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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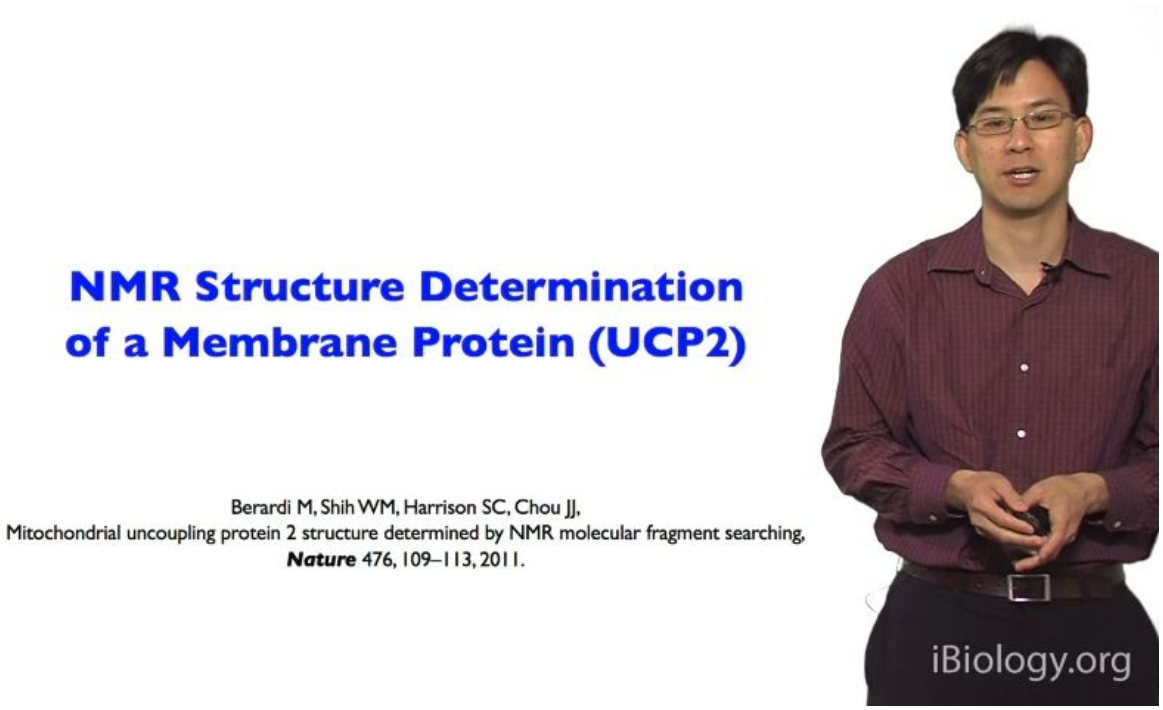
1. Keywords and Terms

