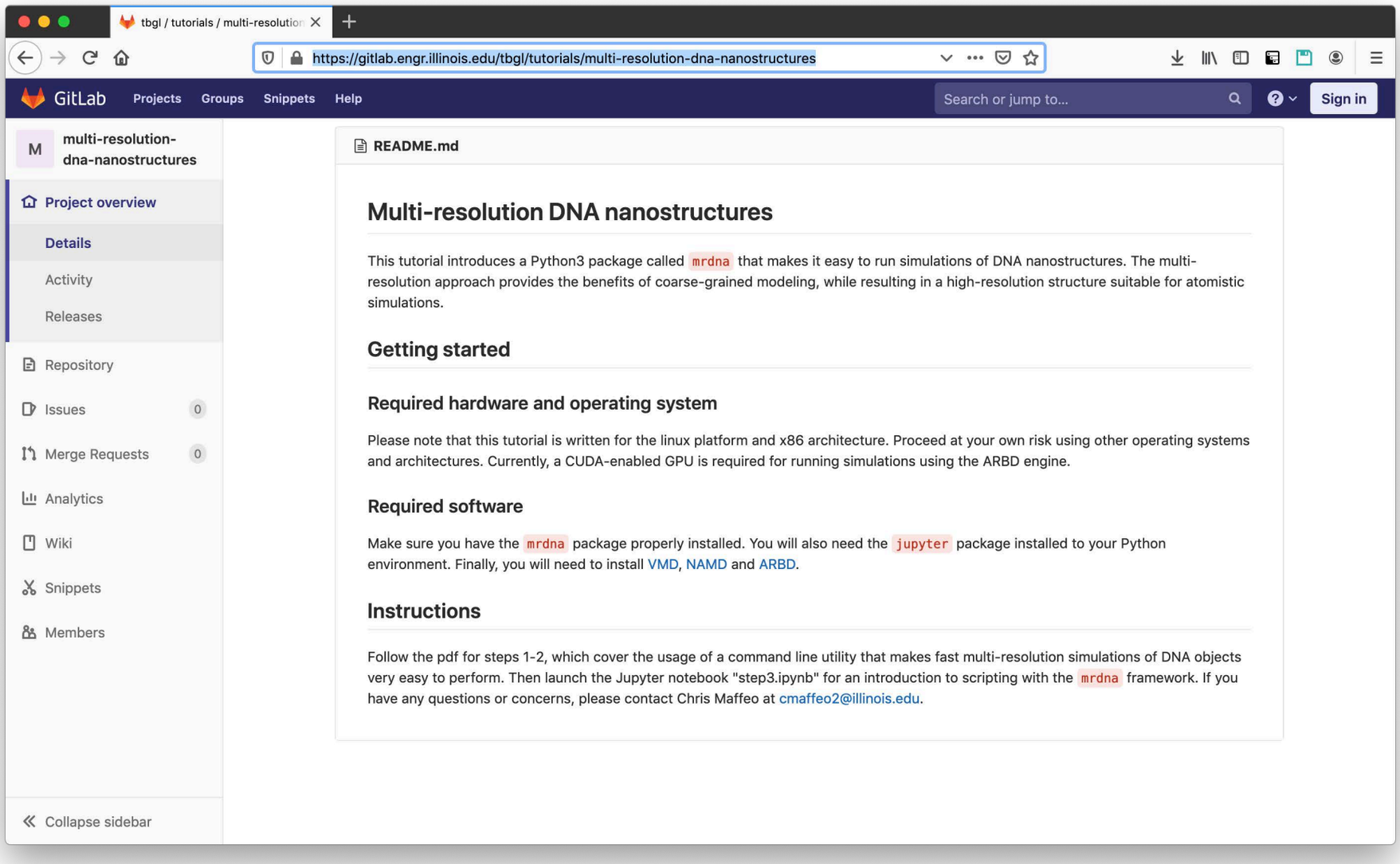
Web service

bionano.physics.illinois.edu/origami-structure-prediction



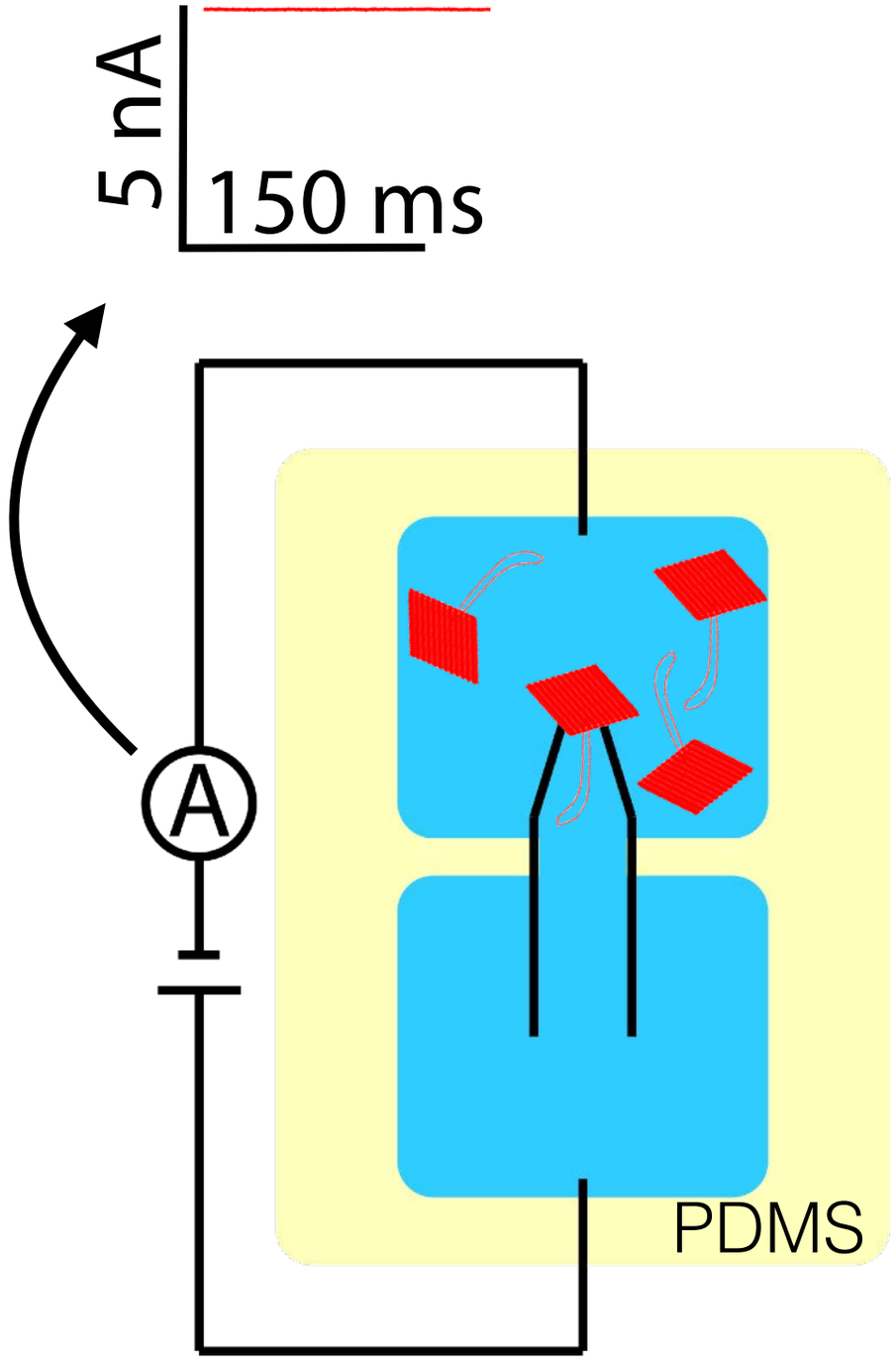
Tutorial: Multi-resolution simulations of self-assembled DNA nanostructures

https://gitlab.engr.illinois.edu/tbgl/tutorials/multi-resolution-dna-nanostructures

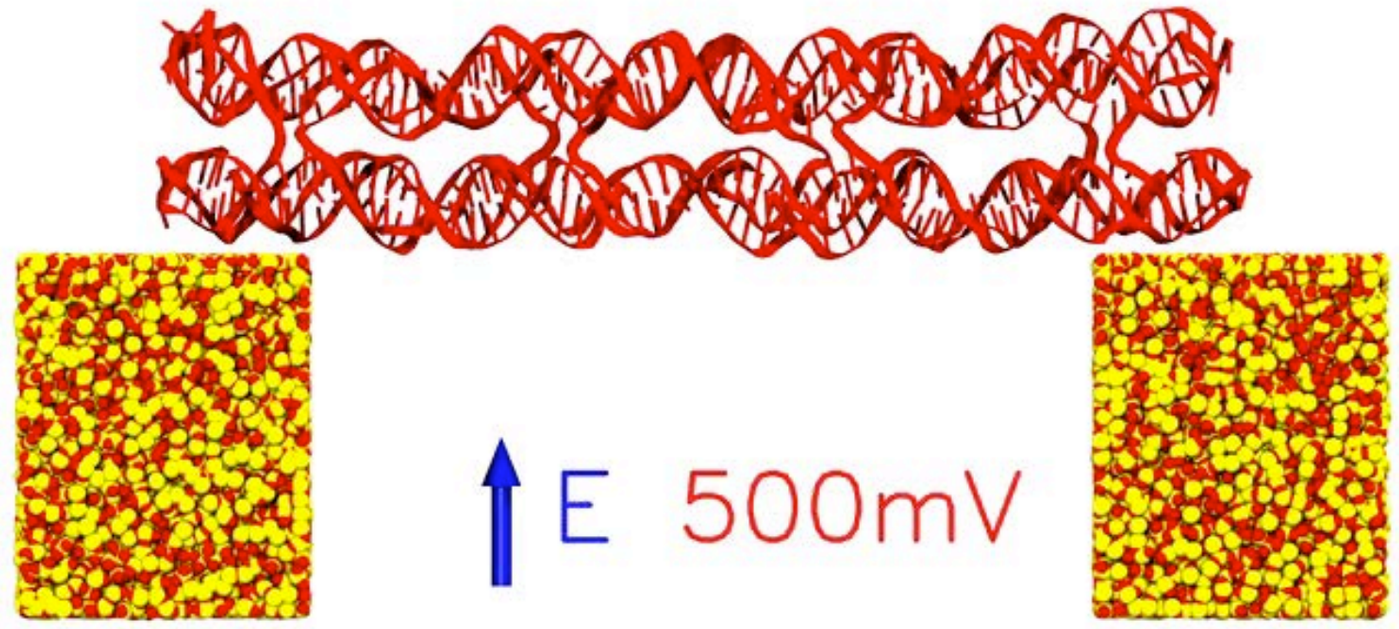


DNA voltage sensor

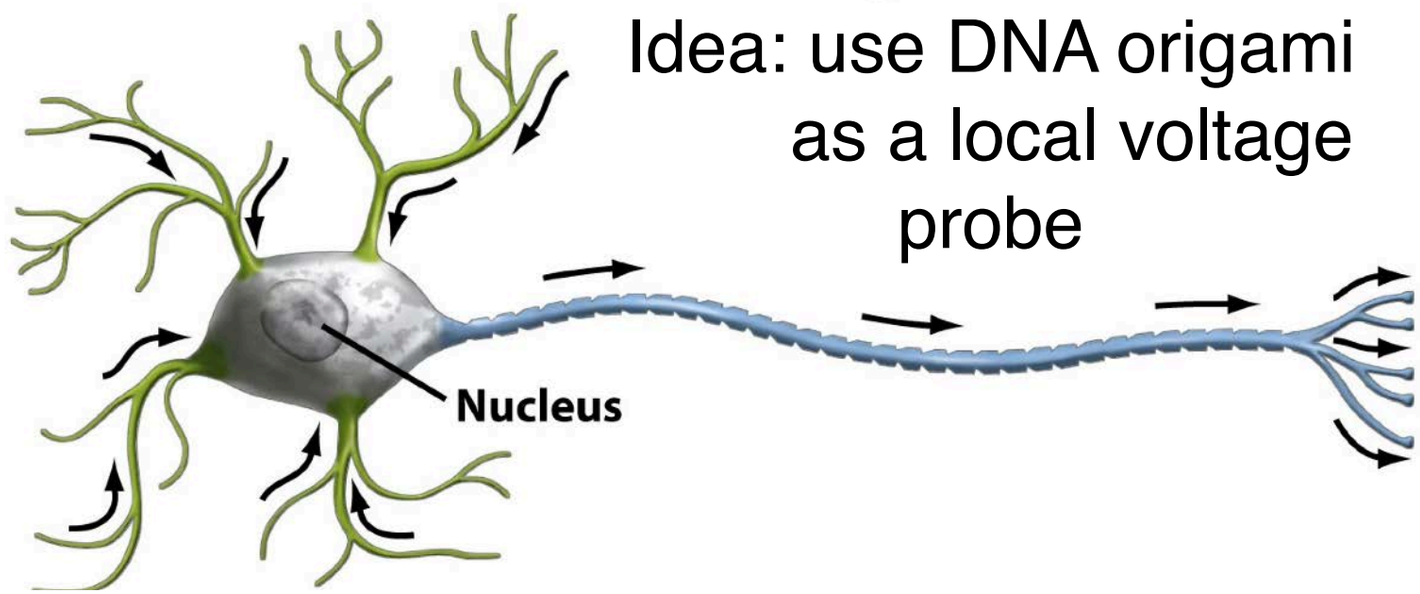
Experimental setup



All-atom MD simulation



ACS Nano 9:1420-1433 (2015)

