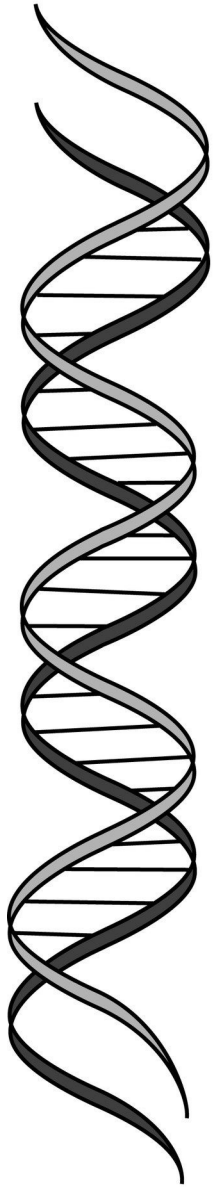
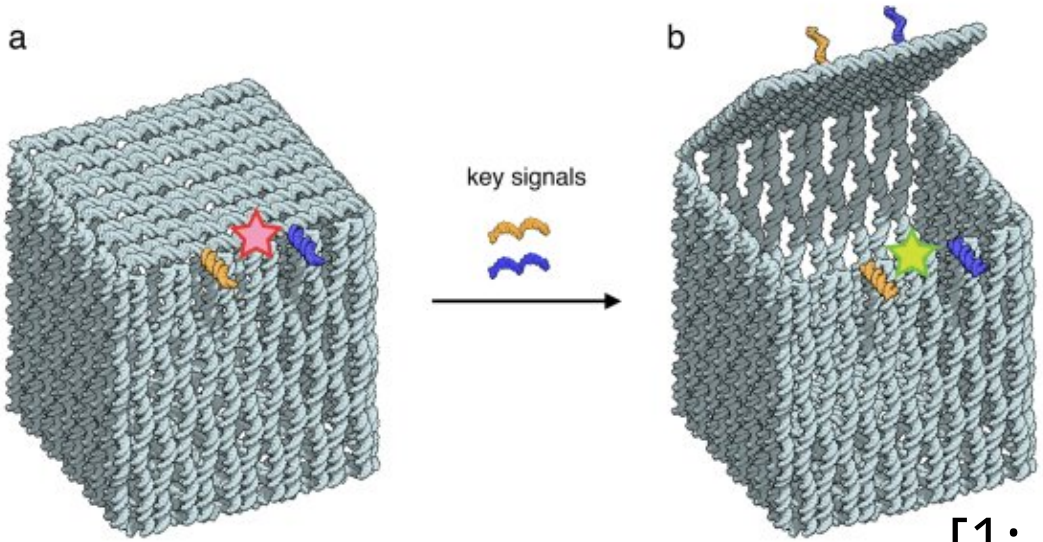
DNA Origami

Philipp R. Steen
Jonas C. Fischer



DNA Origami: Introduction

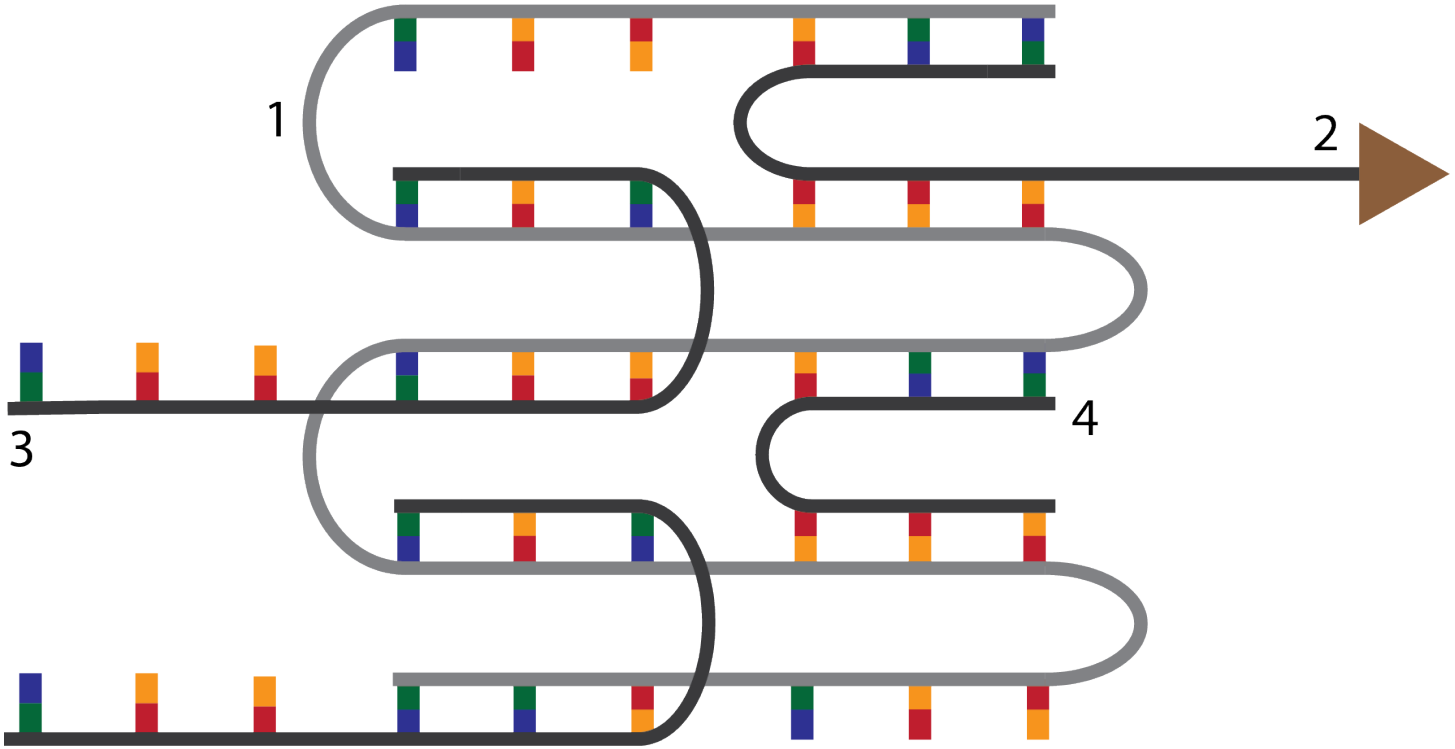
- Build well-defined nanoscale structures
- Complementary nature of DNA bases
- Applications in medicine, synthetic biology and nanoscience research

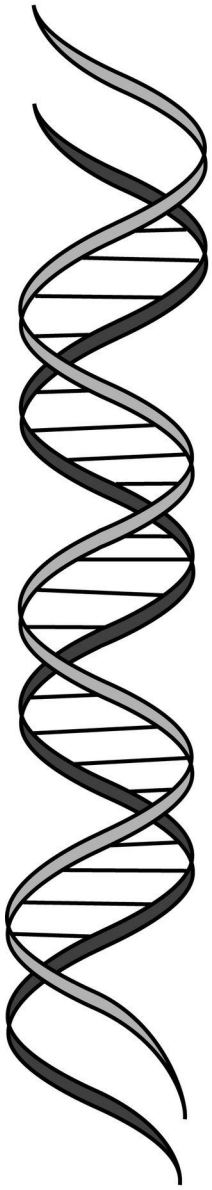


[1: Andersen 2009]