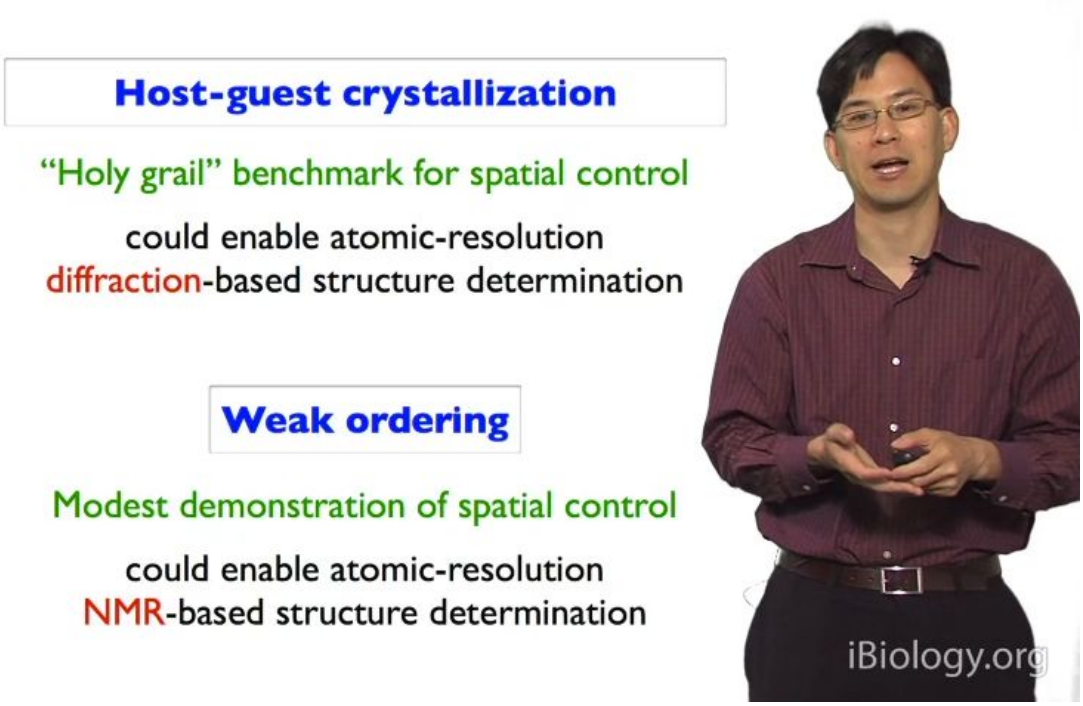
molecular trees (nanotubes), nanobot

2. Lecture Notes



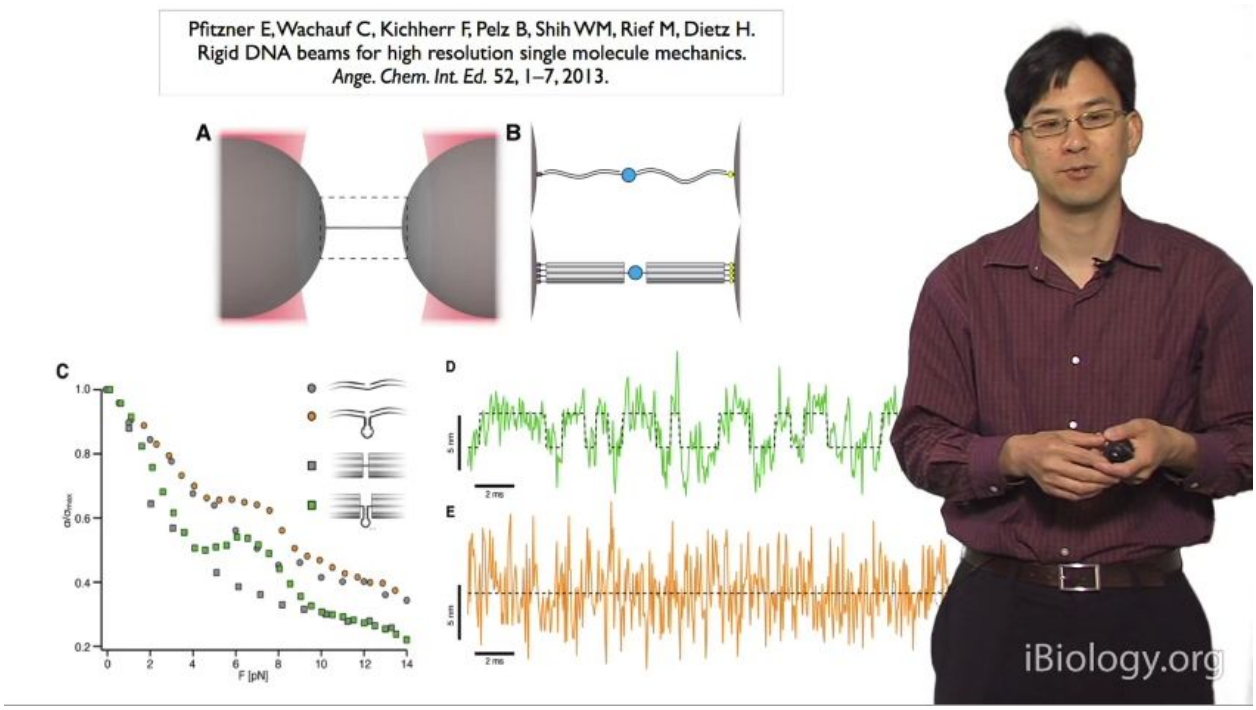
00:59 min

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