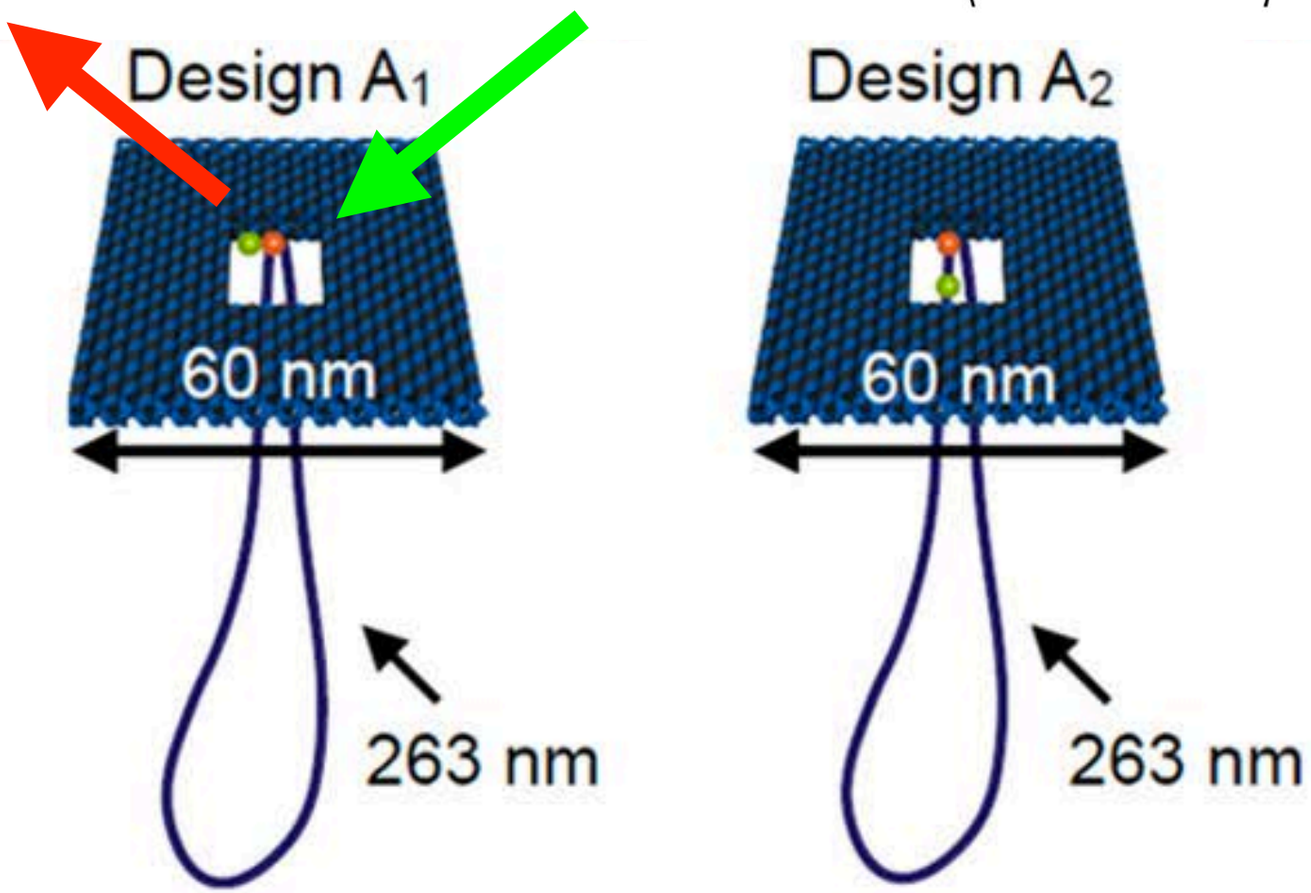


Idea: use DNA origami as a local voltage probe

Design of a nanoscale voltage sensor

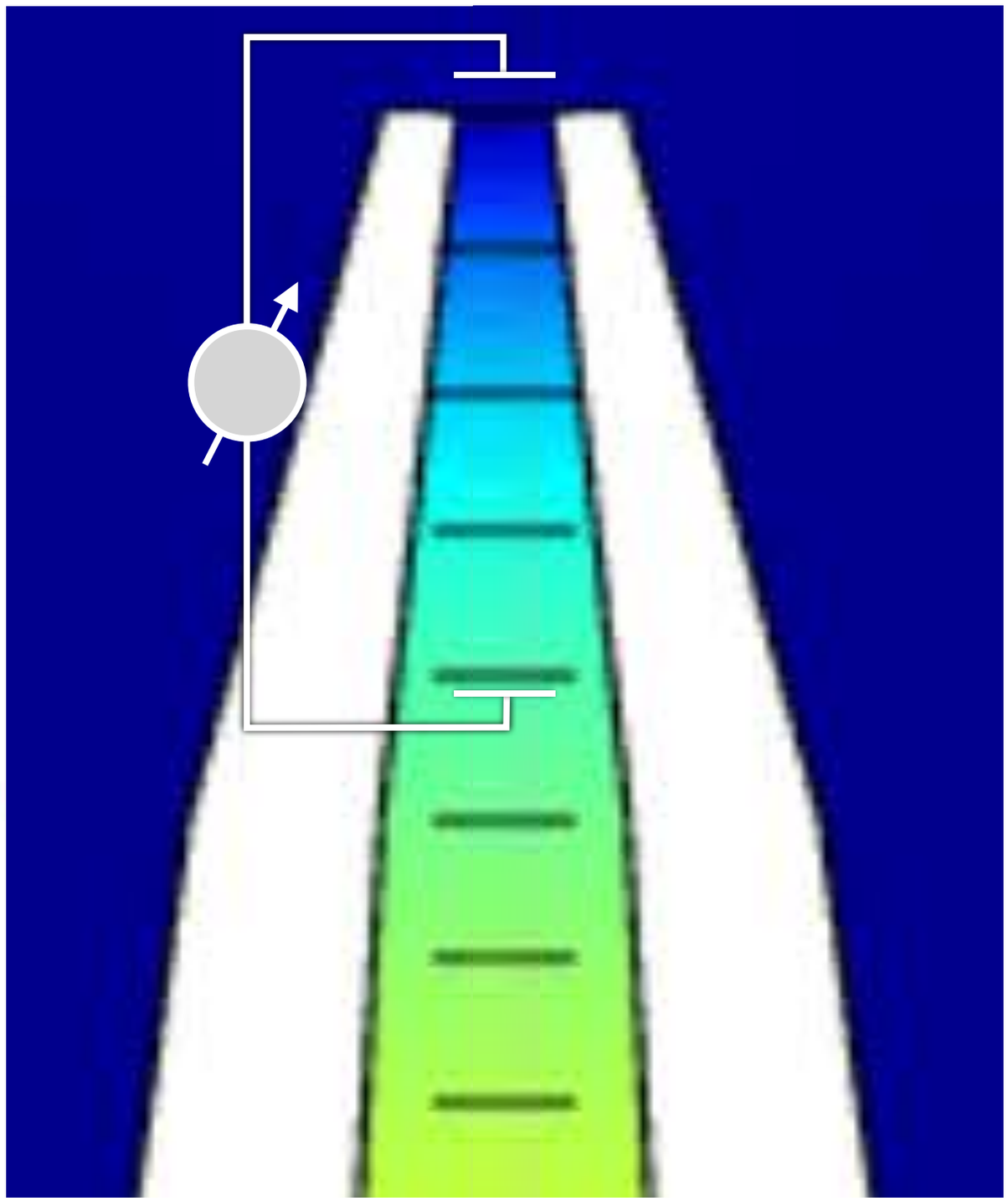
Keyser Group
Cambridge UK

FRET~ $1/\left\langle 1+\left(\frac{r}{R_0}\right)^6 \right\rangle$



E Hemmig, C Fitzgerald, C Maffeo, L Hecker, S Ochmann, A Aksimentiev, P Tinnefeld, and U Keyser. Nano Letters 18:1962 (2018)

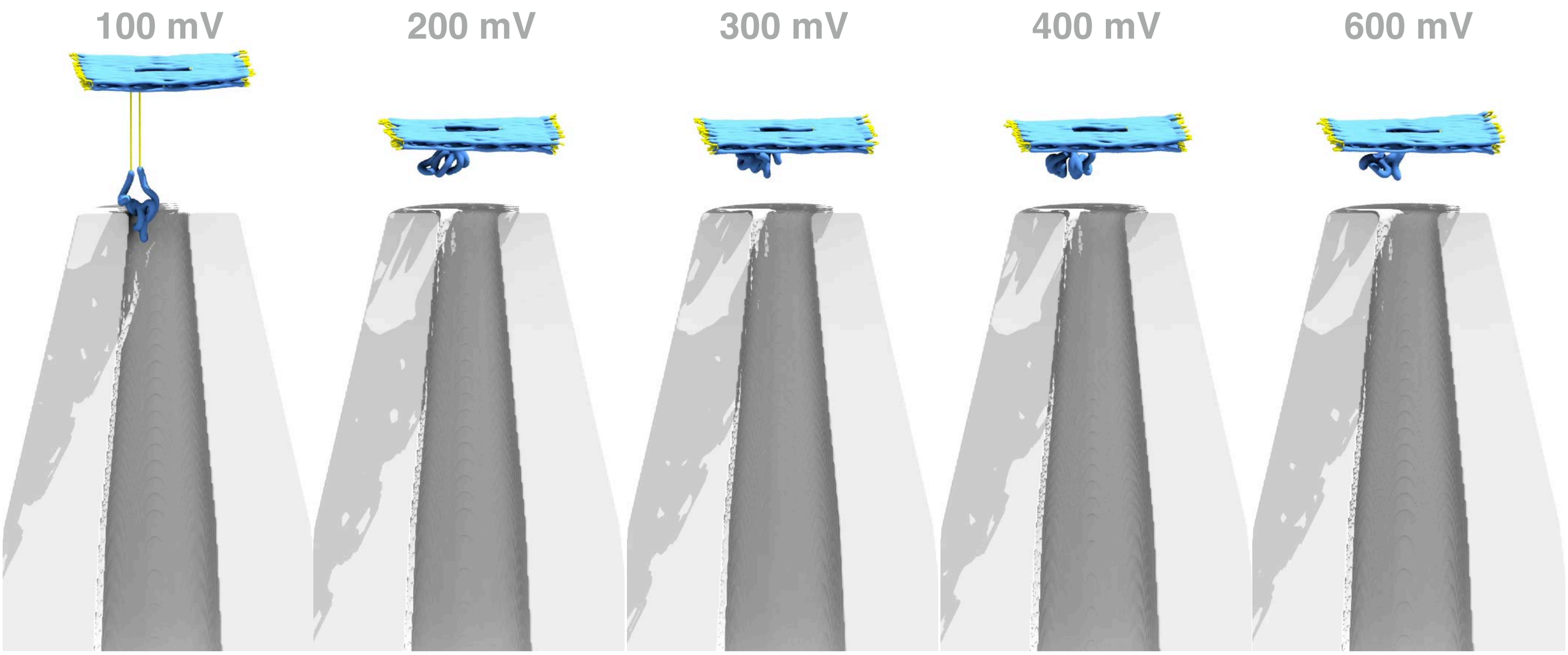
Electrostatic potential in nanopipette from COMSOL



Coarse-grained simulations of a FRET plate capture

~5 bp/bead CG model

40 μs simulations



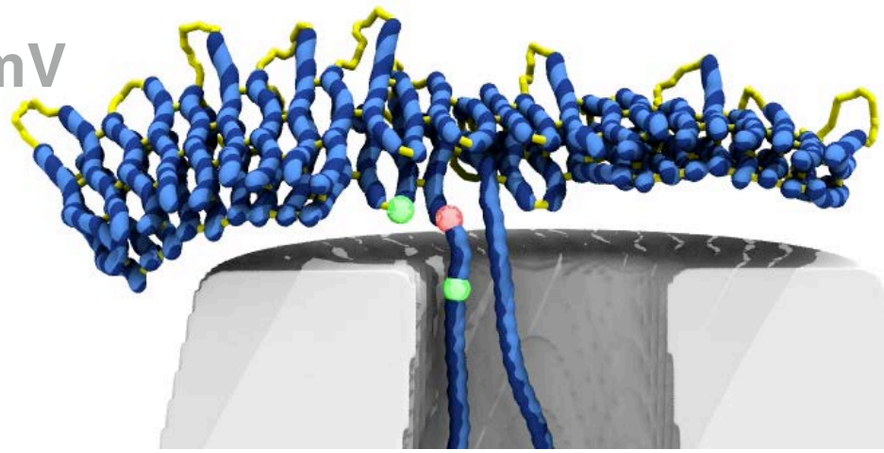
Nano Letters 18:1962 (2018)