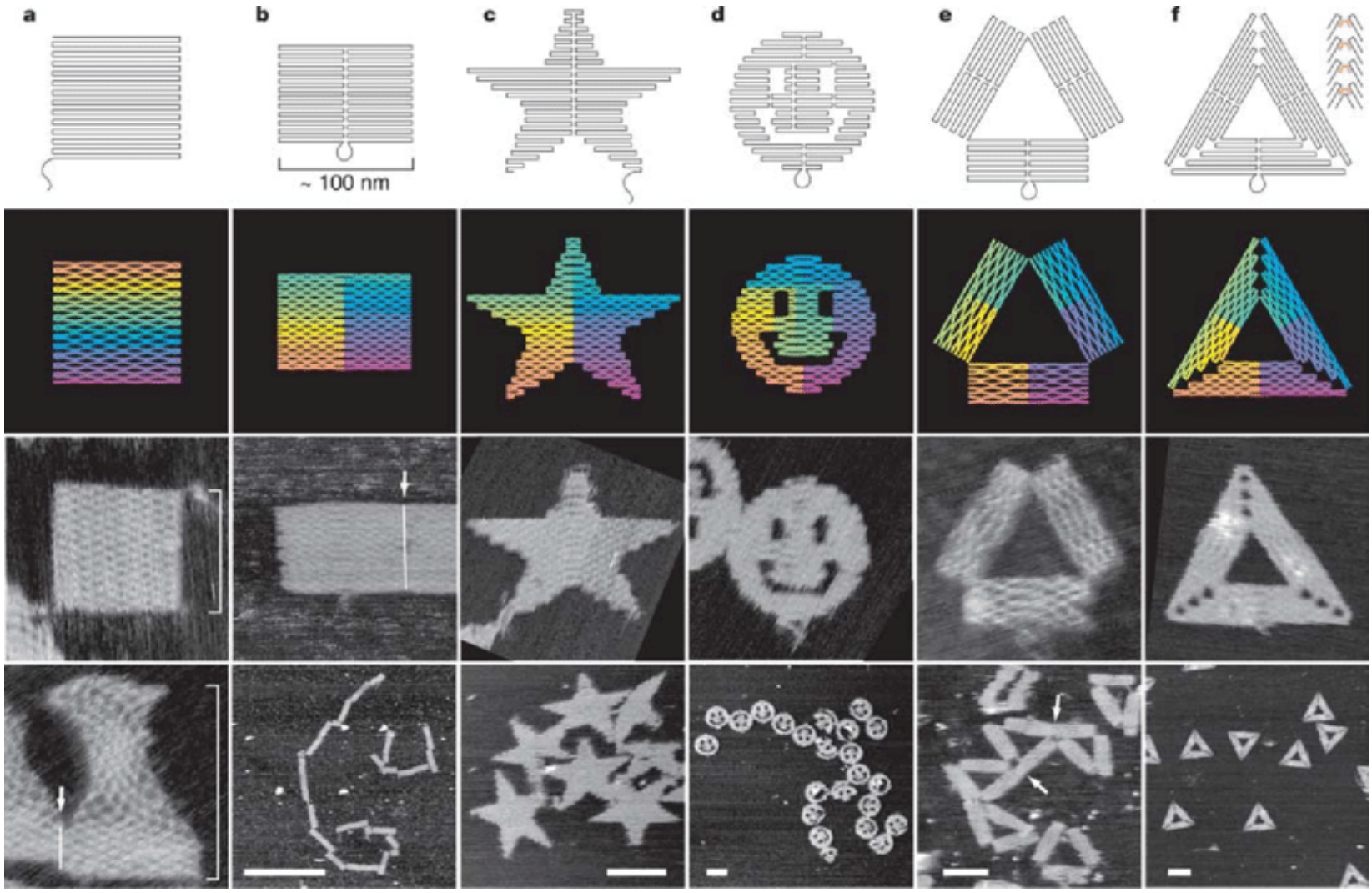
DNA Origami: Theory

- Rothemund [2] principle: Scaffold + Staples





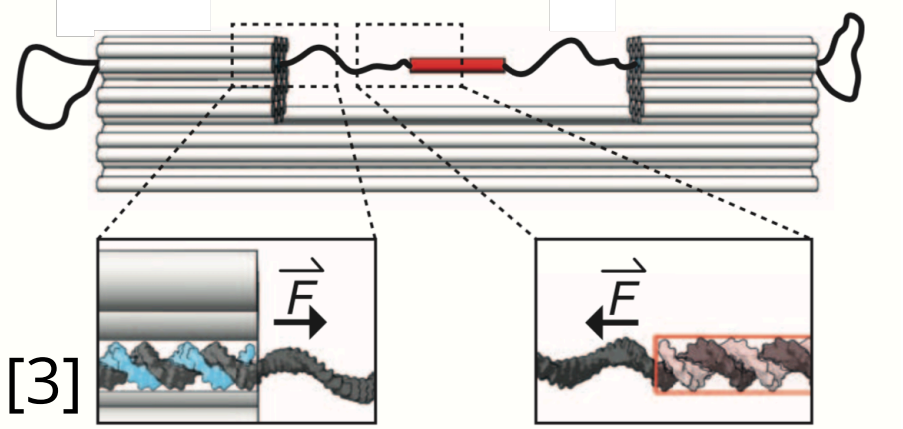
DNA Origami: Practice



[2: Rothemund 2006]

DNA Origami at the LMU

- Membrane-assisted growth of nanostructures (Prof. Liedl, in this talk)
- Nanoscale force clamps (Prof. Liedl)



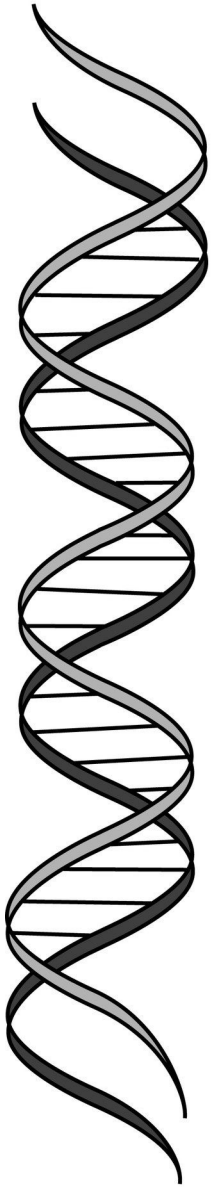
[3]

- Nanocubes (Prof. Lipfert)
- And many more!

Membrane-Assisted Growth of DNA Origami Nanostructure Arrays

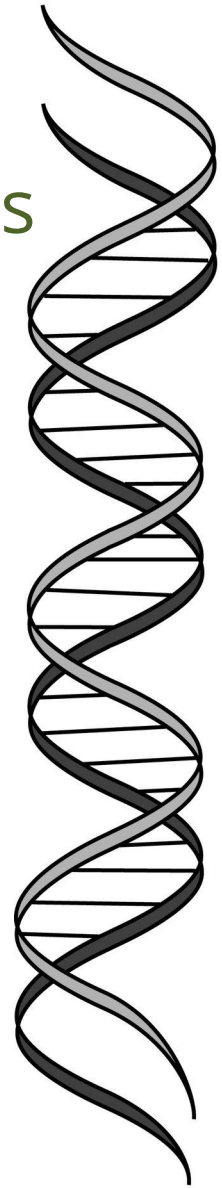
Samet Kocabey, Susanne Kempter, Jonathan List,
Yongzheng Xing, Wooli Bae, Daniel Schiffels, William M. Shih,
Friedrich C. Simmel & Tim Liedl

ACS Nano, 2015



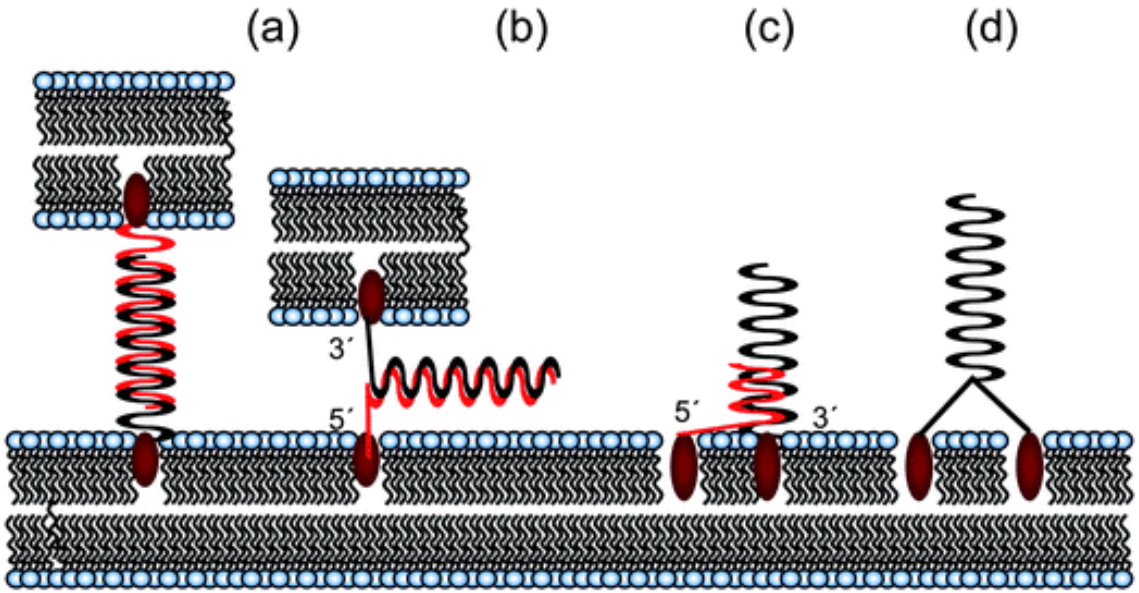
DNA-Superstructures: Intro

- Objective: Control over membrane functions
- Membrane functions:
  - Trafficking of nutrients
  - Compartmentalization
  - Metabolic pathways
  - Cell adhesion
  - Immune response



Adhering Origami to Membranes

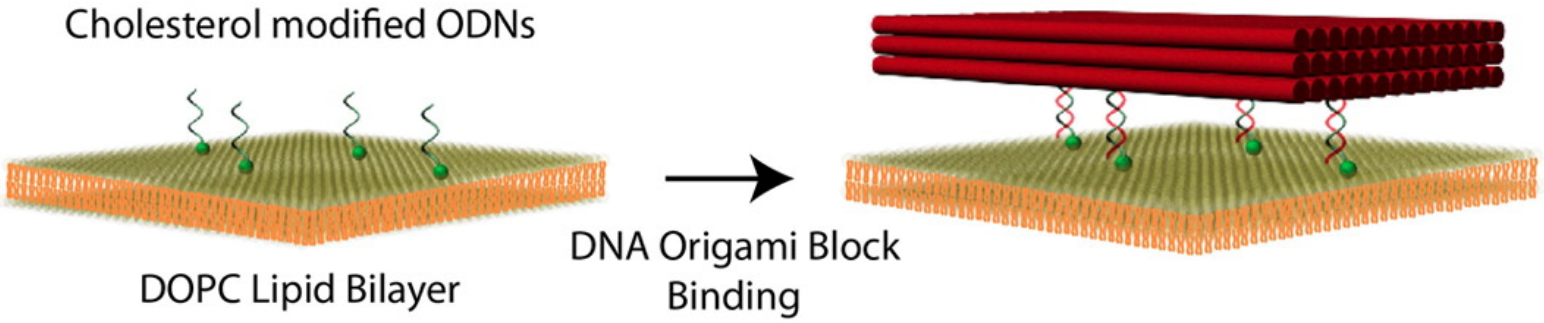
- Anchoring through cholesterol + ssDNA in membrane
- Automatic incorporation into membranes