2:24 min

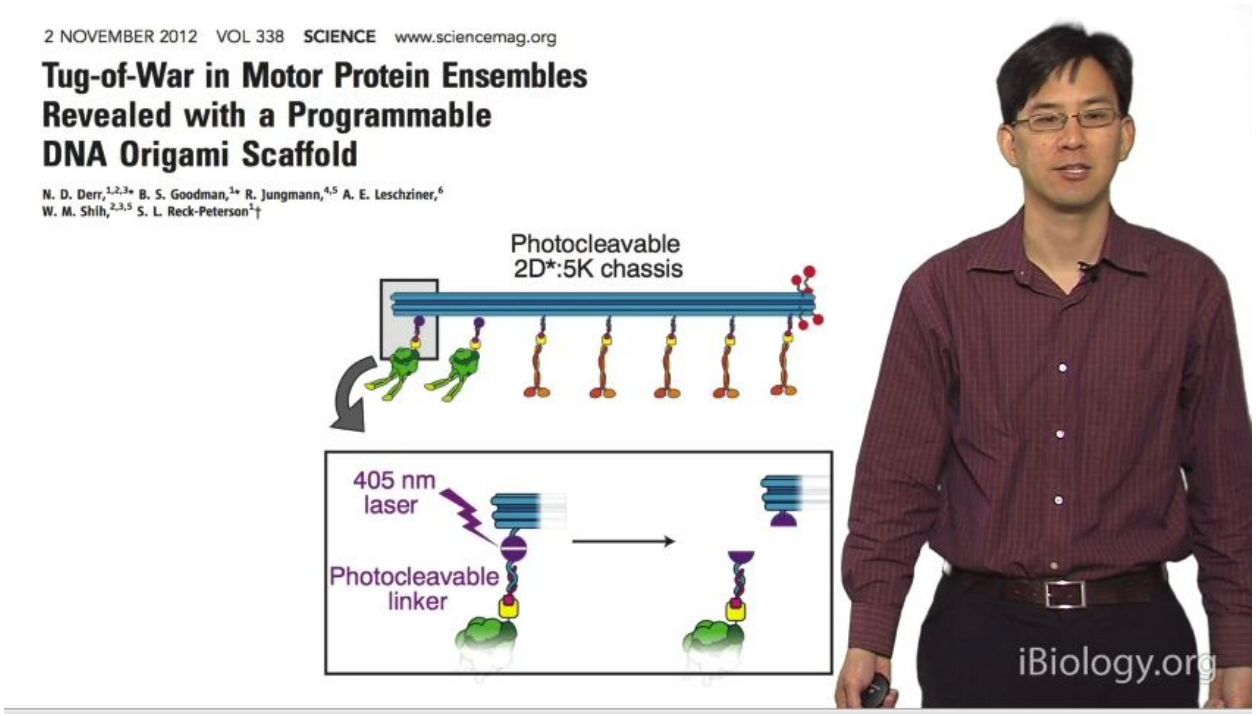
Weak alignment à residual dipolar couplings à global angular restraints à atomic resolution structure (of at least the backbone chain)