CG simulation of FRET efficiency

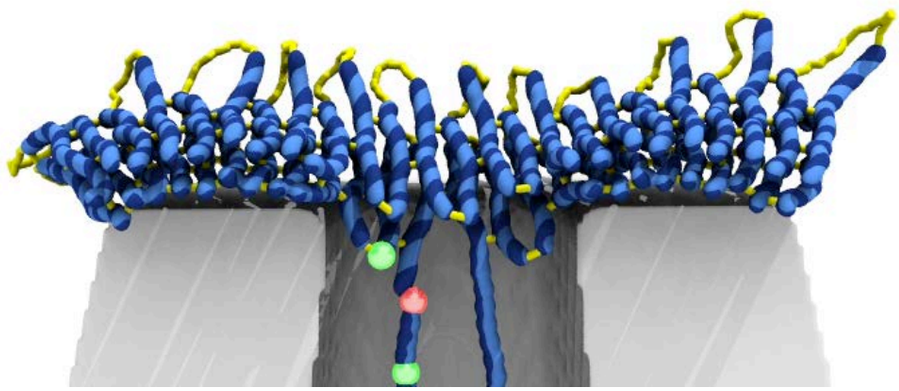
2 beads/bp CG model

1 μs simulations

100 mV

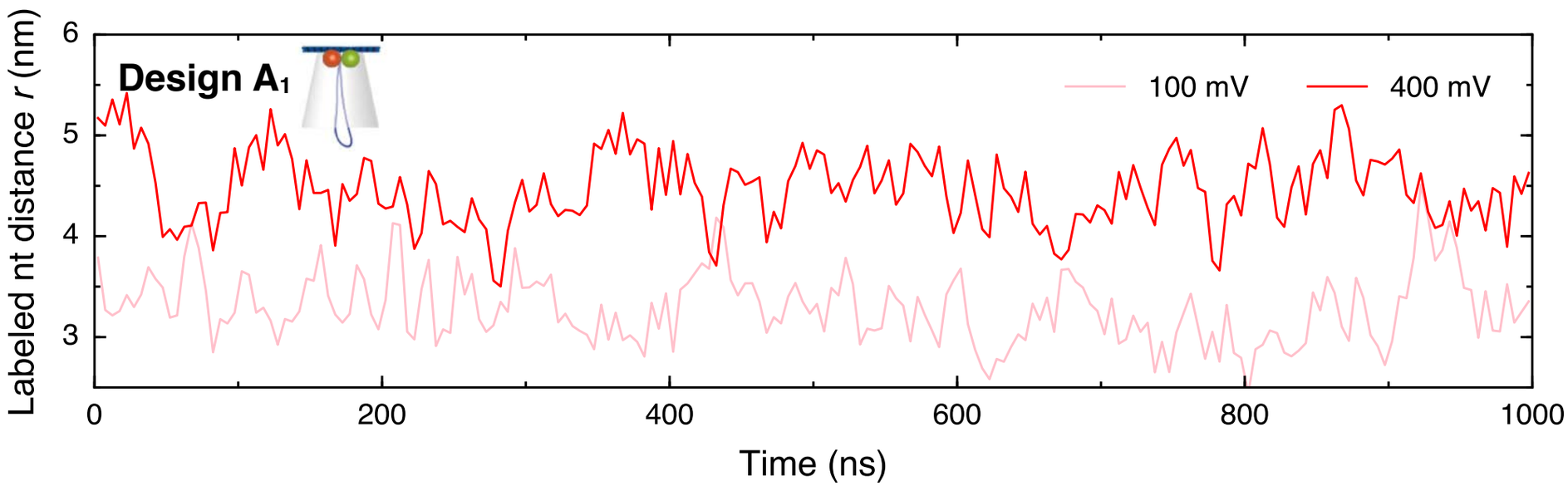


400 mV



200 mV

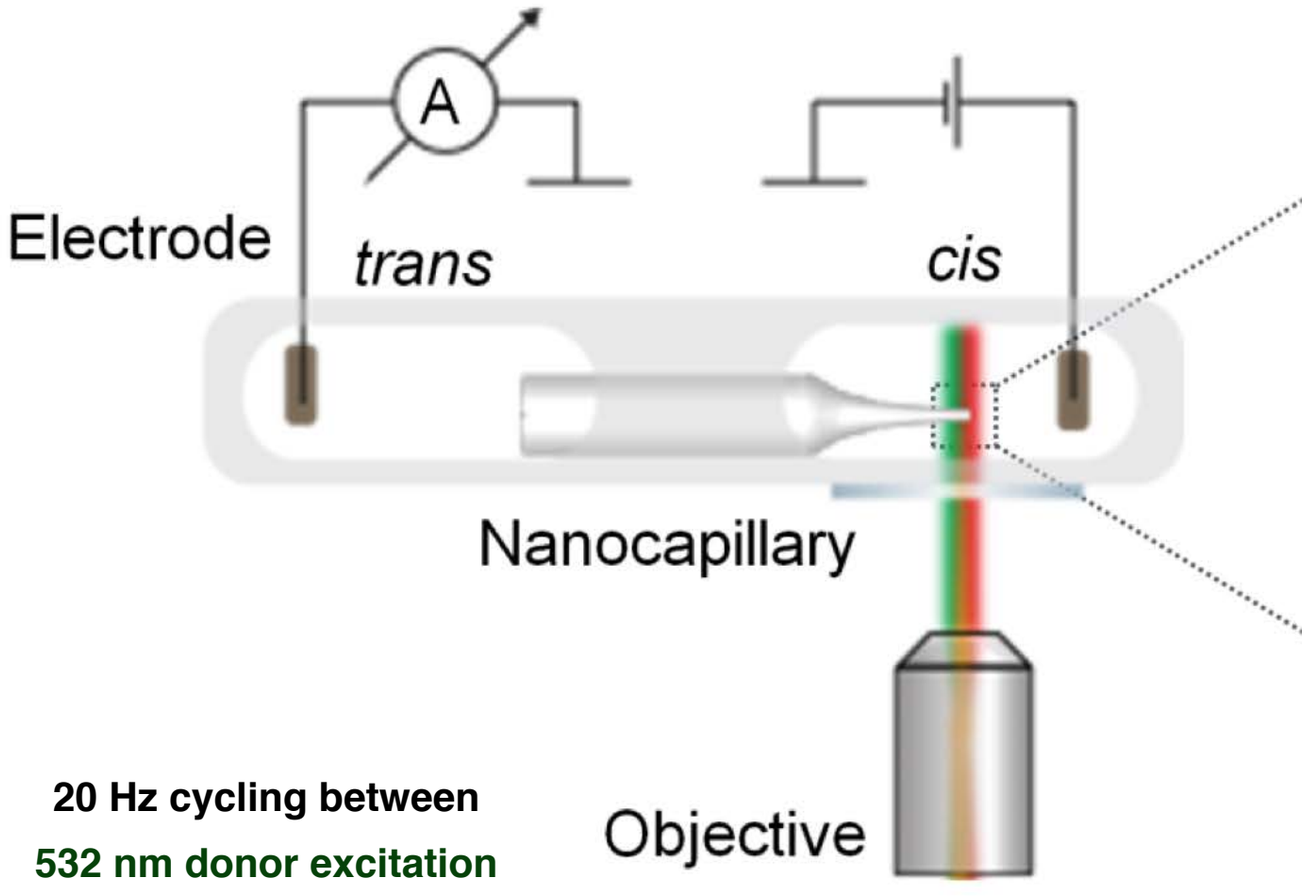
300 mV



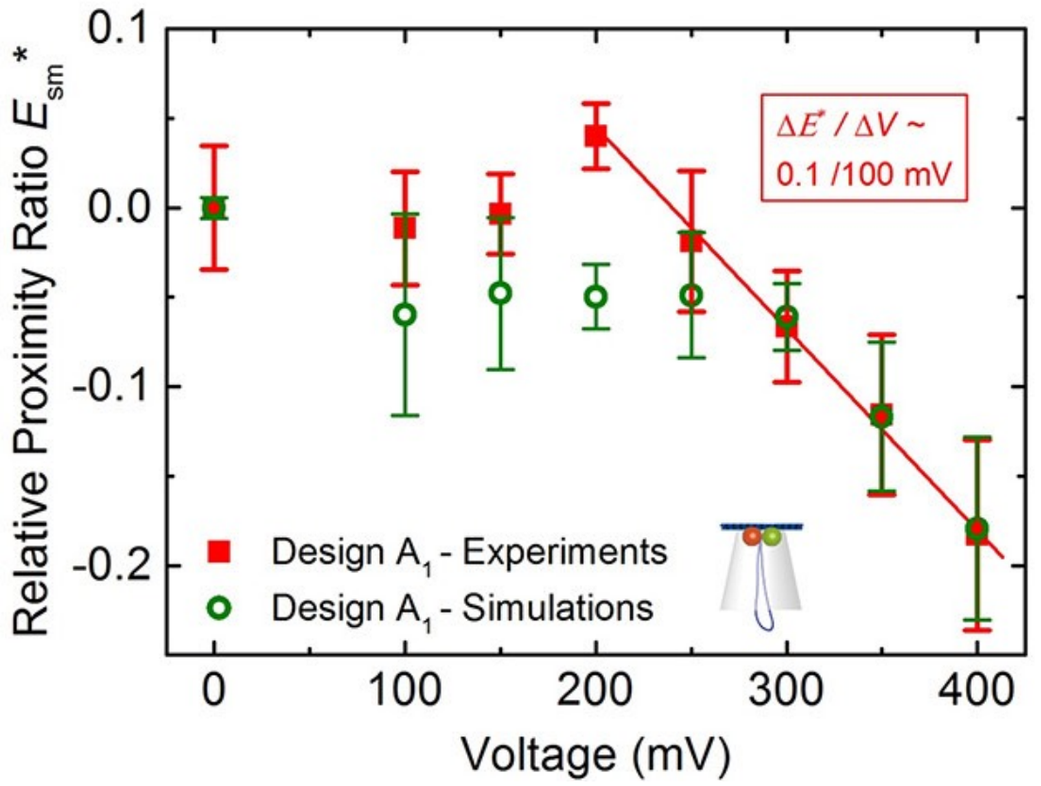
Experimental demonstration

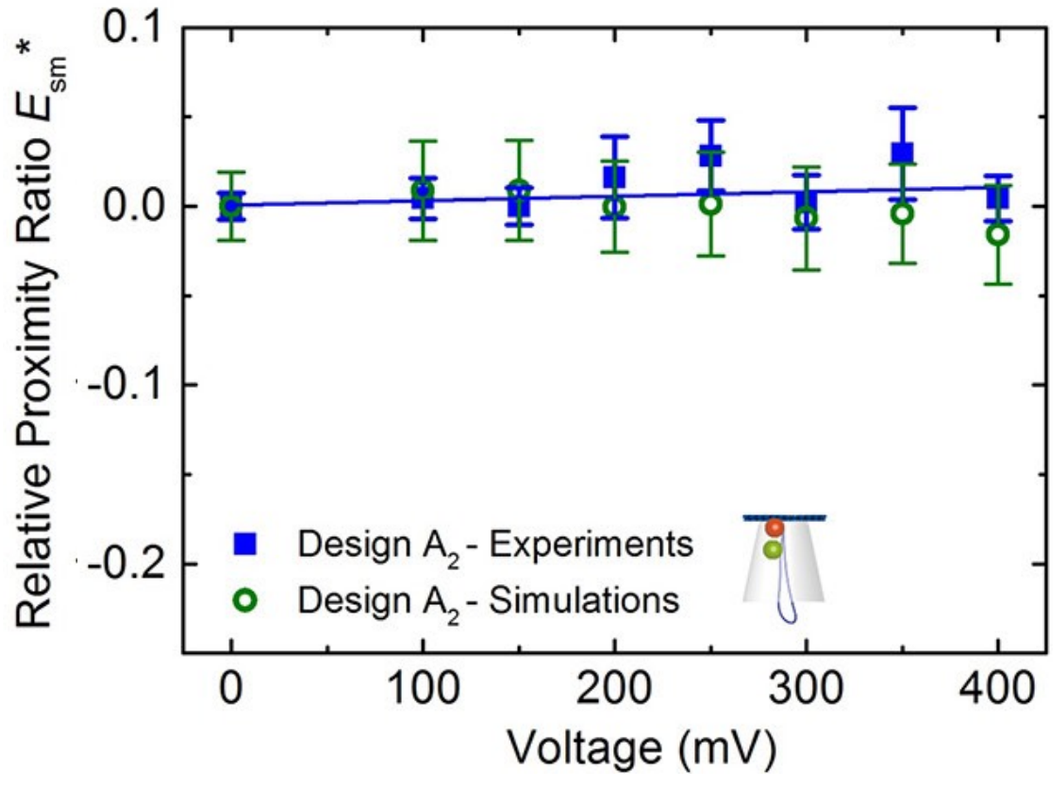
Ulrich Keyser
Cambridge, UK

Philip Tinnefeld