[1: Achalkumar 2010]

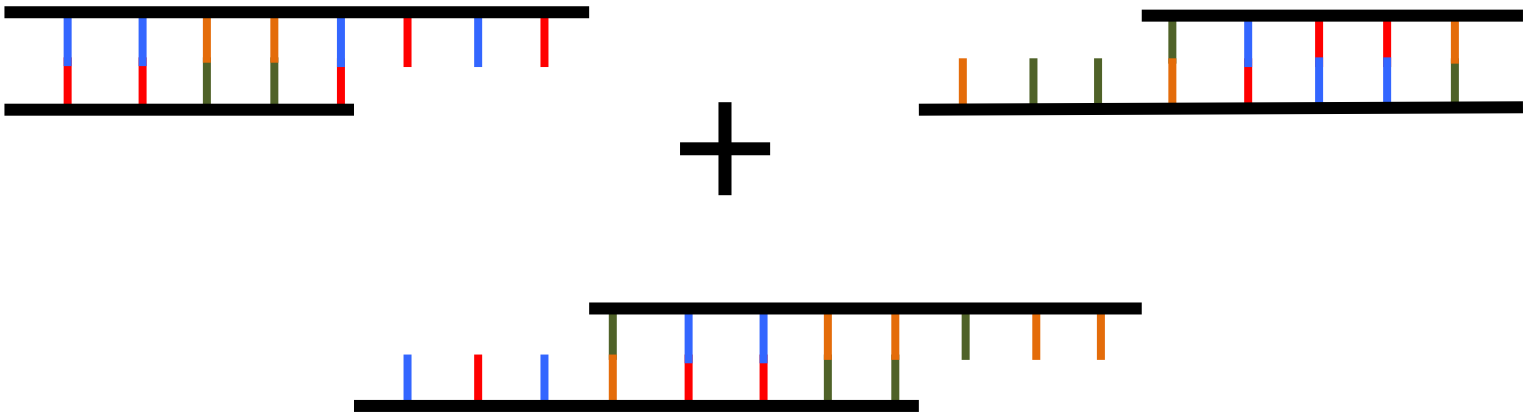
Adhering Origami to Membranes

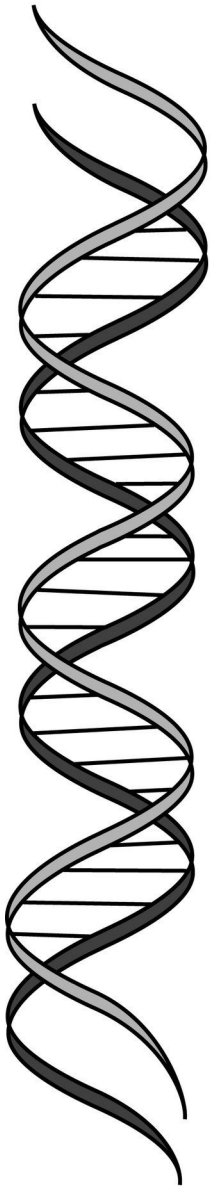
- Anchoring through cholesterol + ssDNA in membrane
- Or directly incorporating into membrane
- Dimensions: 60nm x 35nm x 8nm
- Diffusion coefficient: 0.4 ± 0.1 μm^2 / s



Multimerization of Origami

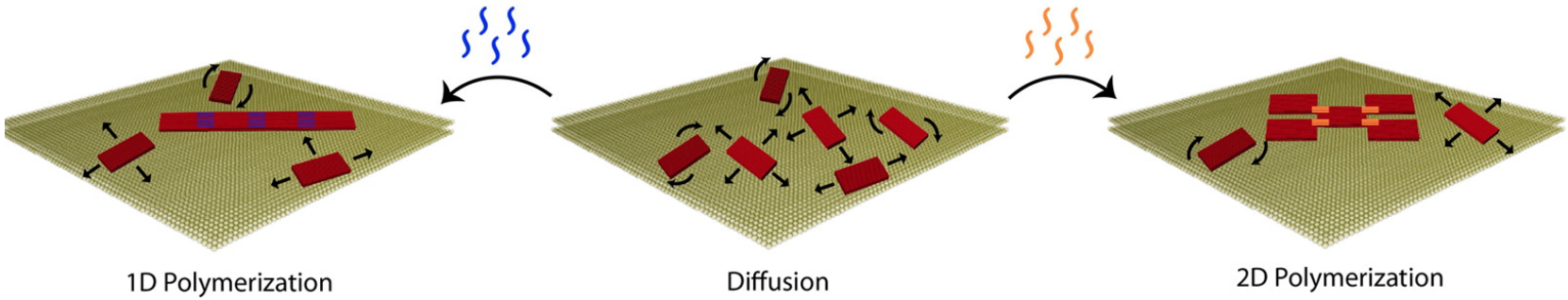
- Sticky ends and multimerization oligonucleotides form connections between single DNA origami blocks