12:24 min

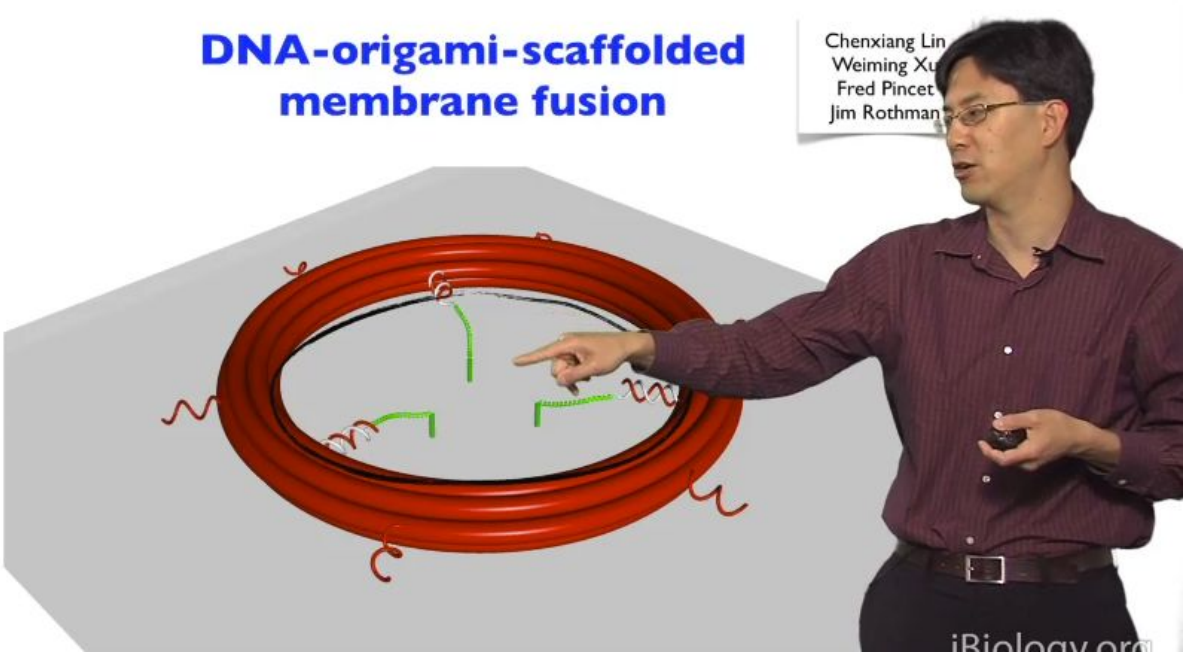
DNA nanotechnology as a tool for single molecule biophysics