LMU



20 Hz cycling between
532 nm donor excitation
and
638 nm acceptor excitation

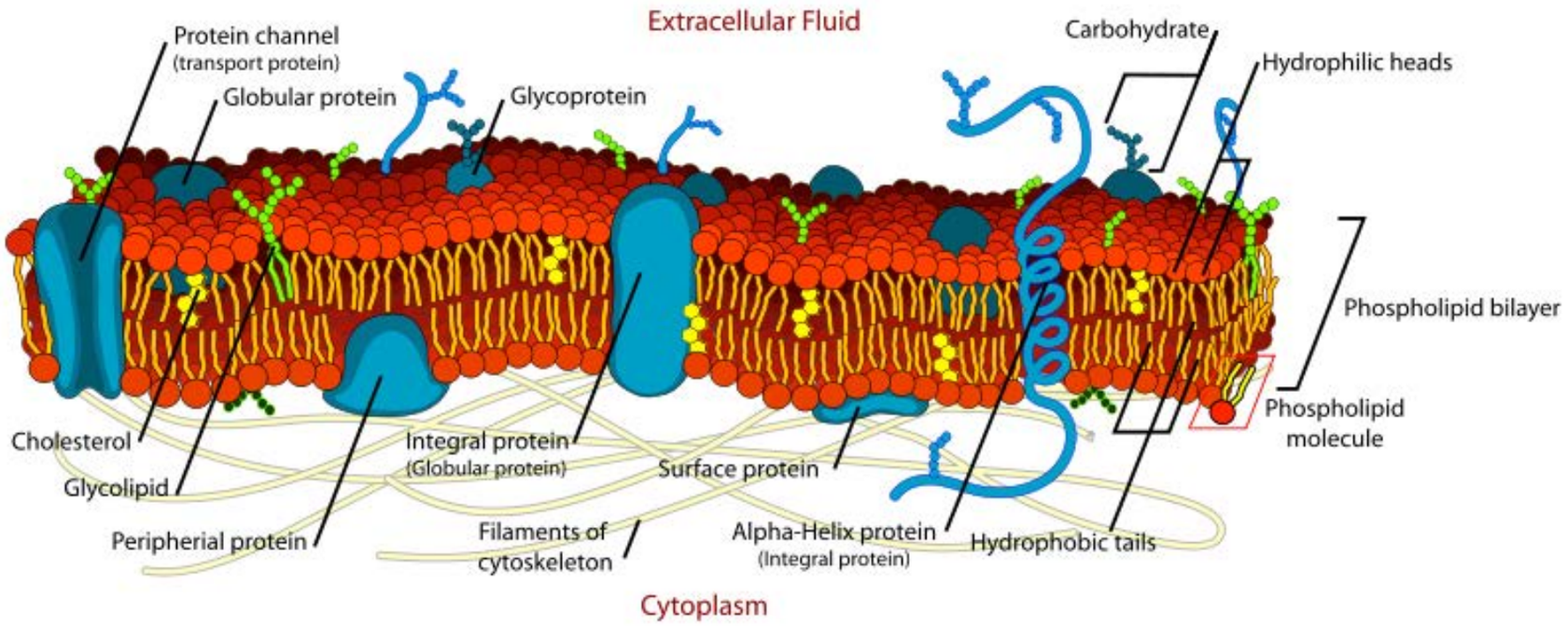


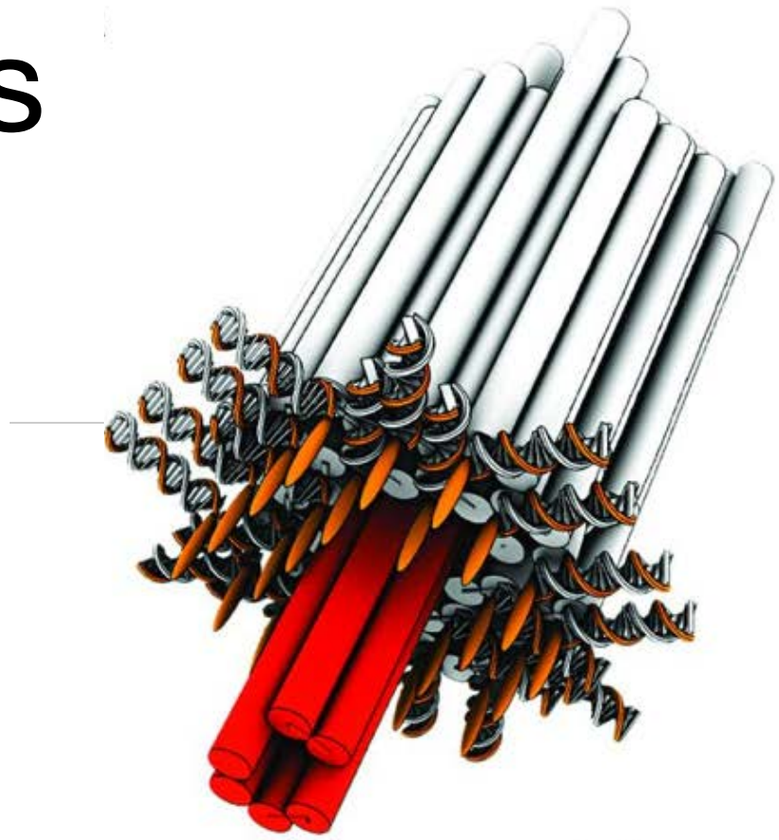


DNA membrane channels

Dr. Ulrich F. Keyser
Cambridge, UK

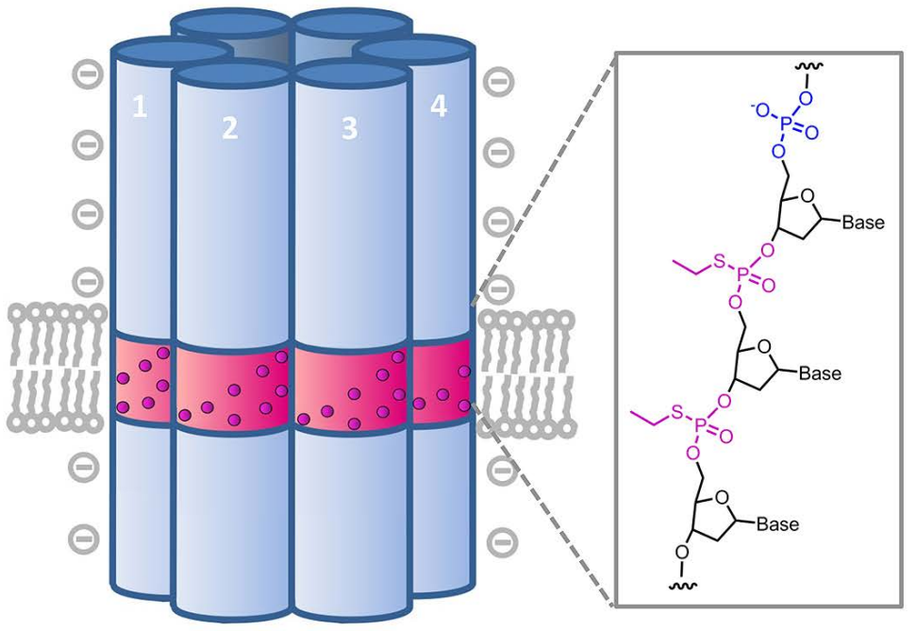
DNA Ion Channels



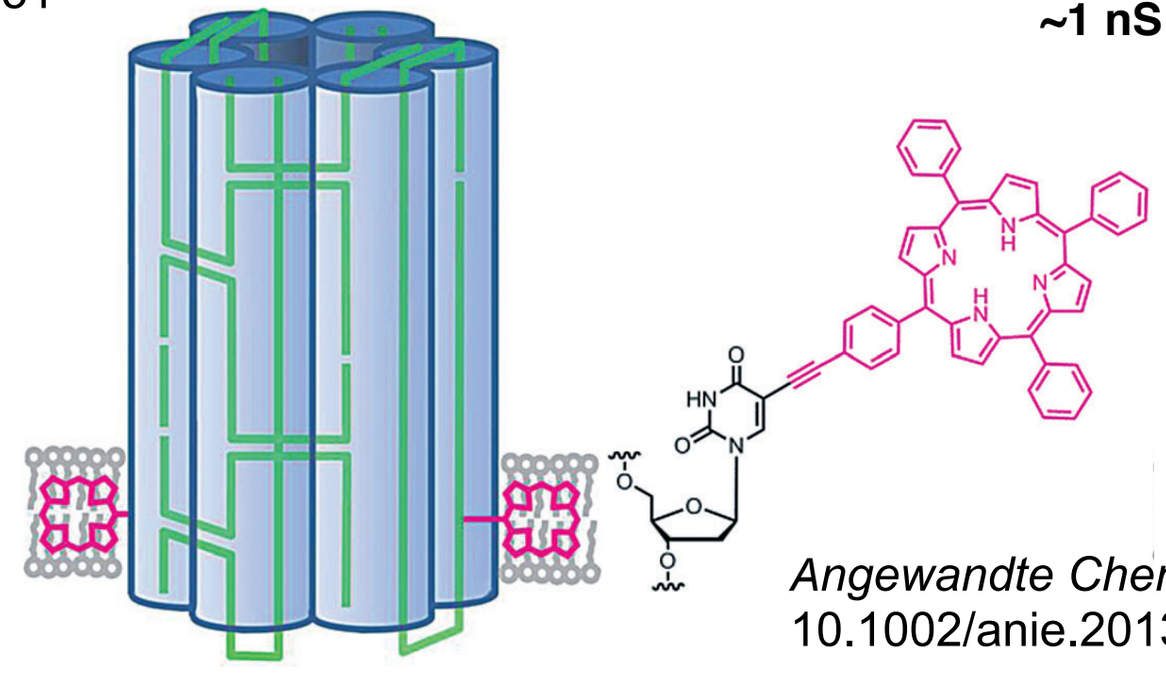


Langecker, M. *et al*., *Science*: 338, 932-936.