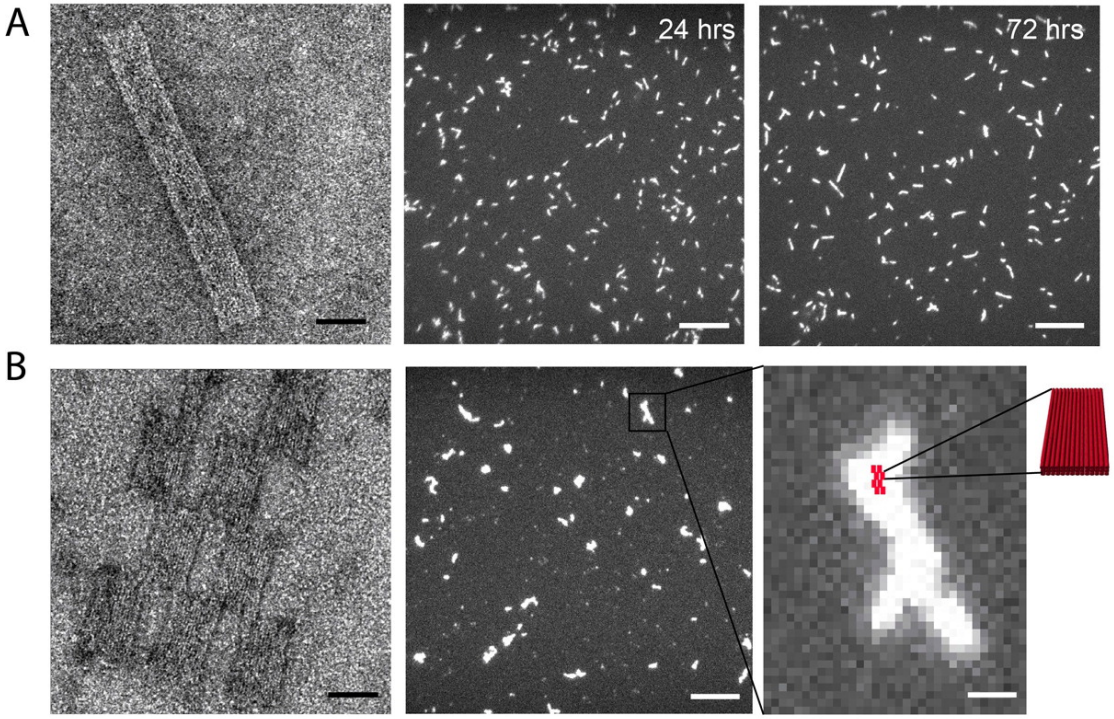
Multimerization of Origami

- Different multimerization styles are possible with the same Origami blocks

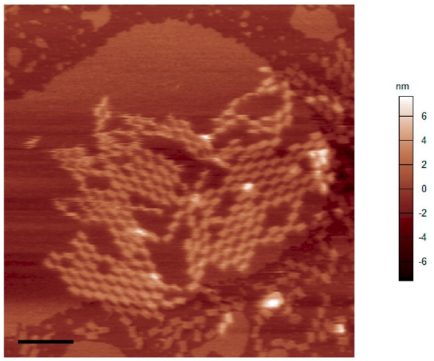


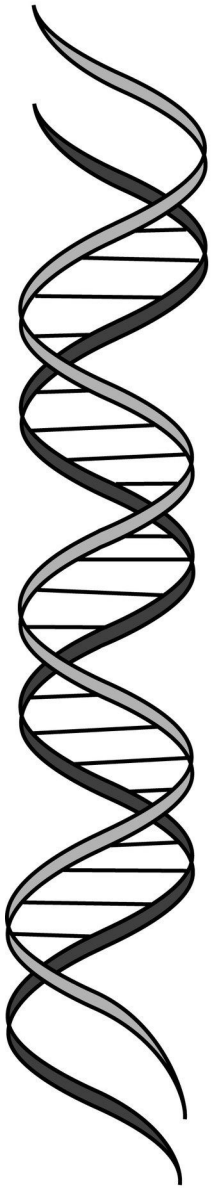
Multimerization of Origami

TEM and TIRFM images



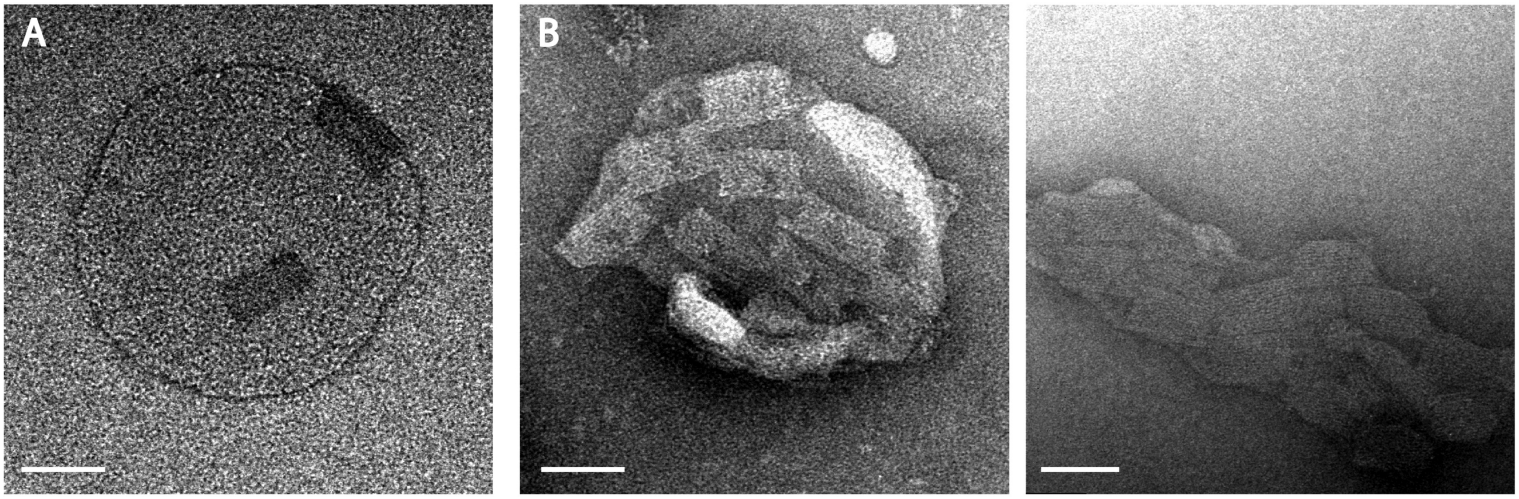
AFM image





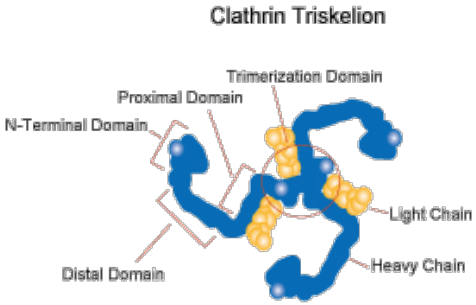
DNA-Origami: Shaping Vesicles

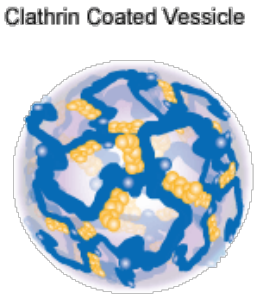
- Polymerization of DNA origami blocks exerts force on SUVs
- Destruction and deformation



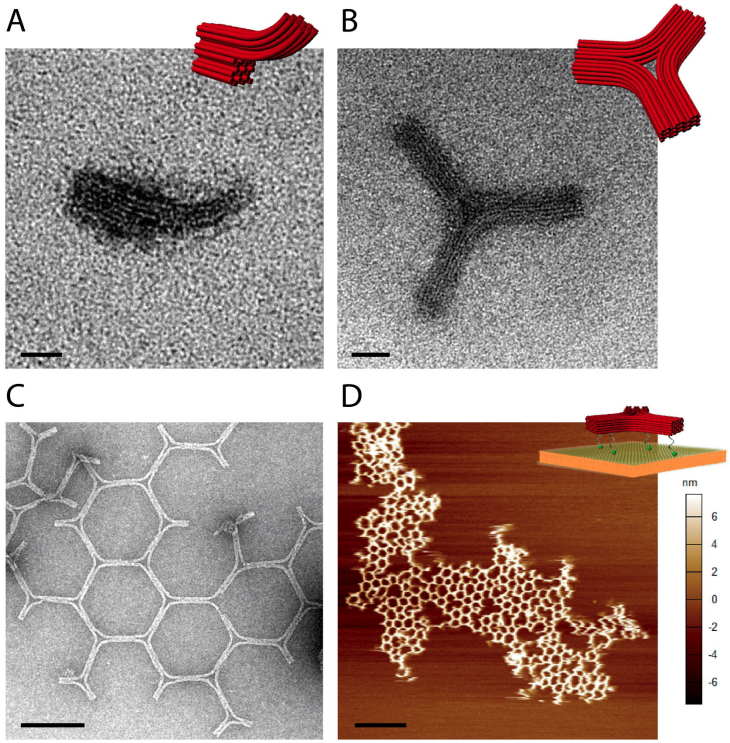
DNA Origami Triskelions

- Form of origami resembles protein that helps bud off vesicles





[2: Sigma-Aldrich]

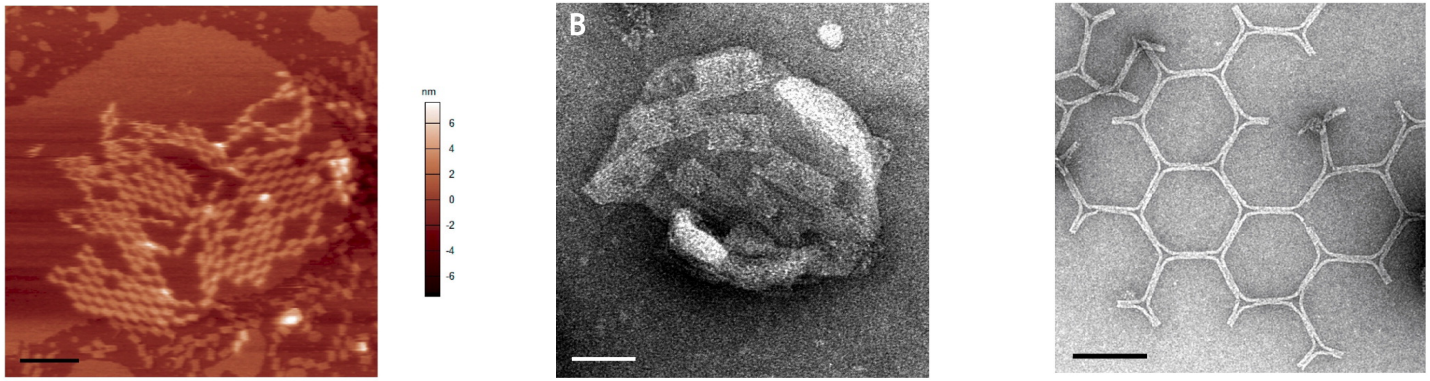


DNA-Superstructures: Summary

- Highly organized membrane structuring
- Inter origami forces can change vesicle shape

❖ Synthetic and structural biology

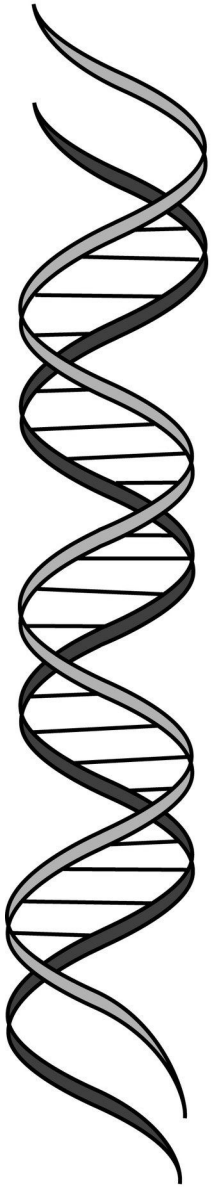
❖ Organized nanostructures



Molecular transport through large-diameter DNA nanopores

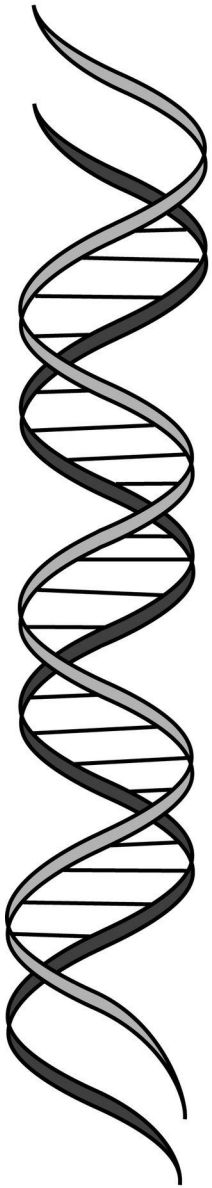
Swati Krishnan, Daniela Ziegler, Vera Arnaut, Thomas G. Martin, Korbinian Kapsner, Katharina Henneberg, Andreas R. Bausch, Hendrik Dietz & Friedrich C. Simmel