Optical tweezers to look at conformational changes in molecule of interest



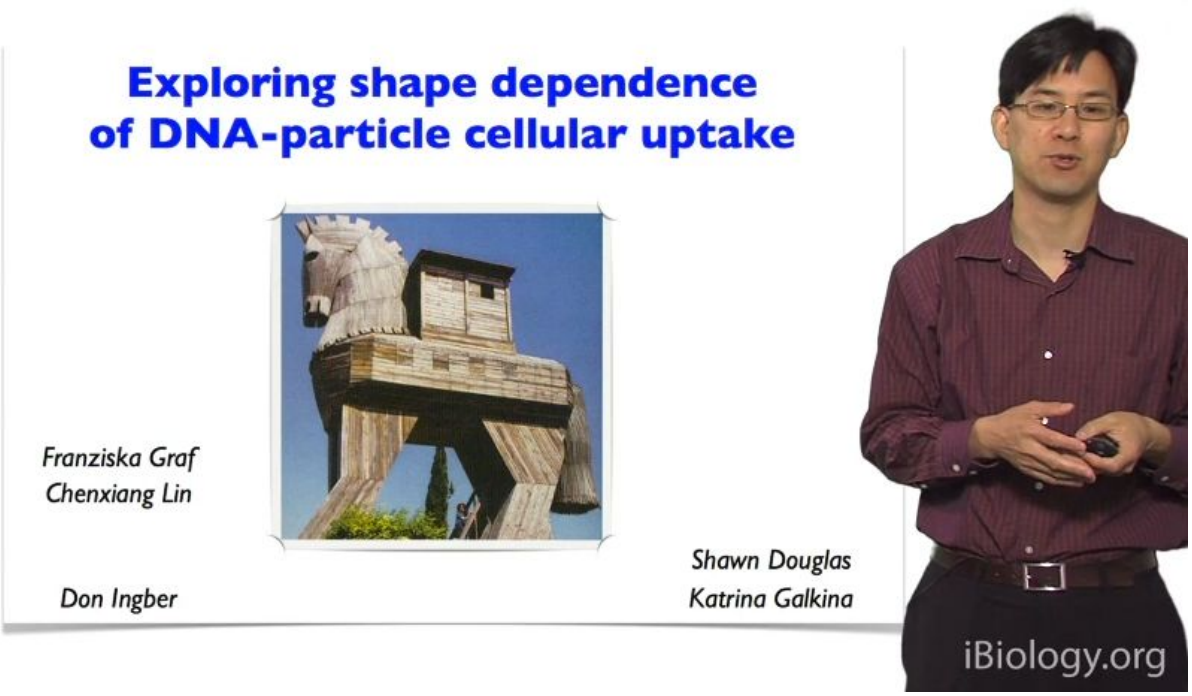
16:58 min

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



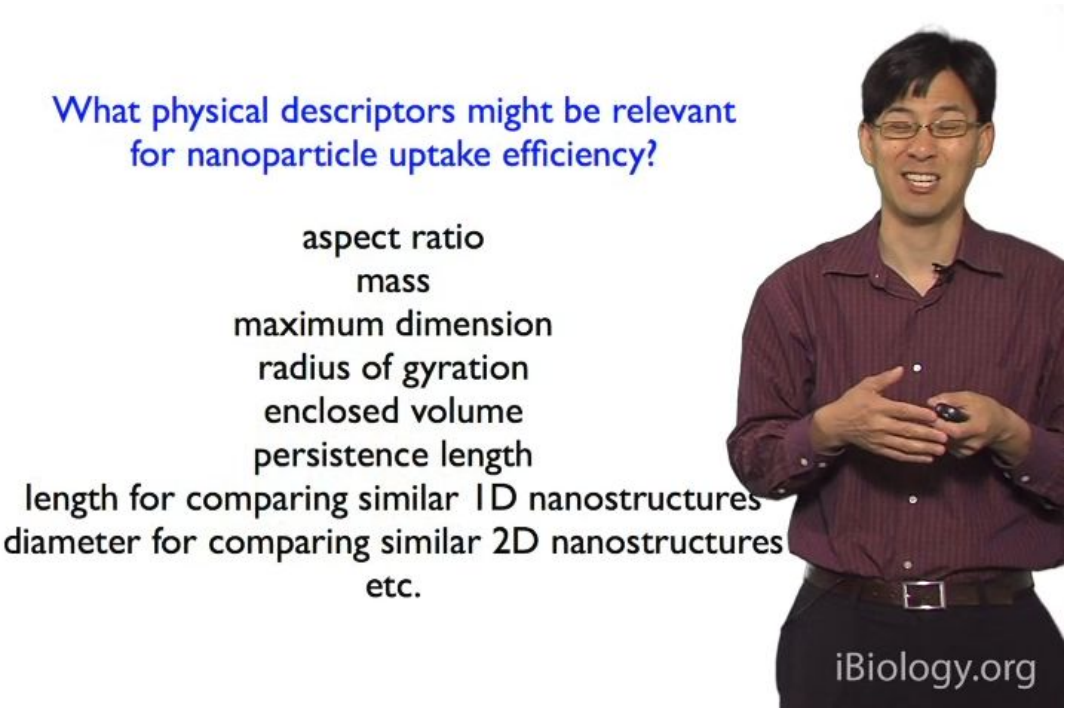
22:01 min

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.