Nano Letters, *13:* 2351

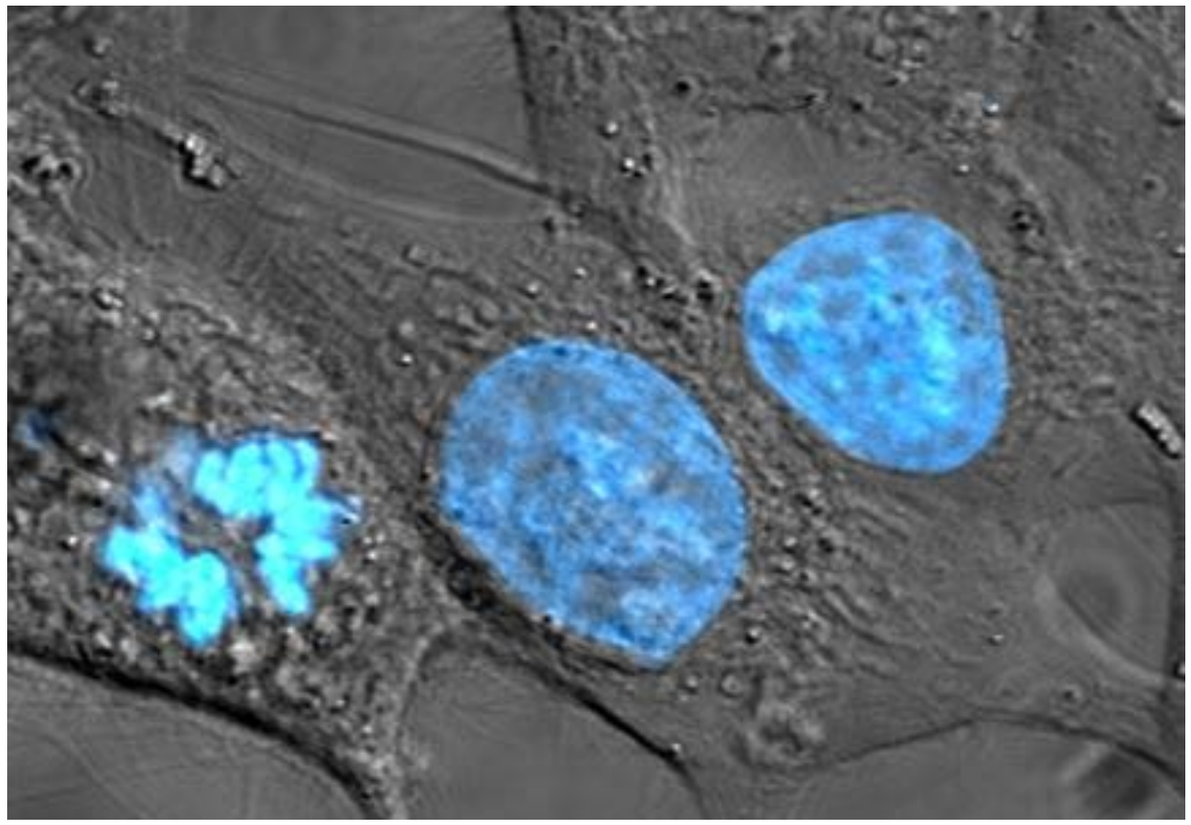


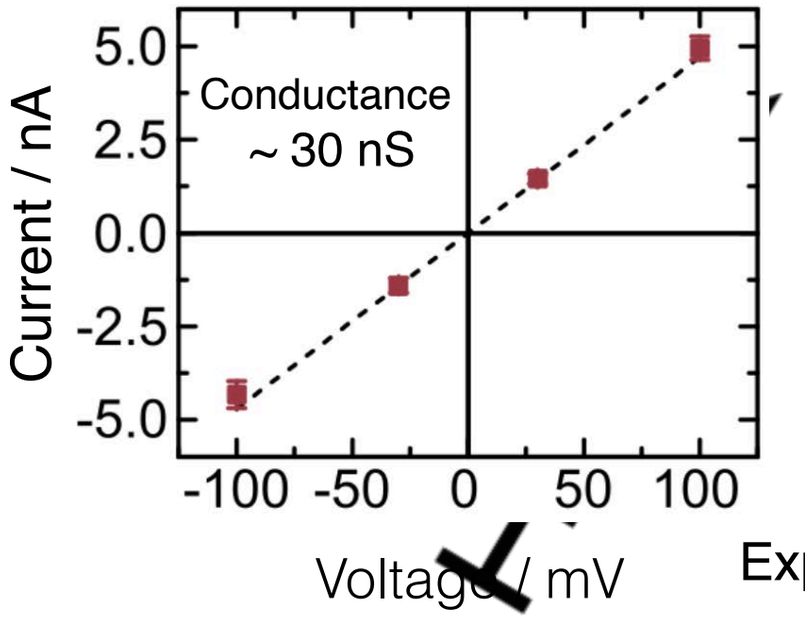
~1 nS conductance

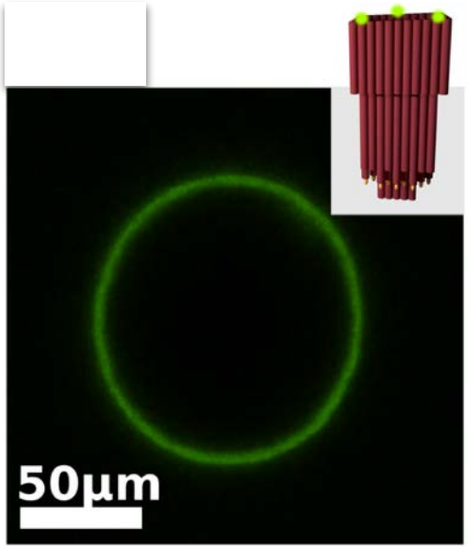


Angewandte Chemie
10.1002/anie.201305765

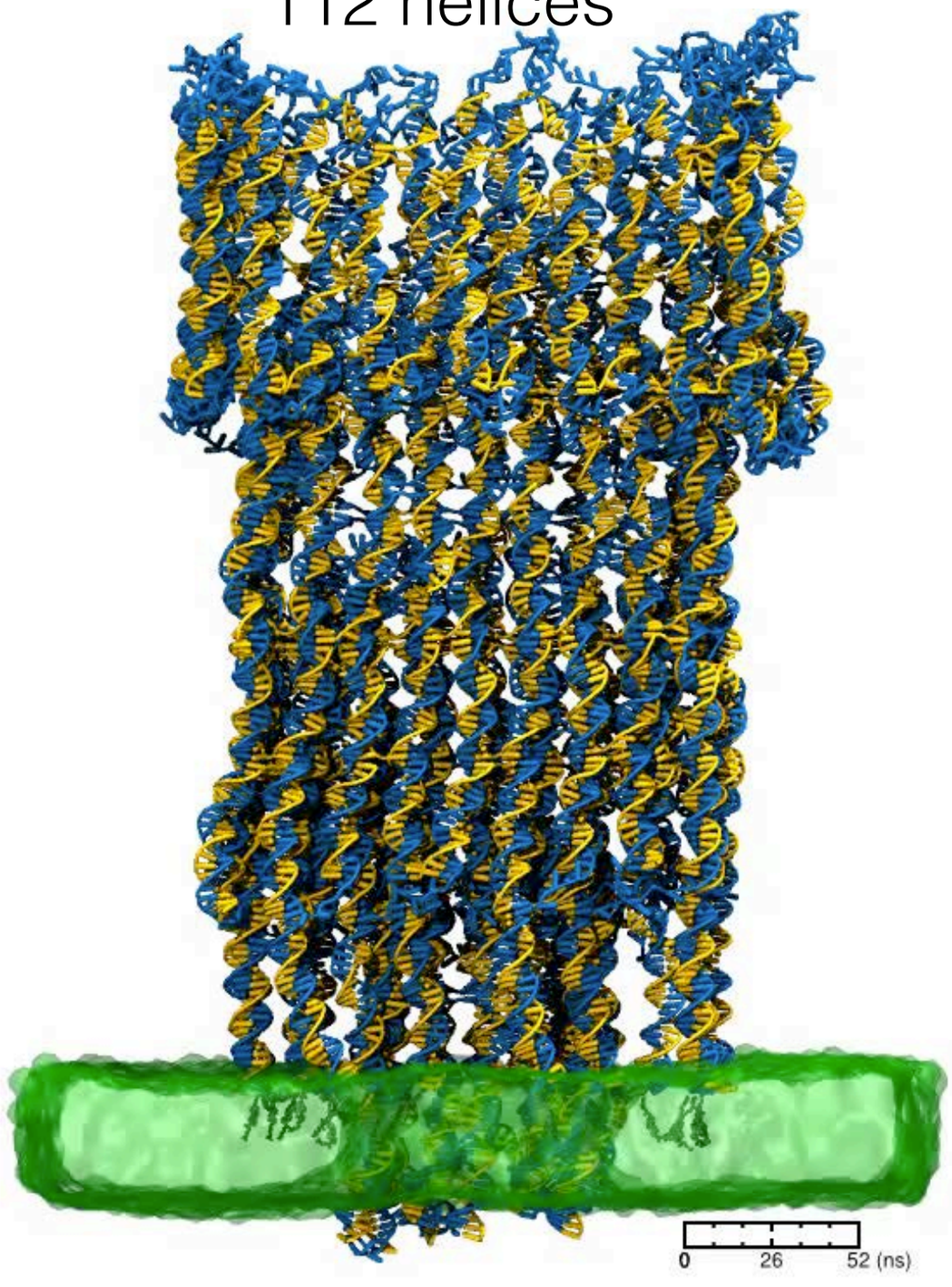
DNA origami syringe







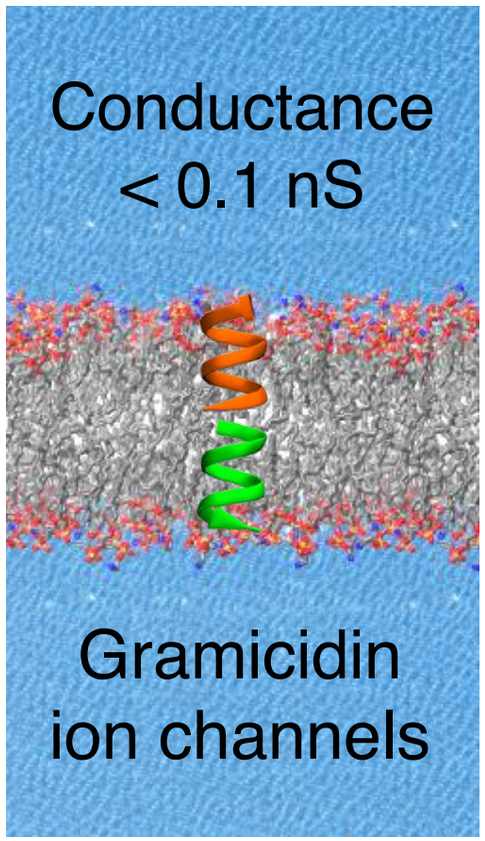
112 helices

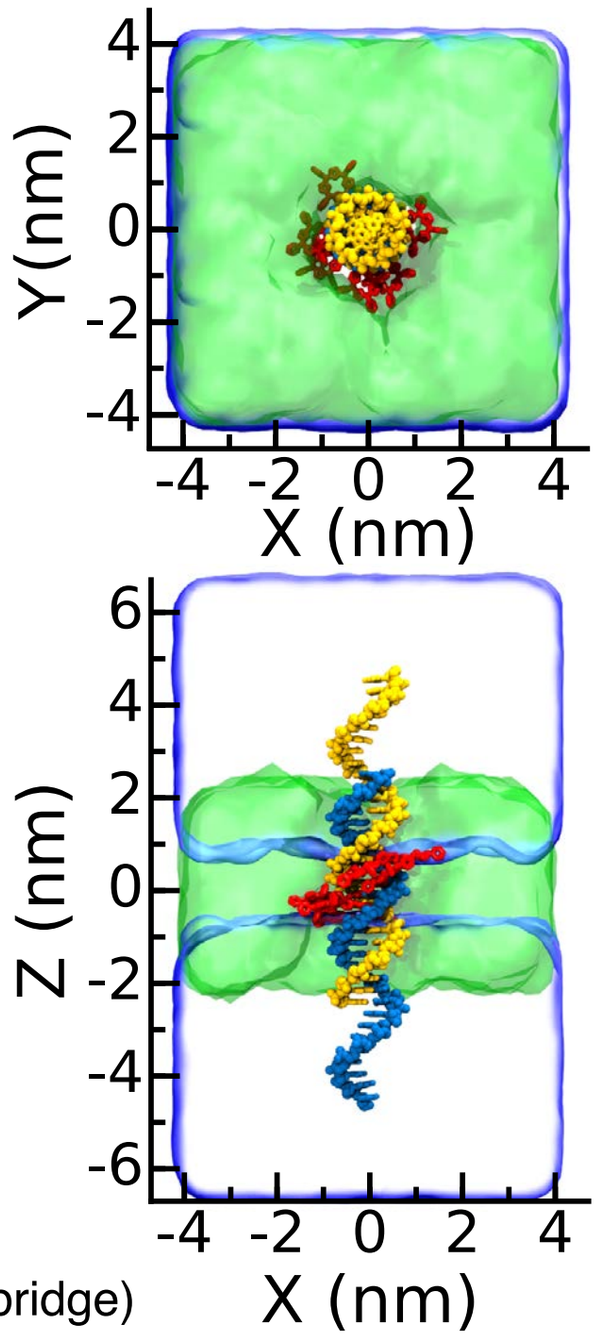


Experiment: Keyser lab (Cambridge, UK) *ACS Nano* 10:8207-8214 (2016)

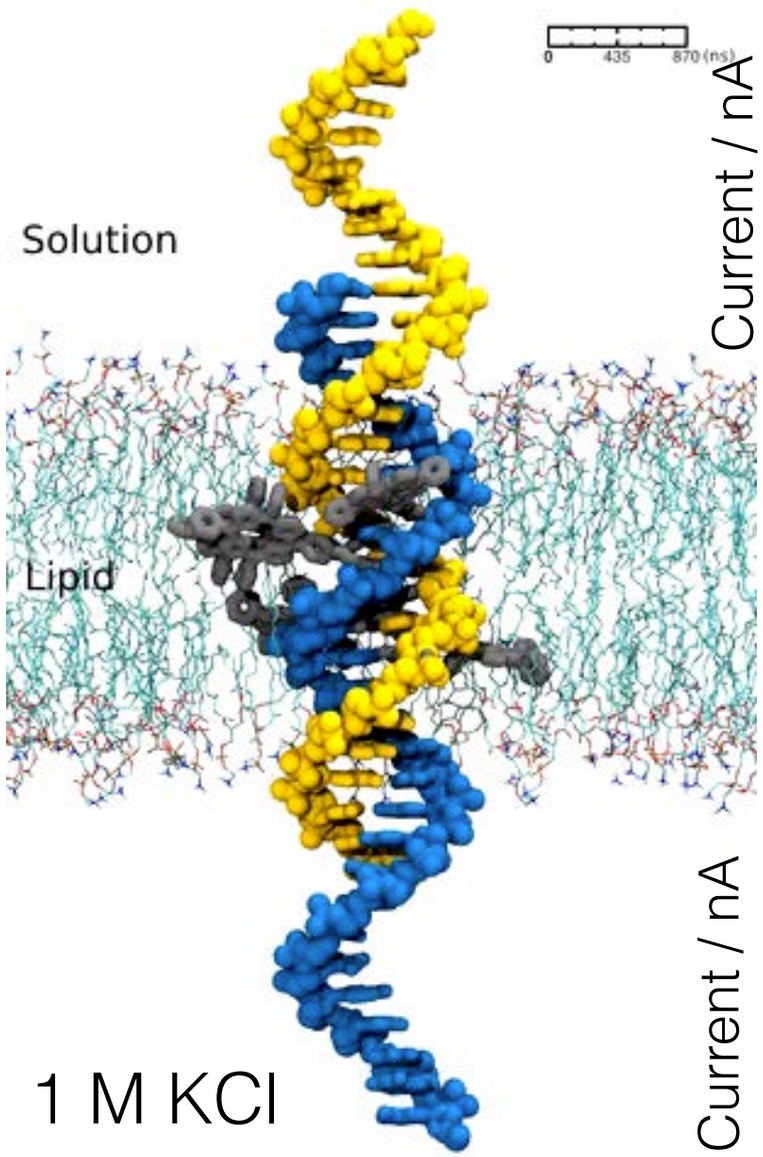
Small conductance DNA channel

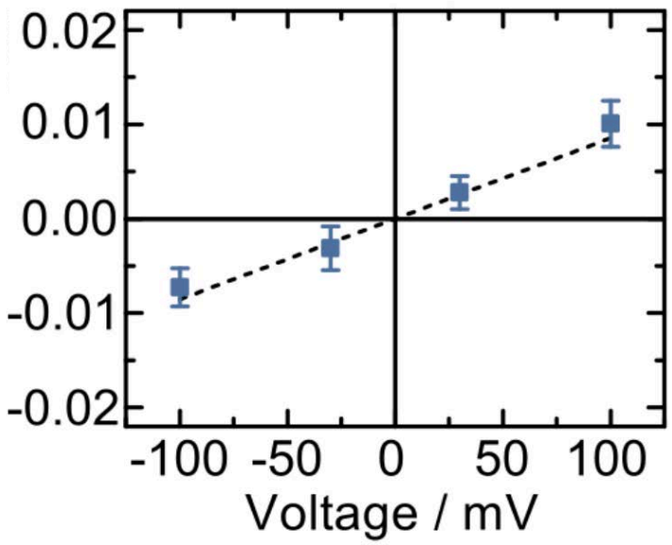
Chen Yu Li



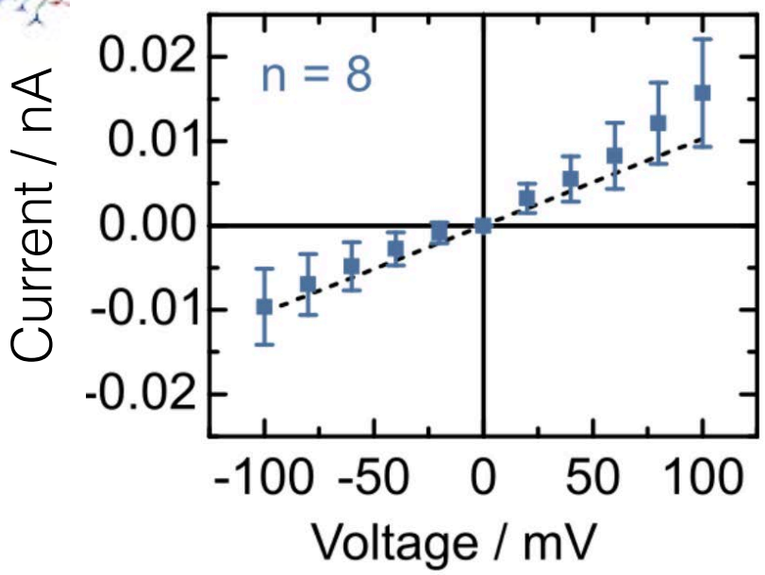


1 helix
140,000 atoms





Conductance: ~ 0.1 nS



Experiment:
Ulrich Keyser (Cambridge)
Eugen Stulz (Southampton)
Mathias Winterhalter (Jacobs U)

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

DNA nanostructure catalyzes lipid scrambling

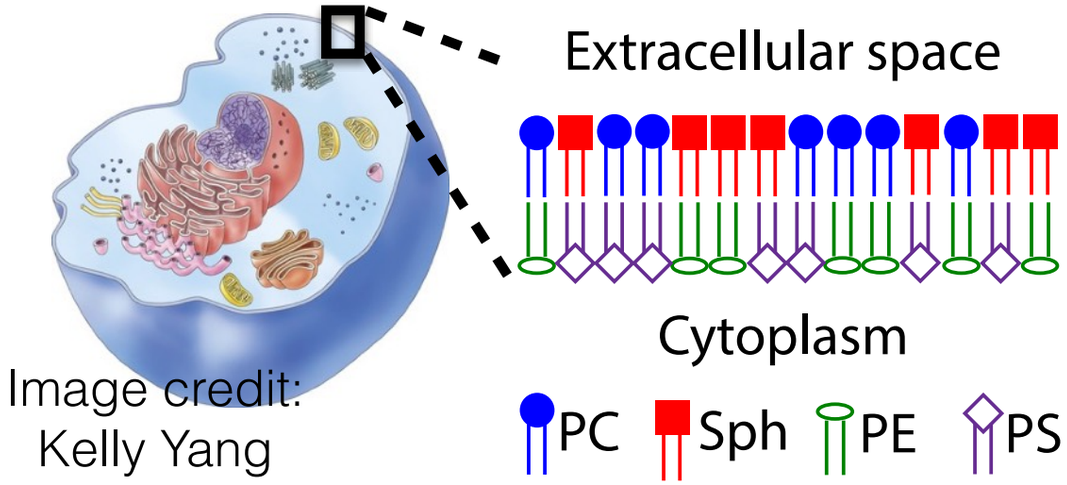
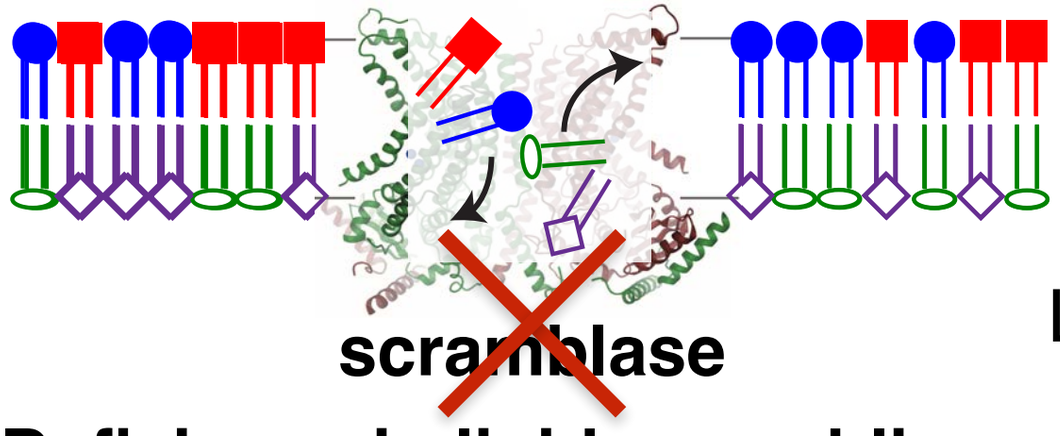