Nature Communications, 2016

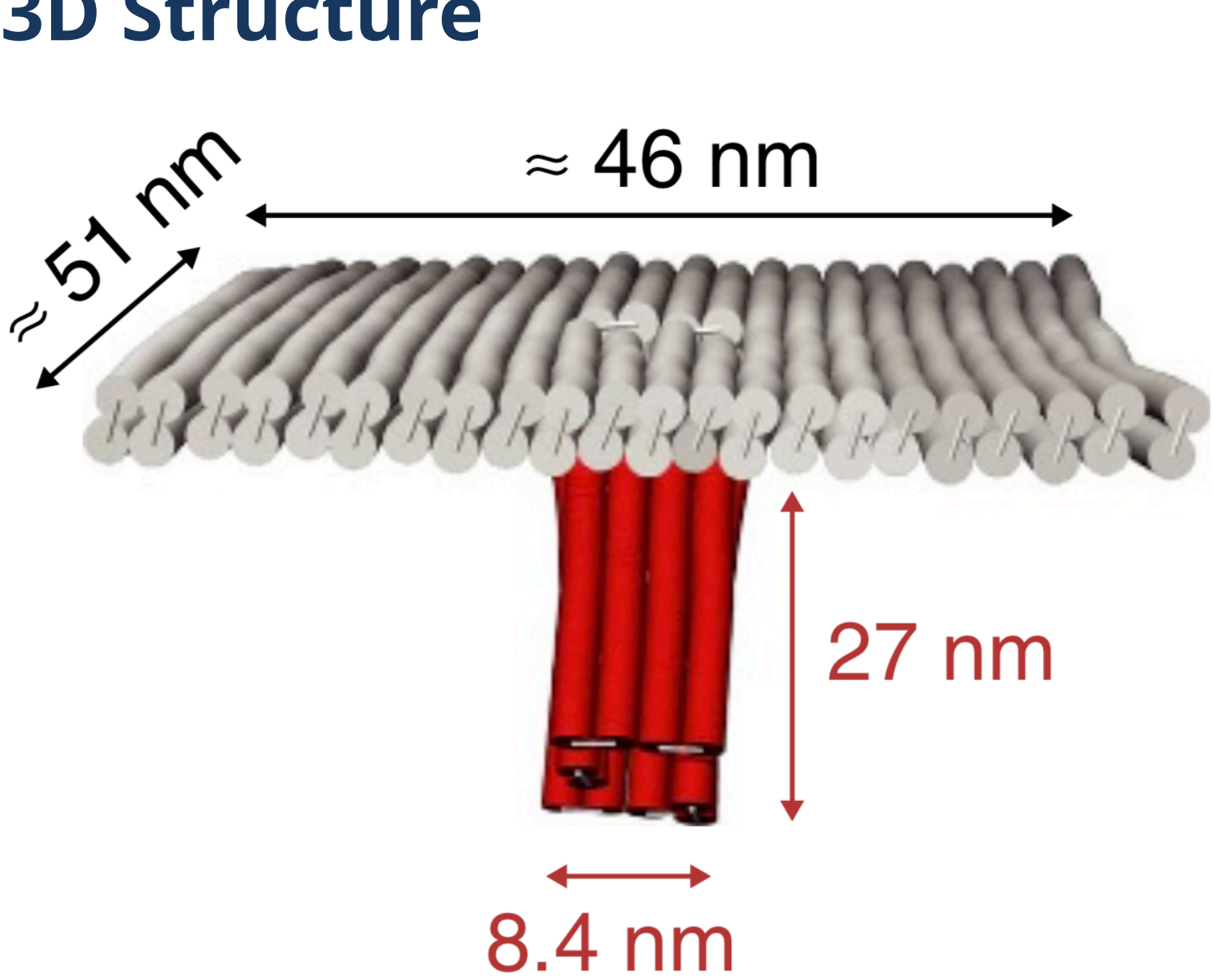


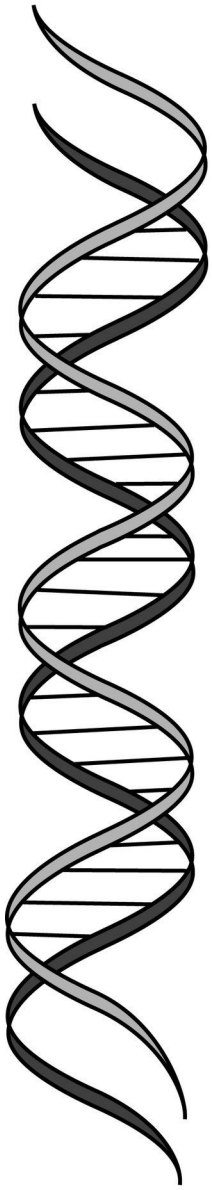
Nanopores: Introduction

- Mimicry of naturally occurring membrane channels
- Applications in synthetic biology and medicine
- Until now: Artificial nanopores don’t reliably self-integrate, not nm-precise
- Now: Addressing these issues
- Constructed using specialized CAD [1]

