

# **iBiology.org Teaching Tools**

## **William Shih's Lecture Part 3:**

### **DNA-nanostructure tools for molecular biophysics and therapeutics**

Teaching Tools were prepared by Karen Cheng and William Shih.

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#### **1. Keywords and Terms**

molecular trees (nanotubes), nanobot

#### **2. Lecture Notes**

## NMR Structure Determination of a Membrane Protein (UCP2)

Berardi M, Shih WM, Harrison SC, Chou JJ.  
Mitochondrial uncoupling protein 2 structure determined by NMR molecular fragment searching,  
**Nature** 476, 109–113, 2011.



**00:59 min**

NMR can be used as a tool  
for structure determination by  
introducing a bias in the  
alignment of the membrane  
protein

## Host-guest crystallization

“Holy grail” benchmark for spatial control

could enable atomic-resolution  
diffraction-based structure determination

## Weak ordering

Modest demonstration of spatial control

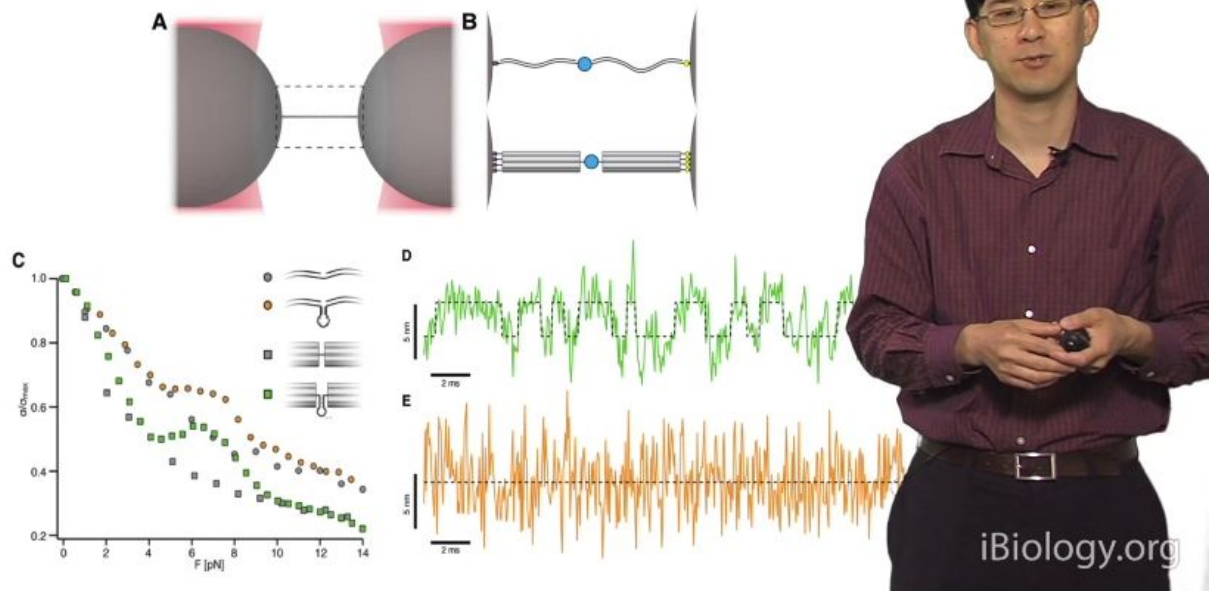
could enable atomic-resolution  
NMR-based structure determination



**2:24 min**

Weak alignment → residual  
dipolar couplings → global  
angular restraints → atomic  
resolution structure (of at  
least the backbone chain)

Pfützner E, Wachauf C, Kichherr F, Pelz B, Shih WM, Rief M, Dietz H.  
Rigid DNA beams for high resolution single molecule mechanics.  
*Ange. Chem. Int. Ed.* 52, 1–7, 2013.

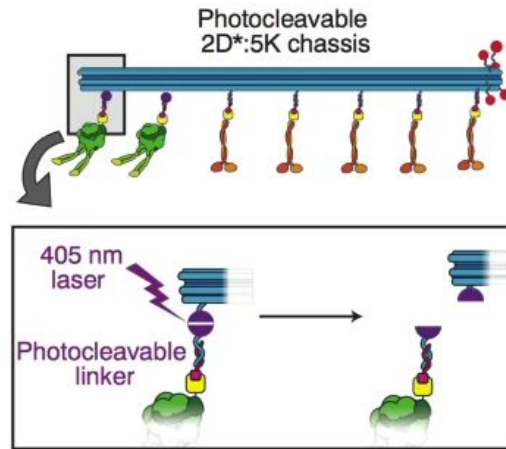


**12:24 min**

DNA nanotechnology as a  
tool for single molecule  
biophysics  
Optical tweezers to look at  
conformational changes in  
molecule of interest