Image credit:
Kelly Yang

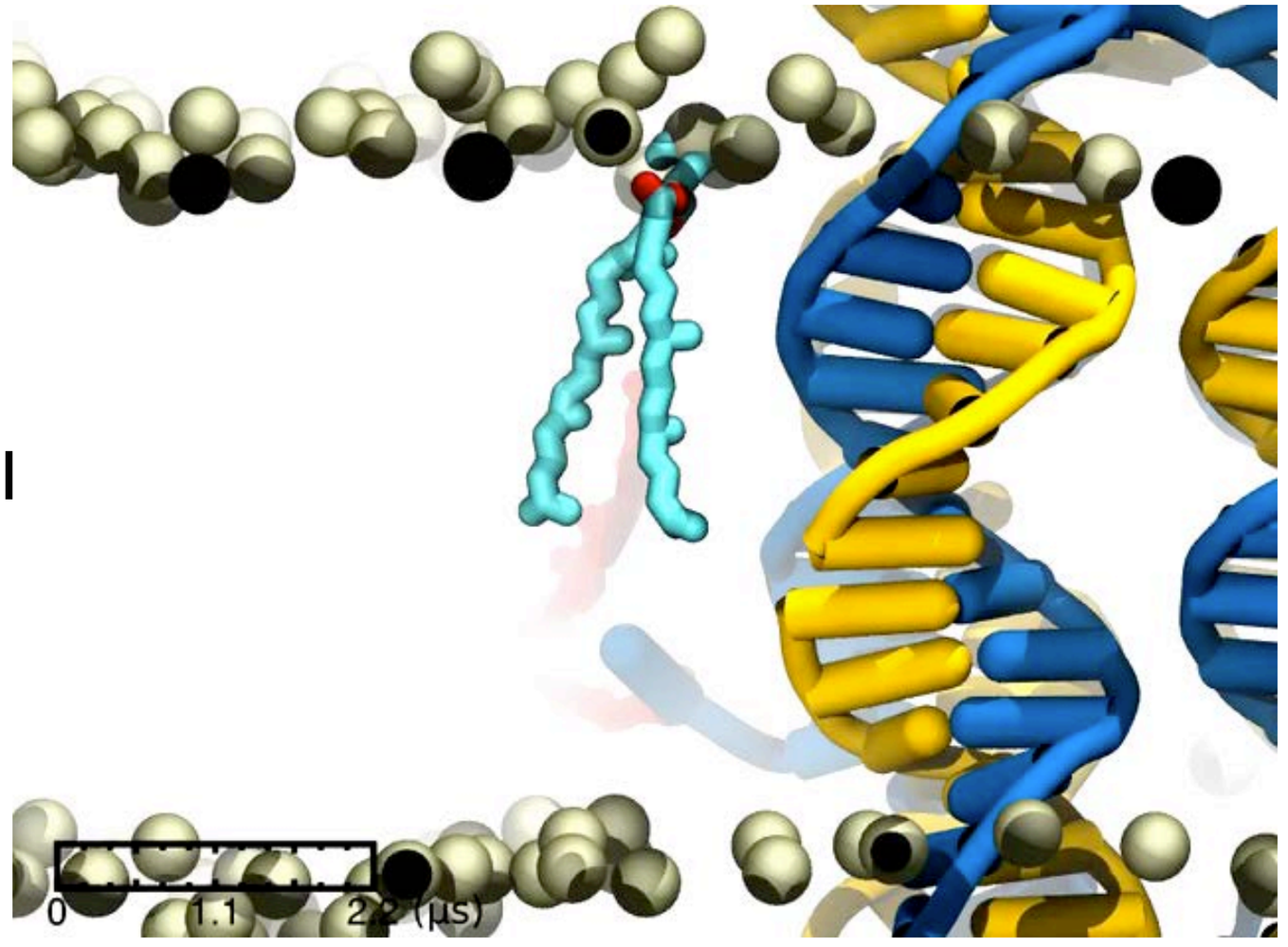
Lipid molecules are asymmetrical distributed in the cell membrane.

apoptosis or thrombin formation:



scramblase

Deficiency in lipid scrambling could result in autoimmune response or Scott syndrome.

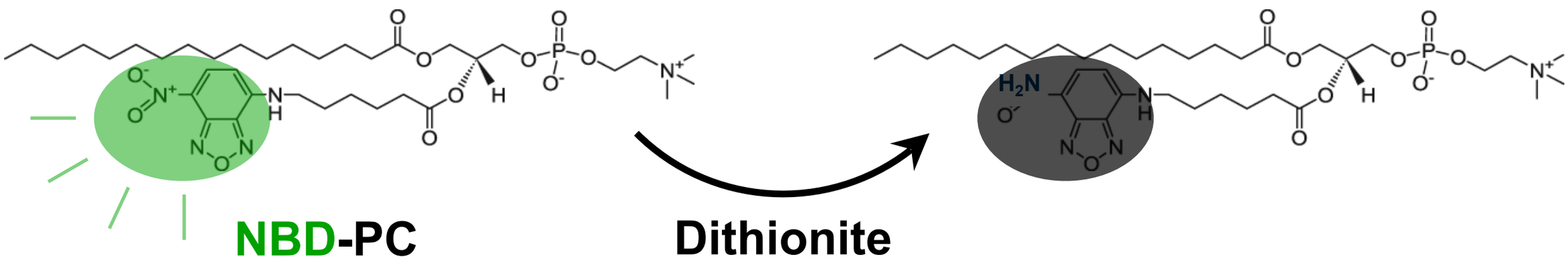


Lipid translocation through toroidal pores is very fast: 10^7 lipids per second, 10,000 times faster then for a protein enzyme

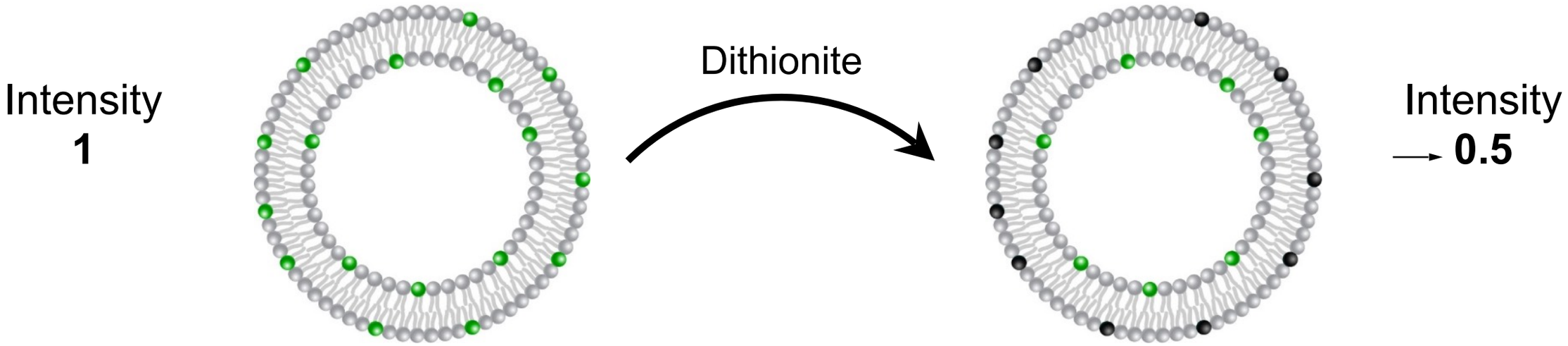
Nature Communications 9:2426 (2018)

Dithionite reduction assay can visualize lipid scrambling

Alex Ohmann



$Na_2S_2O_4 \leftrightarrows 2\ Na^+ + \mathbf{S_2O_4^{2-}}$ ← Membrane impermeable