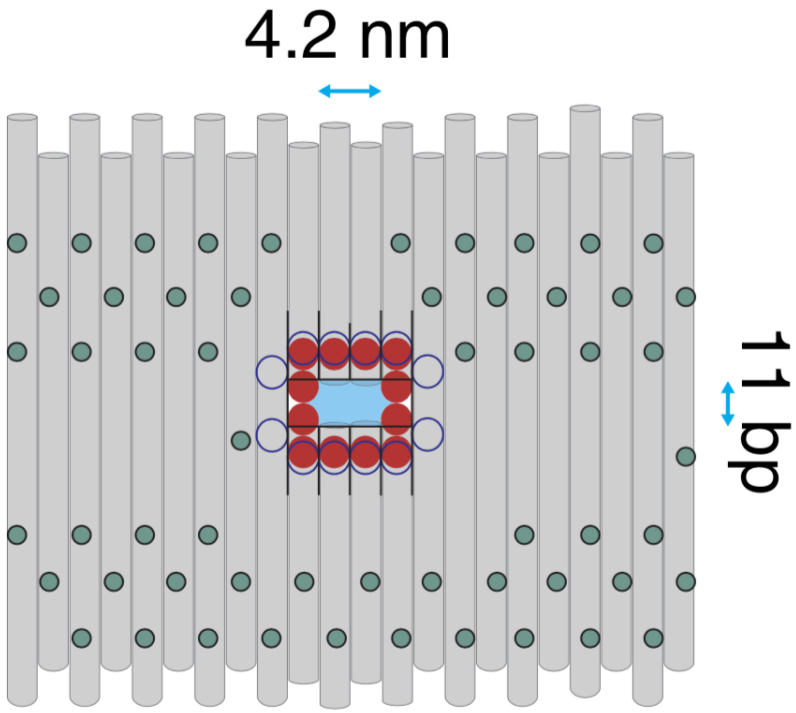


3D Structure



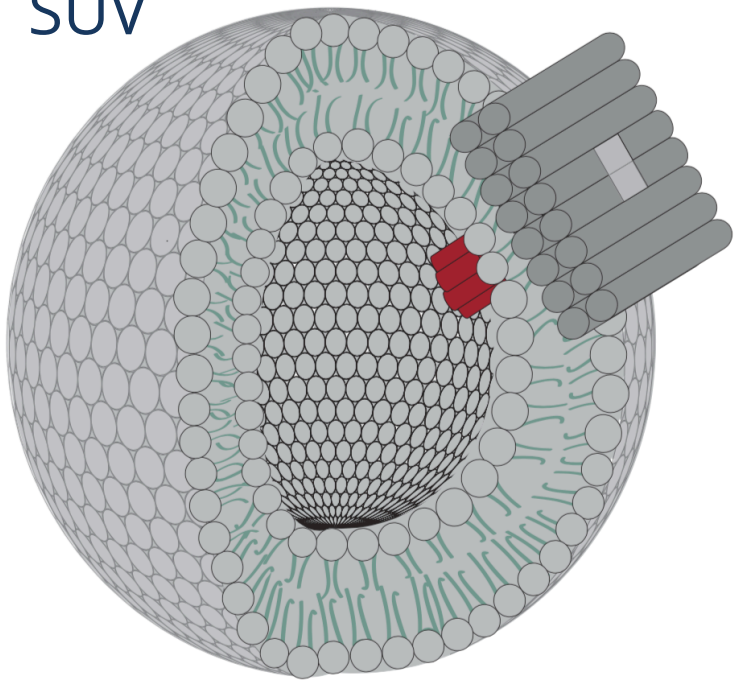


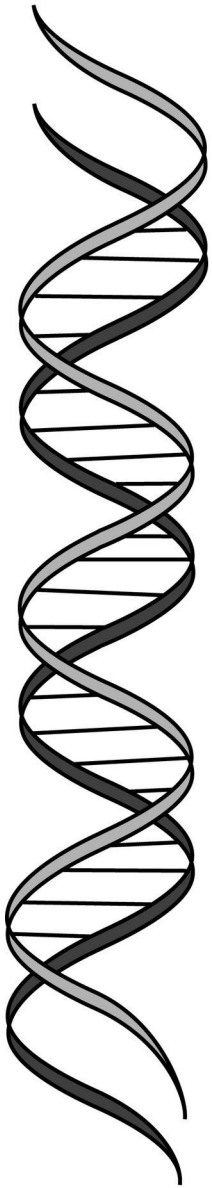
3D Structure



Green: 57 Membrane-interacting modifications

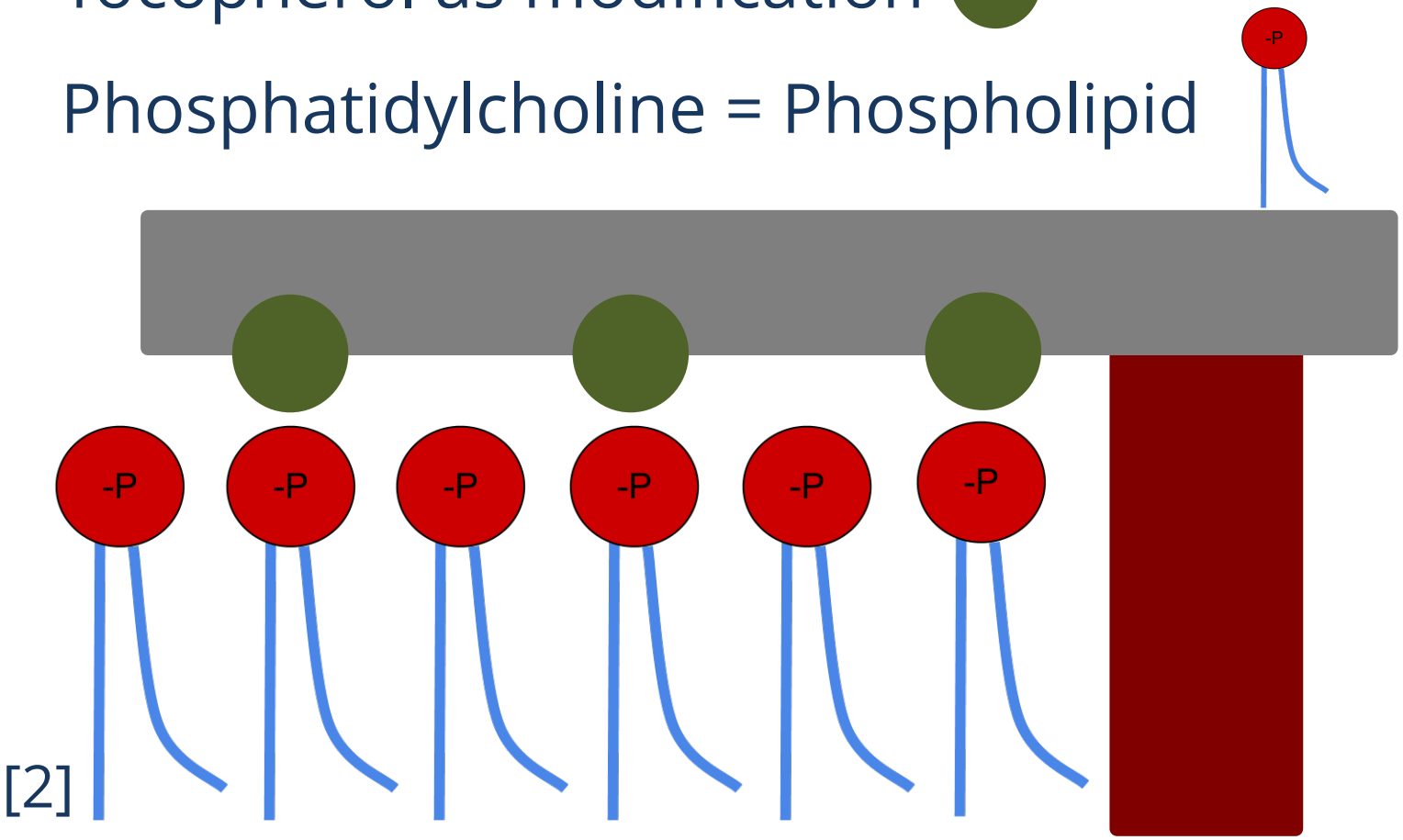
Interaction with a SUV



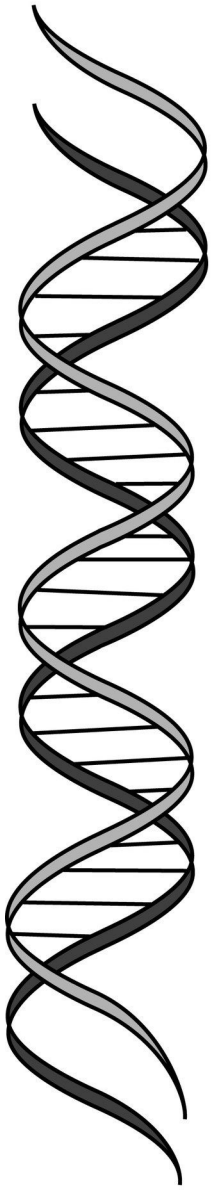


Hydrophobic Integration

- Hydrophobic interactions
- Tocopherol as modification
- Phosphatidylcholine = Phospholipid

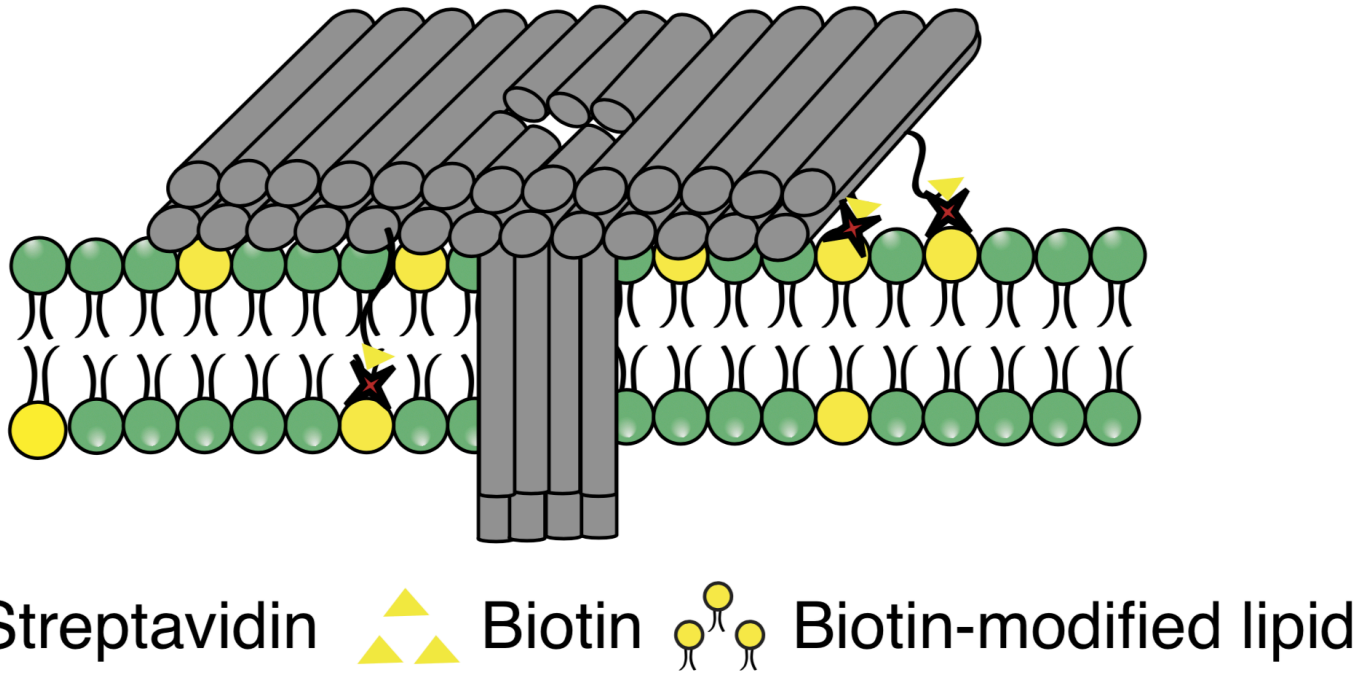


[2]