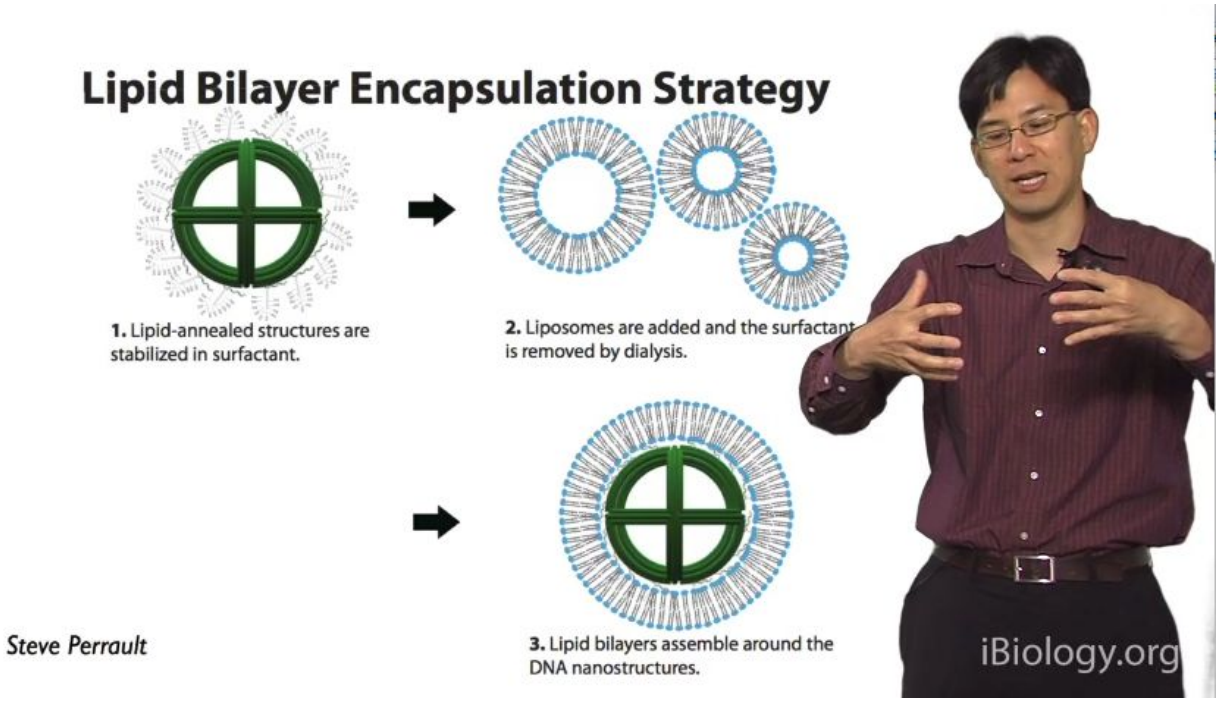


27:36 min

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

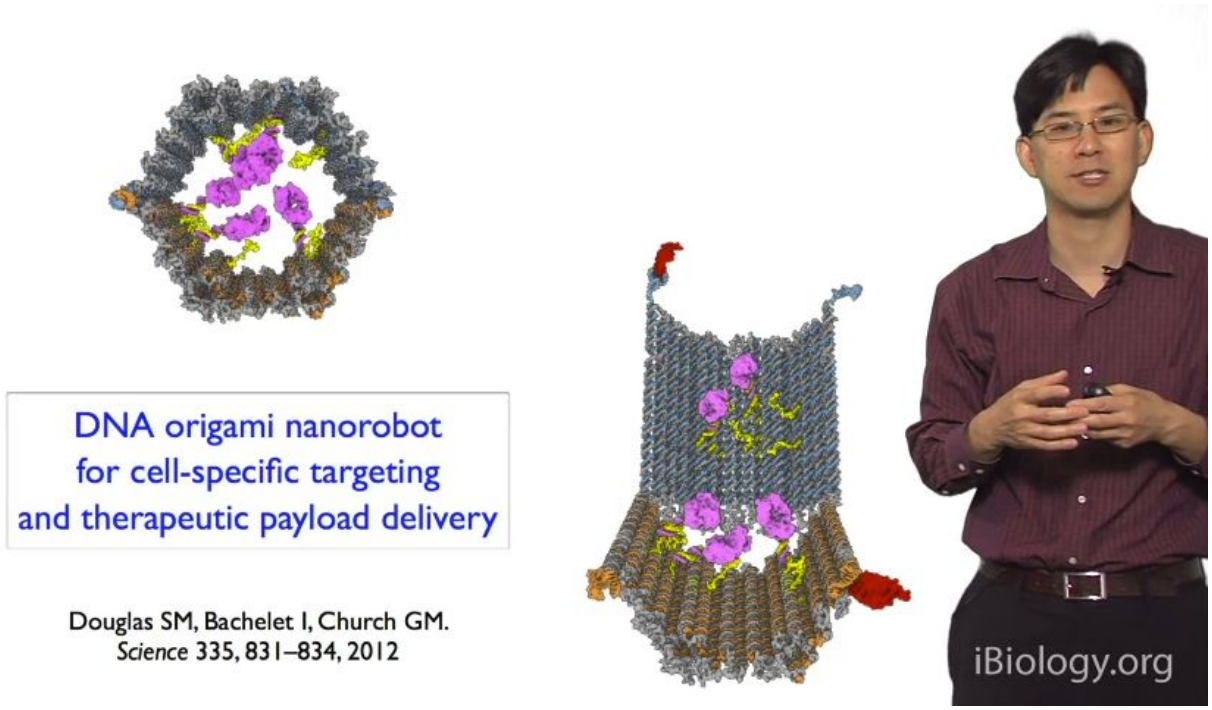


28:01 min
Parameters that might affect
nanoparticle uptake into a cell



33:18 min

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



36:24 min
A DNA origami nanobot 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 nanobot 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?