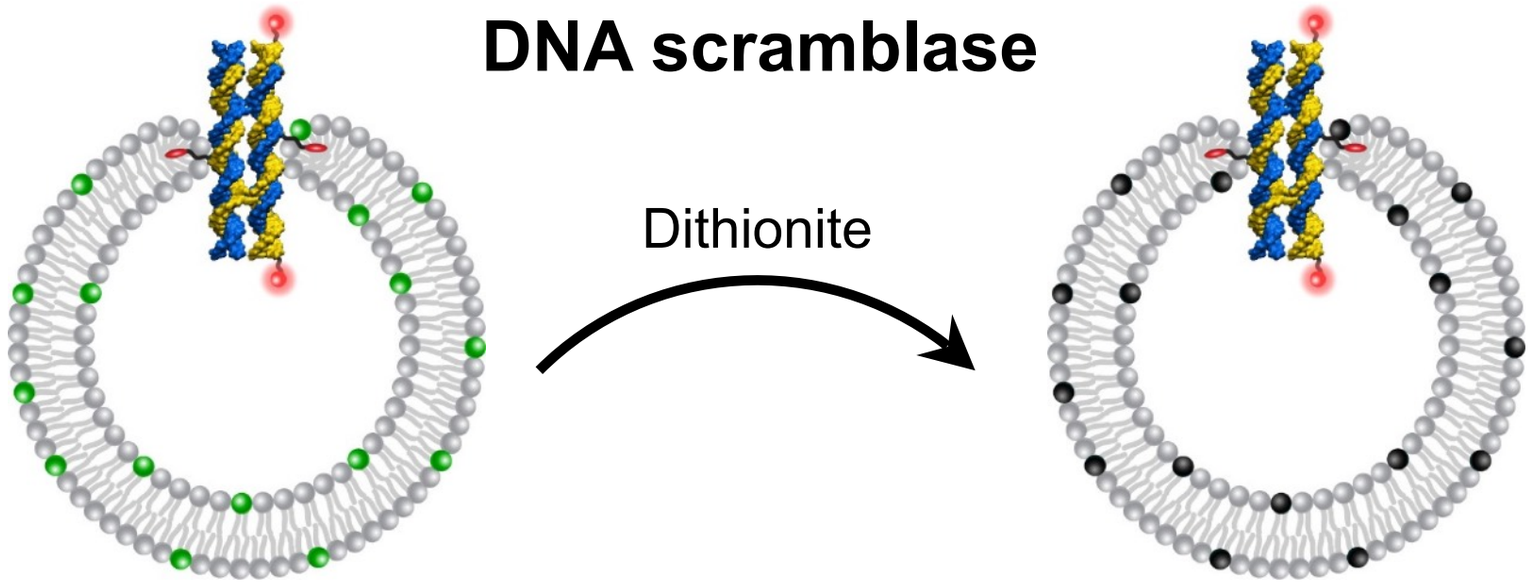


Dithionite reduction assay can visualize lipid scrambling

Alex Ohmann

DNA scramblase

Intensity
1

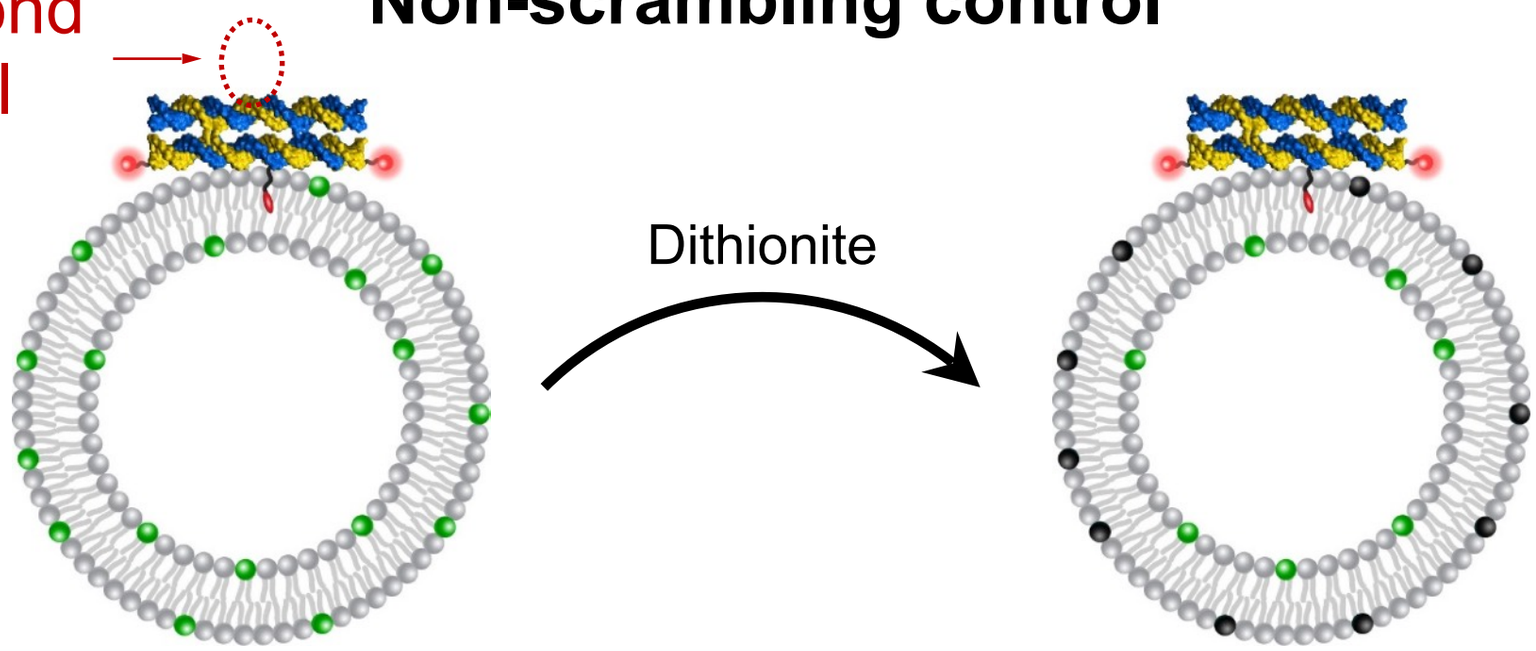


Intensity
→ 0

Non-scrambling control

Missing second cholesterol

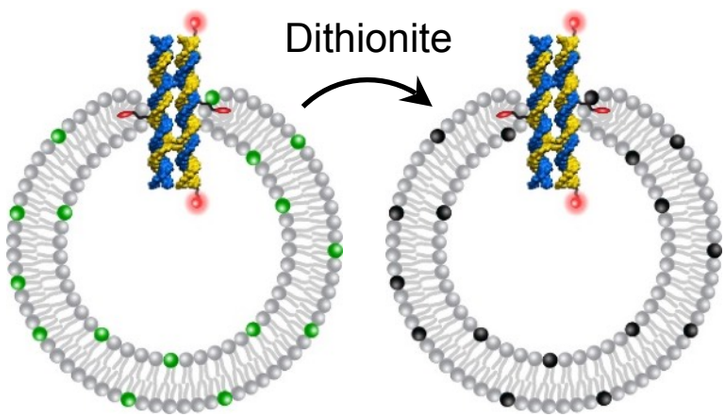
Intensity
1

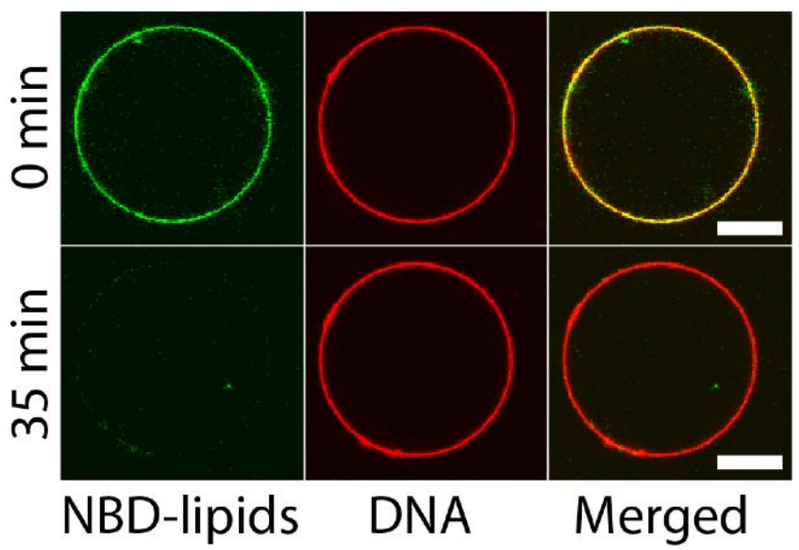


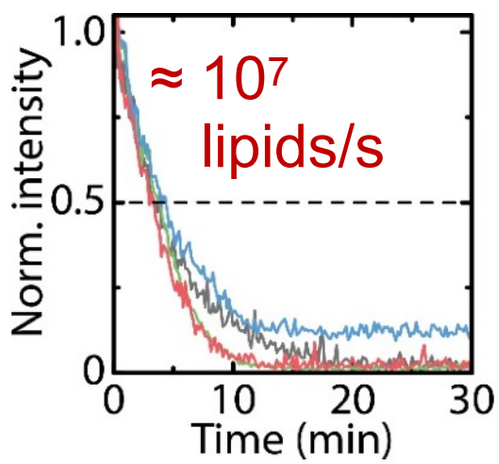
Intensity
→ 0.5

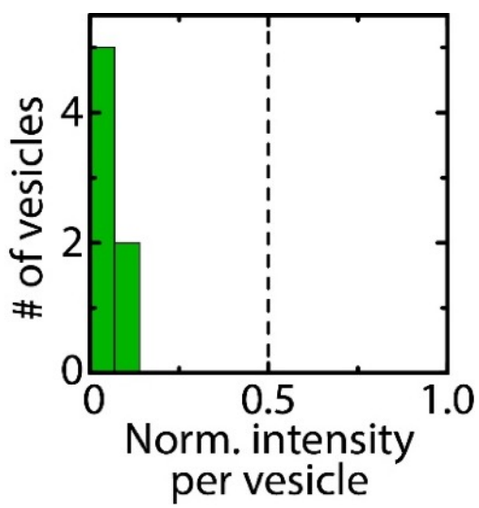
Membrane-inserting DNA structures can scramble but non-inserting structures do not

DNA scramblase

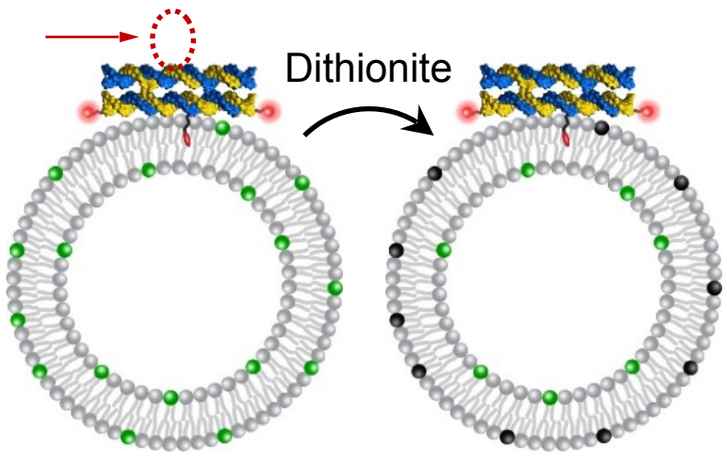


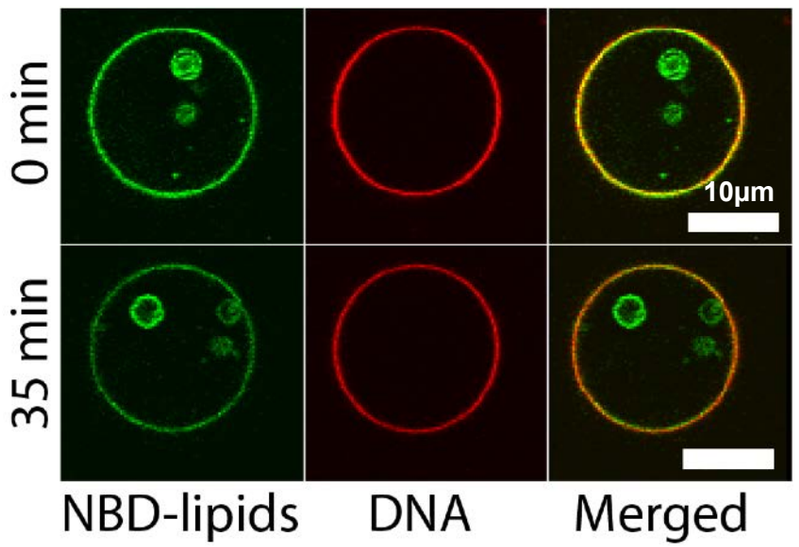


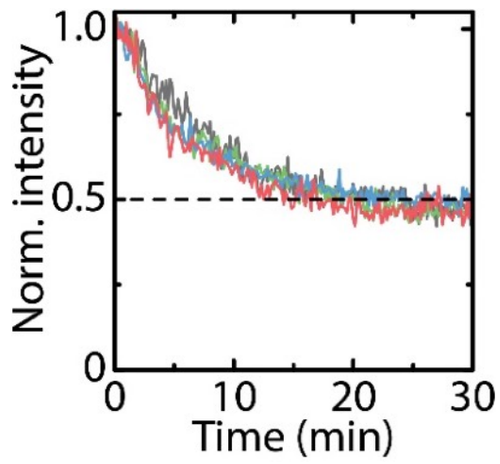


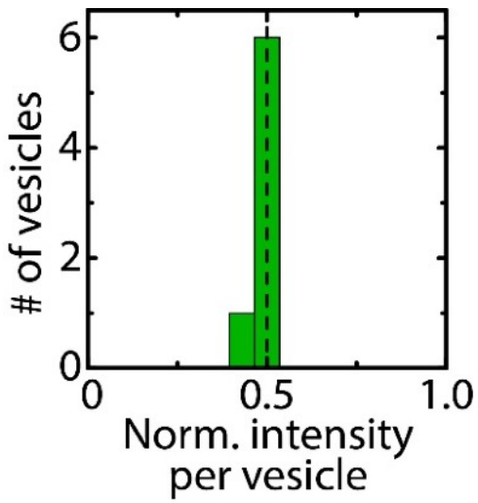


Non-scrambling control







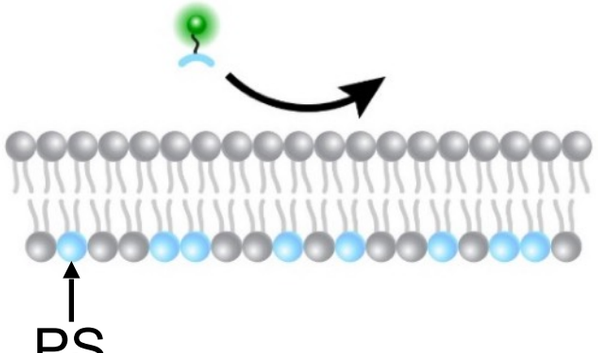


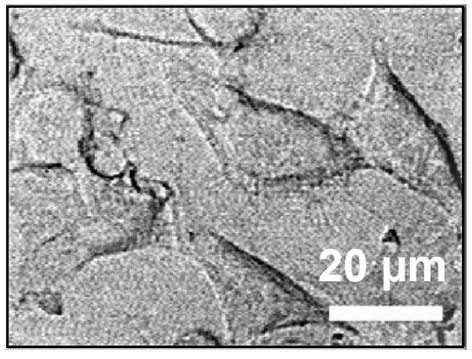
DNA nanostructures induce apoptosis in human cells

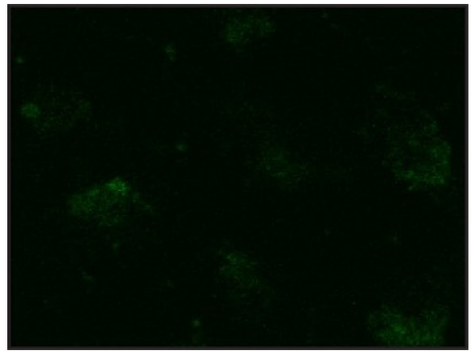
Annexin V

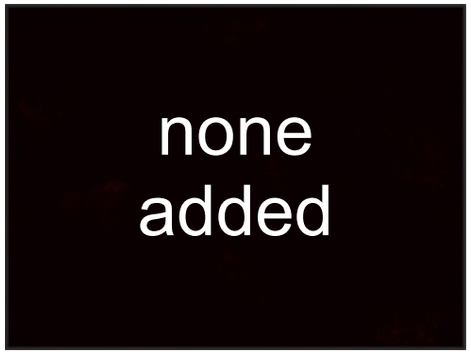
Fixed cells | **Annexin V** | **DNA scramblase**