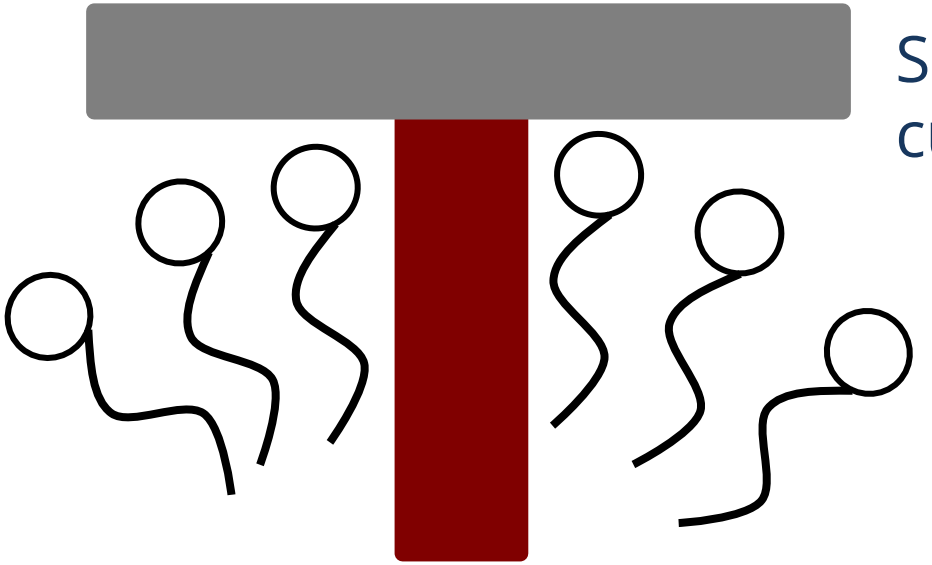
Protein-Ligand Integration

- Biotin-Streptavidin interactions

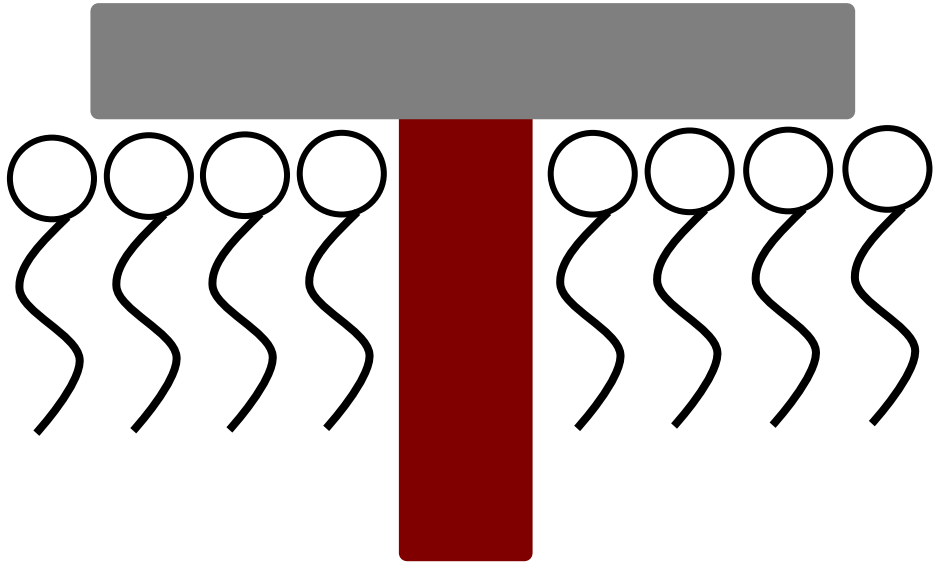


- Slower incorporation kinetics

From SUV to GUV Insertion

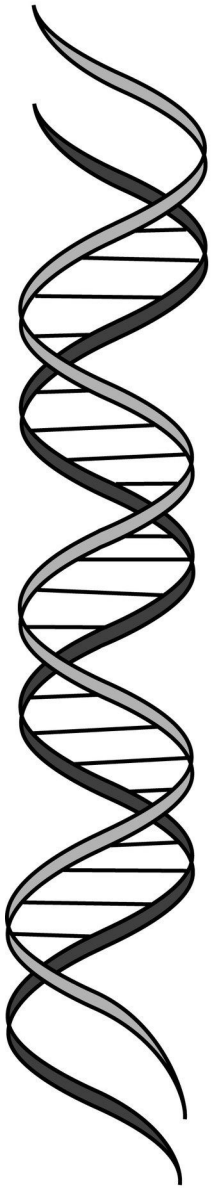


Small radius ➔ Locally curved membrane



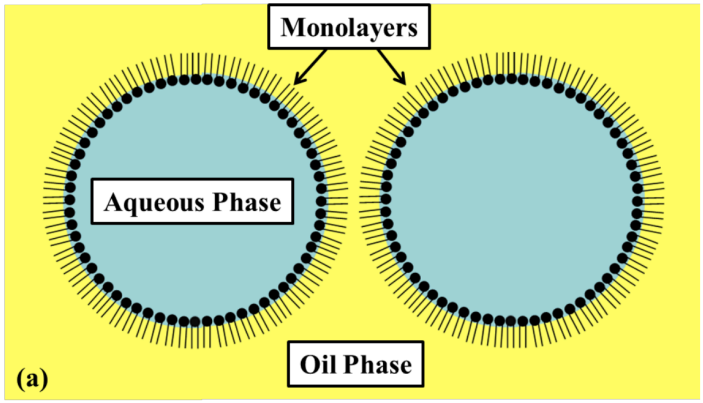
Large radius ➔
Locally flat membrane

Instantaneous insertion

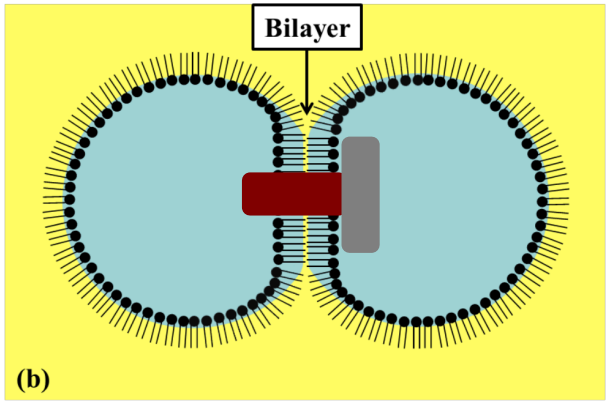


Electrical Characterization

- Droplet interface bilayer (DIB) [3]
- Introduction of T-Pore into bilayer



- Apply voltage



[4]

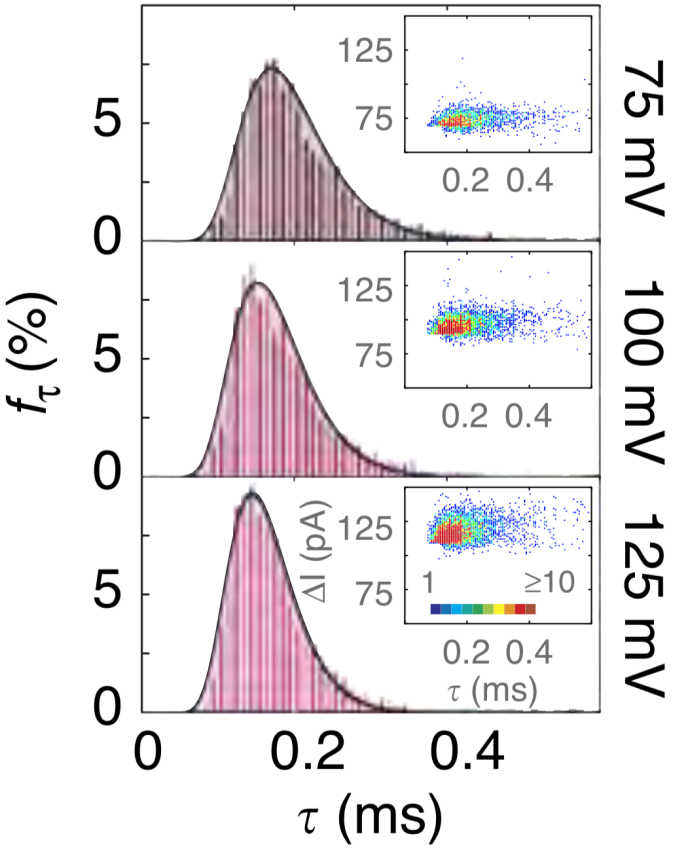
Ionic Conductance:

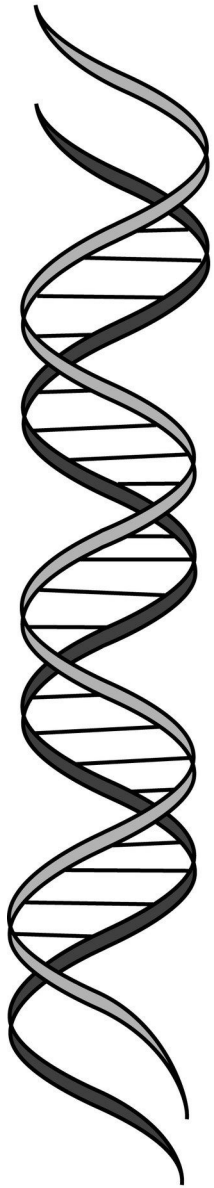
G = 3.1 ± 0.3 nS

Consistent with dimensions

DNA Translocation

- Transmembrane voltages
- Electrophoretic translocation
- Current-dependent channel dwell time
- 527 bp dsDNA
- Sensor applications



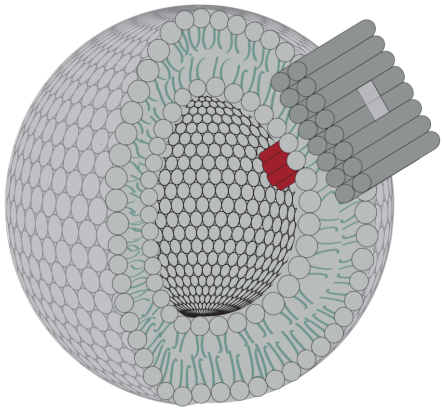


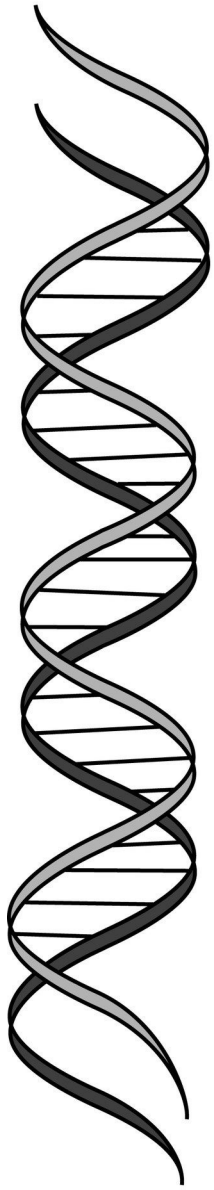
Nanopores: Summary

- Nanometer-precise, tunable pores
- Extremely fast insertion
- Electrically driven DNA translocation