## Tug-of-War in Motor Protein Ensembles Revealed with a Programmable DNA Origami Scaffold

N. D. Derr,<sup>1,2,3\*</sup> B. S. Goodman,<sup>1\*</sup> R. Jungmann,<sup>4,5</sup> A. E. Leschziner,<sup>6</sup>  
W. M. Shih,<sup>2,3,5</sup> S. L. Reck-Peterson<sup>1†</sup>

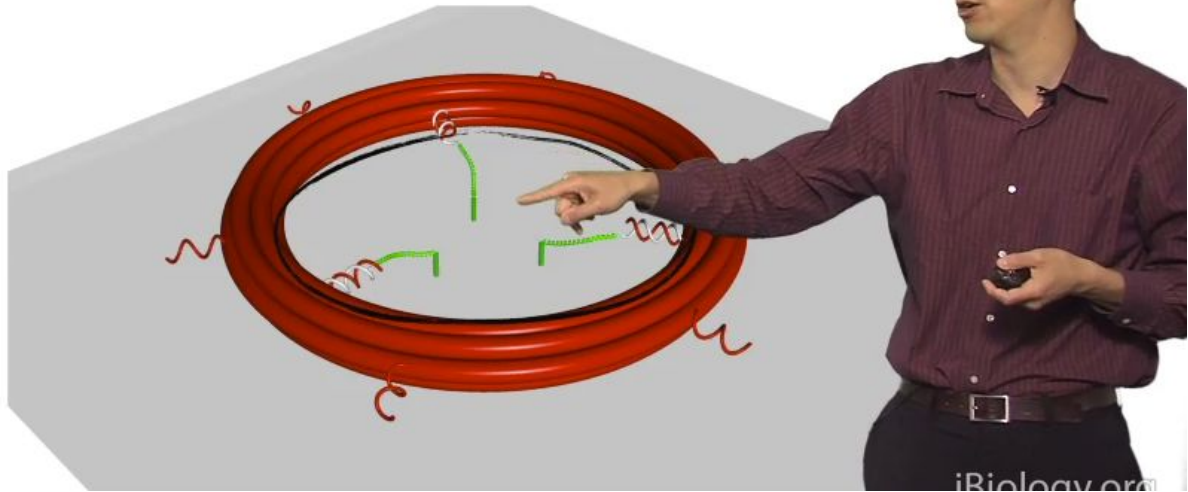


**16:58 min**

Reconstitution of motor protein ensembles using DNA origami scaffold shows that a stalled load can be resolved by removing either the dynein or kinesin motor proteins from the chassis

## DNA-origami-scaffolded membrane fusion

Chenxiang Lin  
Weiming Xu  
Fred Pincet  
Jim Rothman



**22:01 min**

In order to address the question, how many SNARE proteins are required for membrane fusion, DNA origami tubes were used to corral different numbers of SNARE proteins.

## Exploring shape dependence of DNA-particle cellular uptake



*Franziska Graf  
Chenxiang Lin*

*Don Ingber*

*Shawn Douglas  
Katrina Galkina*



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**27:36 min**

How does the shape of DNA  
nanostructures affect their  
uptake into cells?

What physical descriptors might be relevant  
for nanoparticle uptake efficiency?

aspect ratio  
mass  
maximum dimension  
radius of gyration  
enclosed volume  
persistence length  
length for comparing similar 1D nanostructures  
diameter for comparing similar 2D nanostructures  
etc.