No apoptosis induced

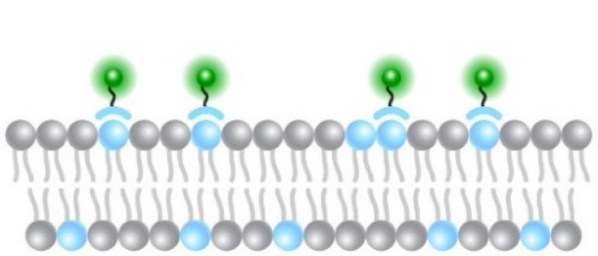


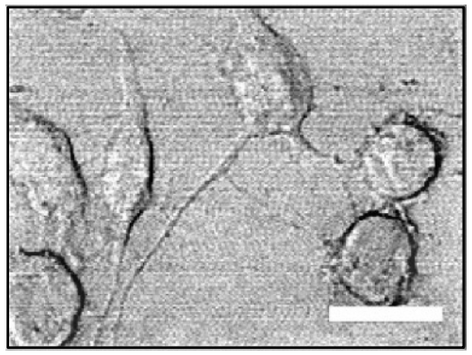


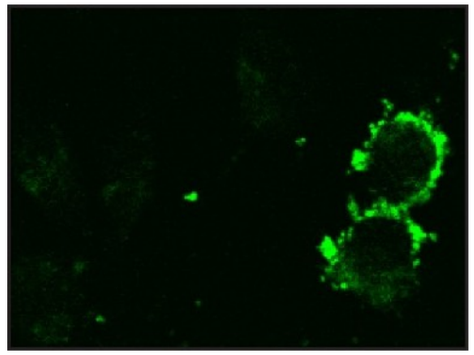




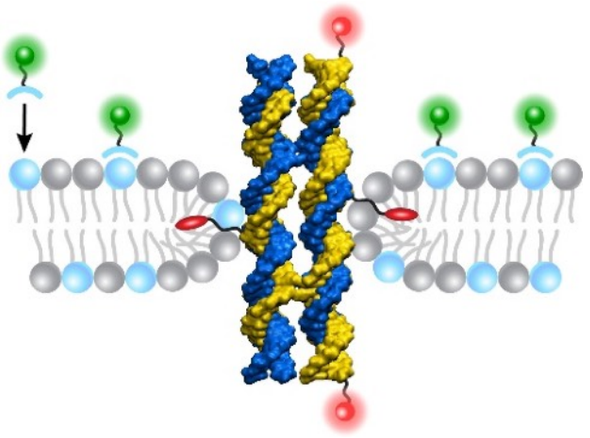
Chemically induced apoptosis

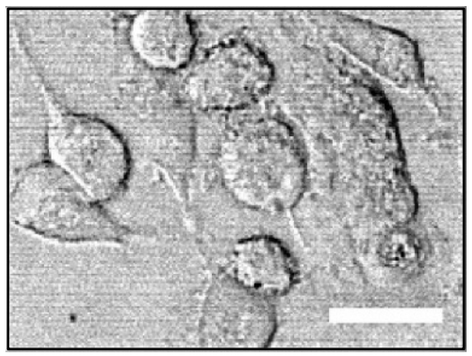


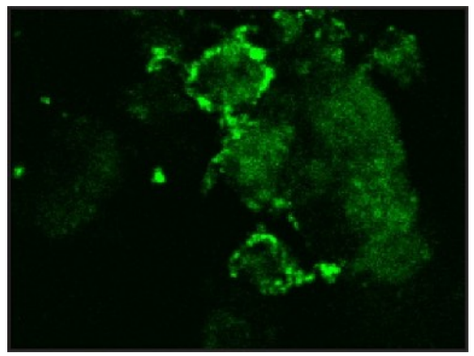


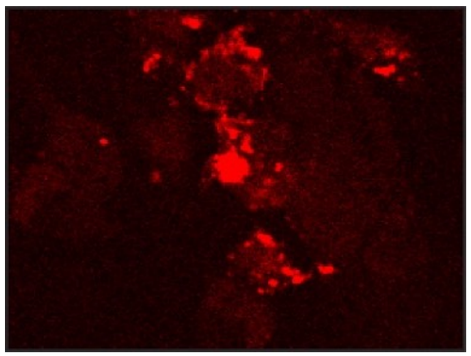


DNA scramblase induced apoptosis



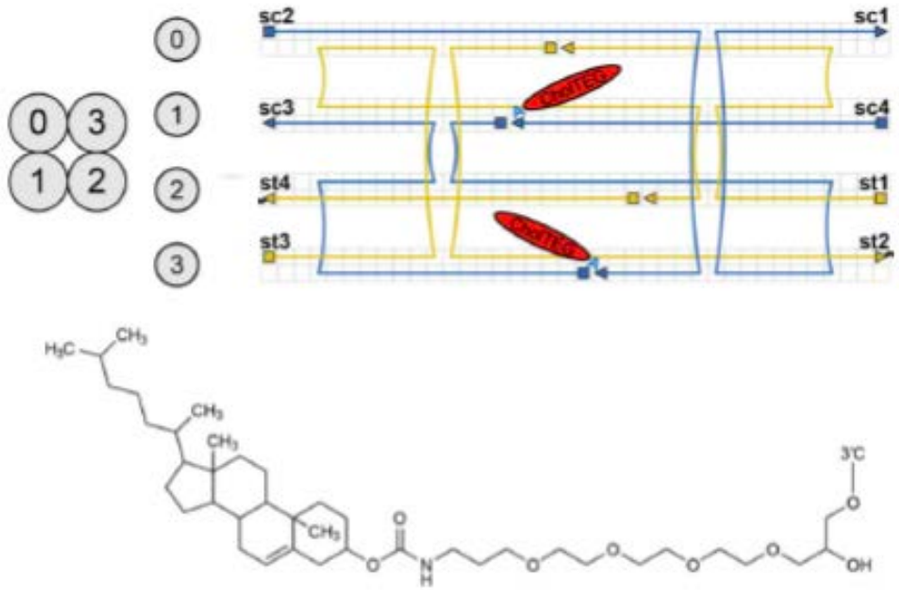


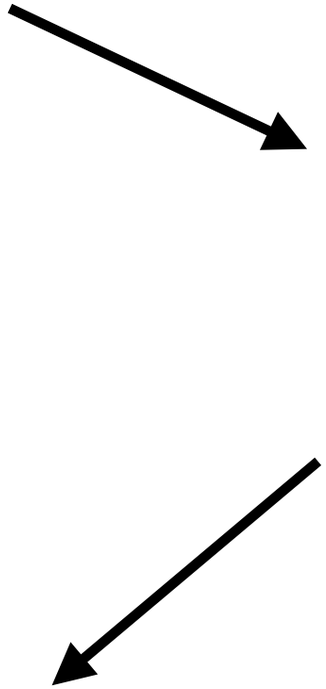


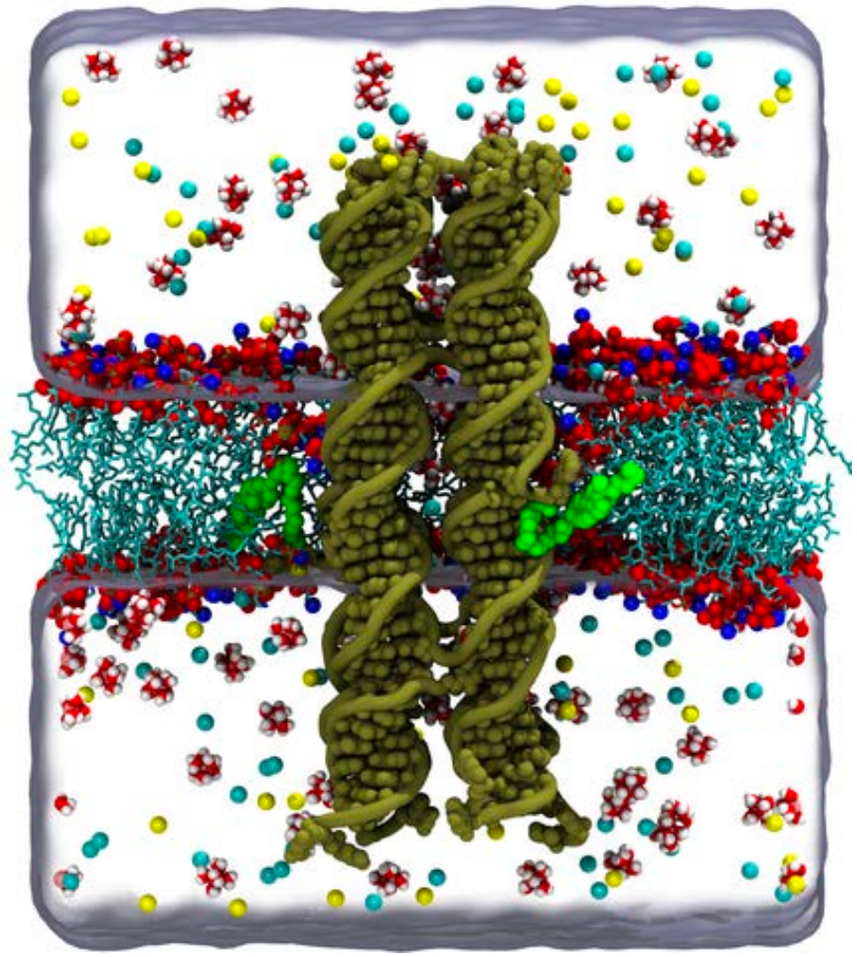


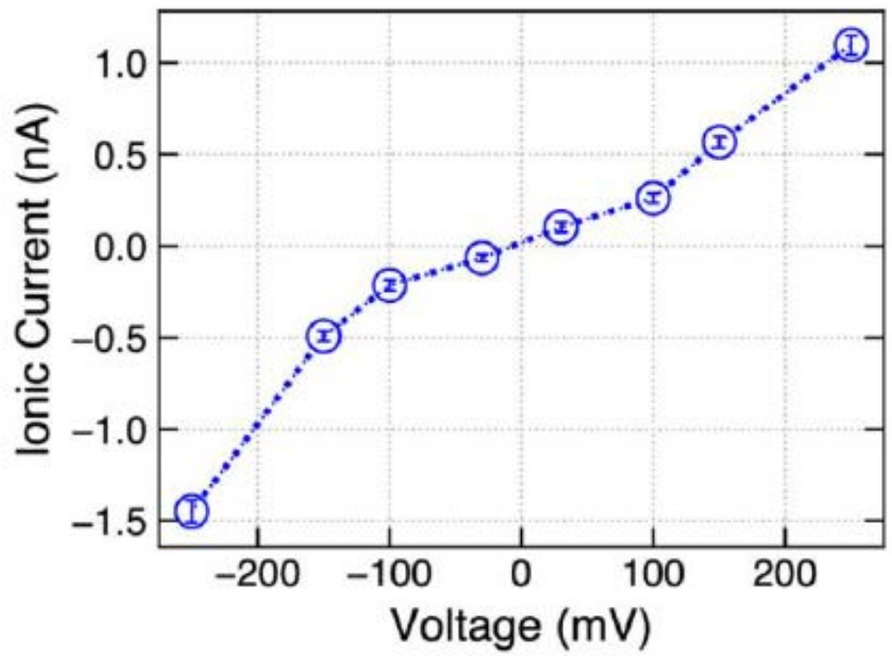
Tutorial: All-atom simulation membrane-spanning DNA nanopores

http://bionano.physics.illinois.edu/tutorials/all-atom-simulation-membrane-spanning-dna-nanopores





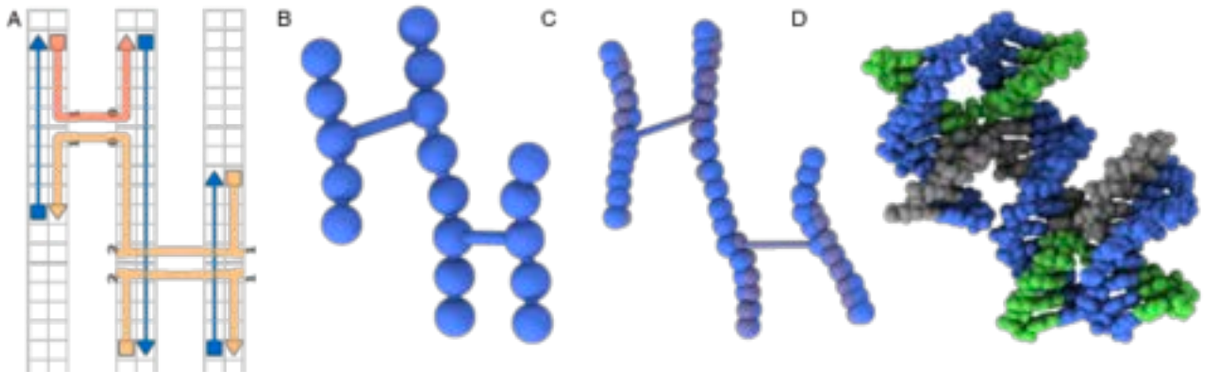




— Walk through the protocol for all-atom simulations of DNA origami nanopores

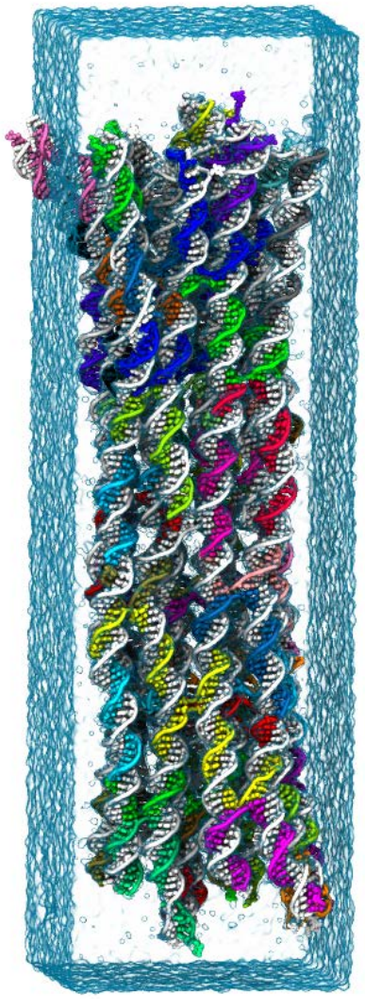
Tutorials overview

Multi-resolution simulations of self-assembled DNA nanostructures

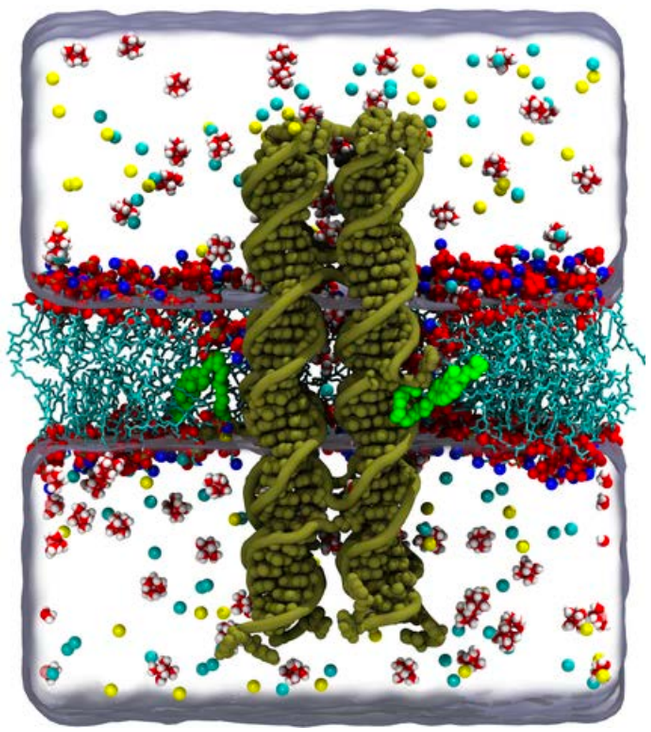


Chris Maffeo

A Practical Guide to DNA Origami Simulations Using NAMD



All-atom simulation membrane-spanning DNA nanopores



Himanshu Joshi

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