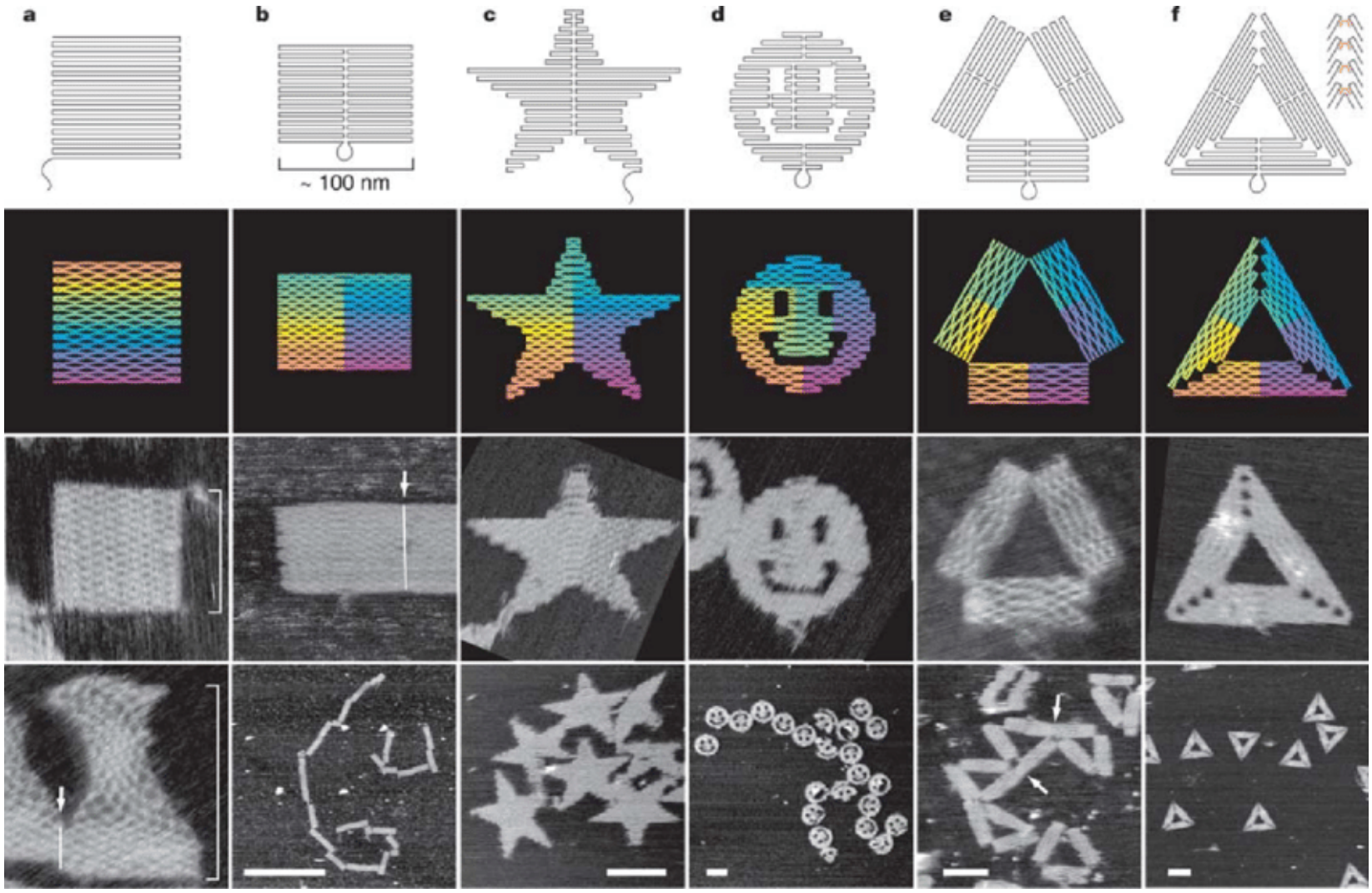
❖ Synthetic biology

❖ Programmable medical applications





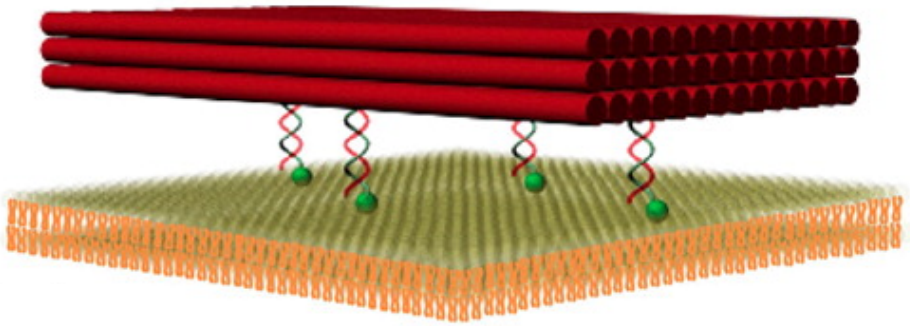
Conclusion: From the Past



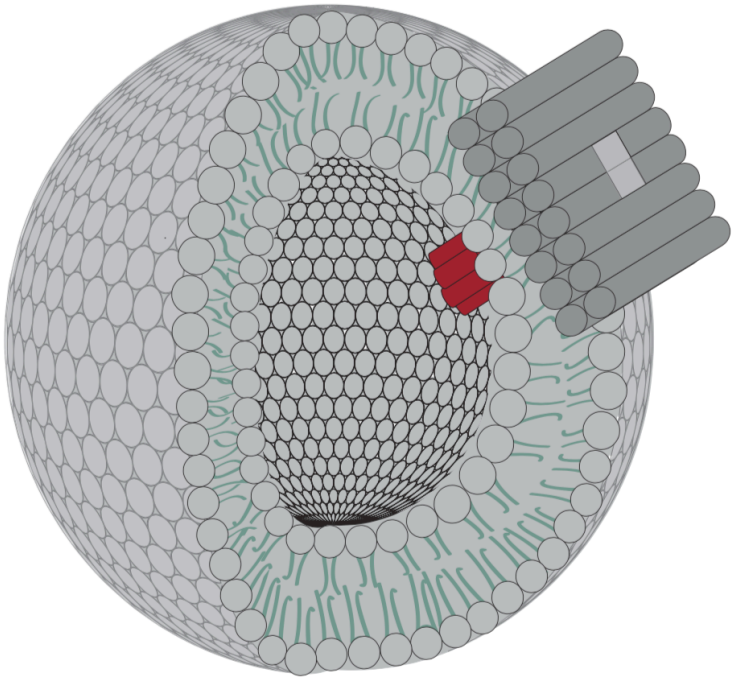
[2: Rothemund 2006]

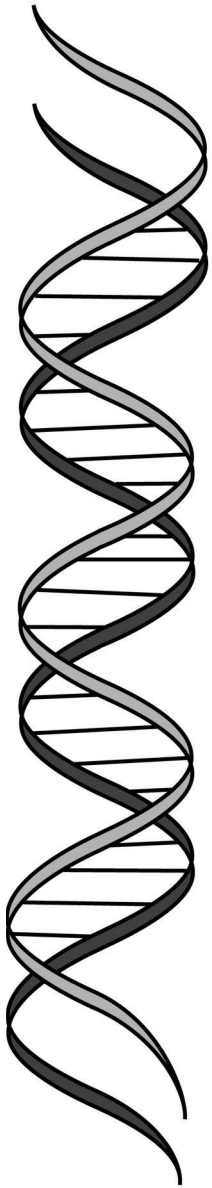
Conclusion: Back to the Future

Superstructures



Nanopores





Citations I

Introduction

[1] Andersen, E., Dong, M., Nielsen, M. et al. Self-assembly of a nanoscale DNA box with a controllable lid. Nature **459**, 73–76 (2009).

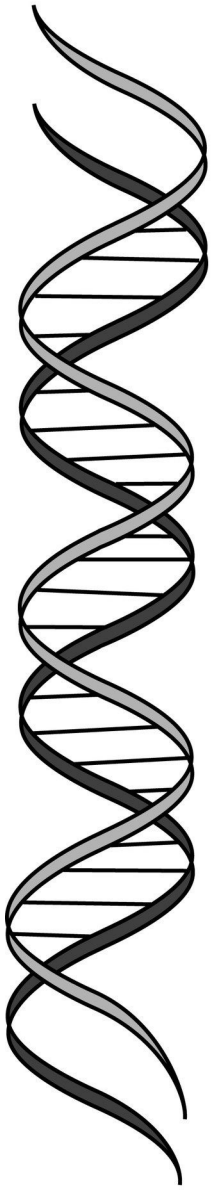
[2] Rothemund, Paul. (2006). Folding DNA to create nanoscale shapes and patterns. Nature. 440.

Assembly on Membranes

[0] Kocabey S, Kempter S, List J, et al. Membrane-assisted growth of DNA origami nanostructure arrays. ACS Nano. 2015;9(4):3530–3539.

[1] Achalkumar A, Bushby R, Evans S, et al. **Cholesterol-based anchors and tethers for phospholipid bilayers and for model biological membranes. Soft Matter. 2010;6;6036-6051.**

[2] Image credit: Sigma-Aldrich: **https://www.sigmaaldrich.com/life-science/metabolomics/enzyme-explorer/learning-center/structural-proteins/clathrin.html**



Citations II

Nanopores
[0] Krishnan, S., Ziegler, D., Arnaut, V. et al. Molecular transport through large-diameter DNA nanopores. Nature Communications 7, 12787 (2016)
[1] Douglas, S. M. et al. Rapid prototyping of 3D DNA-origami shapes with caDNAno. Nucleic Acids Res. 37, 5001–5006 (2009).
[2] Image credit: User Veggiesaur of Wikimedia, CC Attribution-Share Alike 3.0 Unported license
[3] Bayley H, Cronin B, Heron A, et al. Droplet interface bilayers. Mol Biosyst. 2008;4(12):1191–1208.
[4] Alexander James Edgerton. Design and Testing of a Hydrogel-Based Droplet Interface Lipid Bilayer Array System. Masters' thesis, 2015, Virginia Polytechnic Institute.