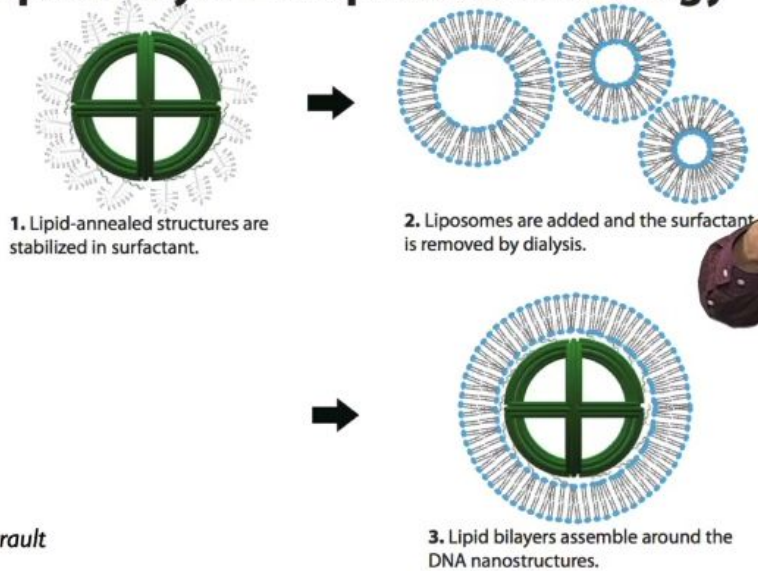


**28:01 min**

Parameters that might affect  
nanoparticle uptake into a cell



## Lipid Bilayer Encapsulation Strategy

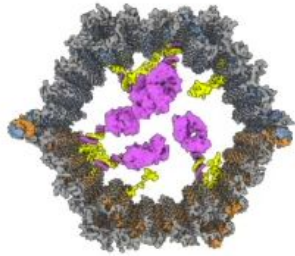


Steve Perrault



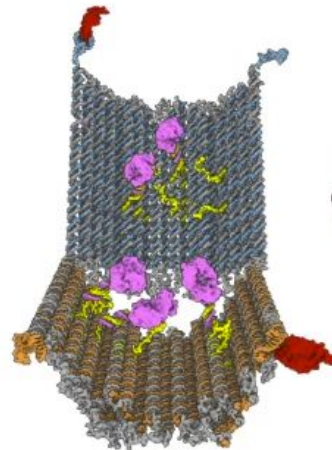
**33:18 min**

Lipid bilayer structures have been constructed to encapsulate the DNA nanostructures to make something that is structurally analogous to enveloped viruses.



DNA origami nanorobot  
for cell-specific targeting  
and therapeutic payload delivery

Douglas SM, Bachelet I, Church GM.  
*Science* 335, 831–834, 2012



**36:24 min**

A DNA origami nanorobot was programmed to open up when it encountered a cancerous cell and selectively induce apoptosis while leaving the healthy cells alone.

### 3. Review Questions

1. True/False: Replacing linkers in the optical tweezer experiments with DNA bundle structures increase the rigidity between the microsphere and molecule of interest and increases experimental noise.
2. Fill in the blank:
  - a. More compact structures are easy/hard for a cell to uptake.
  - b. Long, thin structures are easy/hard for a cell to uptake.
3. Explain how the DNA origami nanorobot uses DNA nanostructures to selectively target cancer cells for apoptosis while leaving healthy cells alone?

#### **4. Answers to Review Questions**

1. False, DNA bundles increase rigidity and decrease experimental noise
2.
  - a. Easy
  - b. Hard
3. Antibodies that bind to receptors that cause receptor clustering that then leads to apoptosis are sequestered inside the DNA barrel and not accessible to cells. The nanostructure opens up when recognizes cancer cell but not healthy cell. It looks for sequences that are enriched in cancer cell lines but not in healthy cells

#### **5. Discussion Questions**

1. How are “DNA bundles” used in optical tweezer experiments to look at changes in conformation of a molecule? Why does this reduce experimental noise?
2. How could the observation that long, thin structures resist uptake into the cell be useful for therapeutic considerations?

#### **6. Answers to Discussion Questions**

1. The microspheres used in optical tweezer experiments undergo Brownian motion and the challenge is to differentiate between what is noise coming from random motion and what are actually meaningful changes in conformation, especially at low force. The DNA bundles that replace the linkers help suppress the Brownian motion and the noise should be suppressed.
2. Designing DNA structures that resist uptake into the cell and stay on the outside of the membrane could act as a signal to recruit other particles or contents to the cell.

#### **7. Explain or Teach These Concepts to a Friend**

Is there something unique about DNA that makes it an attractive molecule to use in the rational design of nanostructures? Could you imagine another class of molecules that could recapitulate the same functions and goals?

## 8. Paper for Journal Club

Derr et al. Tug-of-War in Motor Protein Ensembles Revealed with a Programmable DNA Origami Scaffold (2012) *Science*, 338: 662-665.

Key words: dynein, kinesin, processivity, DNA origami, chassis, synthetic cargo

1. Describe how Derr et al. used DNA origami to design a synthetic cargo that could be attached selectively to kinesin or dynein motor proteins.
2. How do the motile properties of dynein and kinesin cargo transport differ according to the single molecule chassis-motor assays (see Figure 2)?
3. How does the mixed-motor photocleavable chassis experiments support a tug-of-war model for opposite-polarity motor proteins (see Figure 4)?
4. Are you convinced that this engineered chassis-motor protein complex accurately recapitulates motor-protein directed microtubule transport?