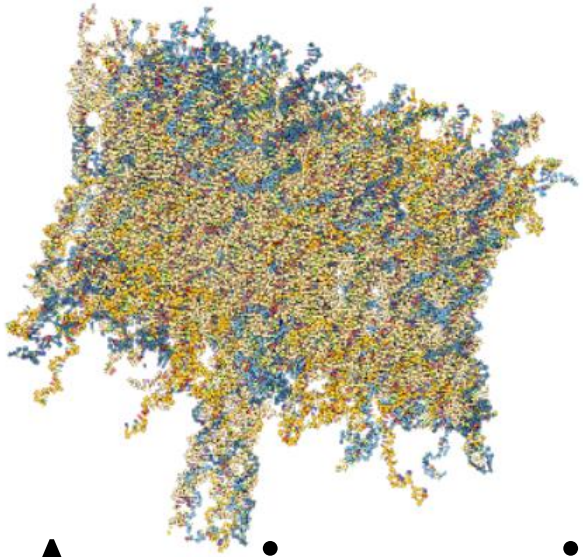
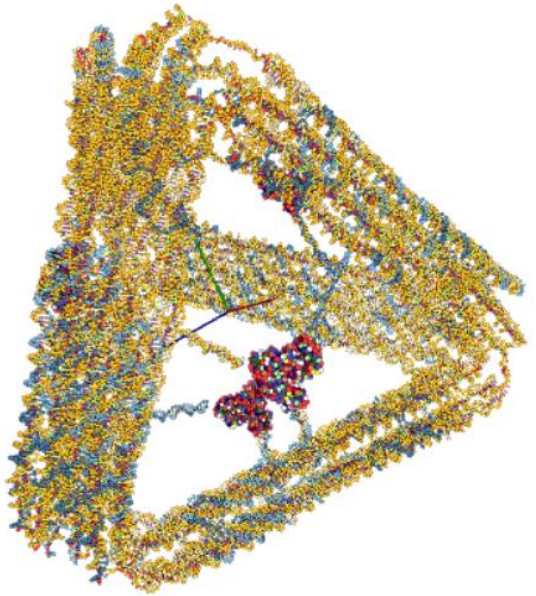
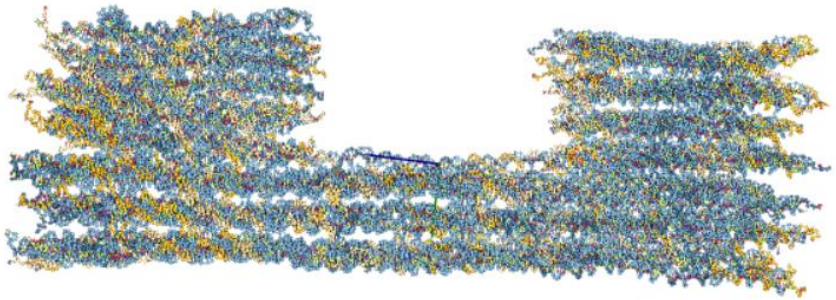
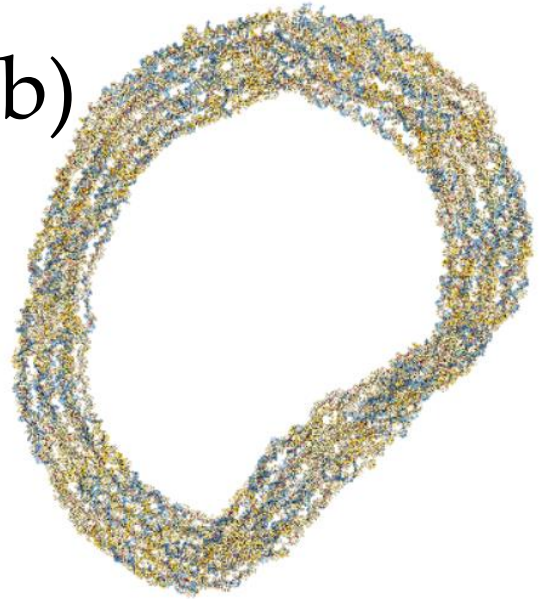




DNA origami

Alessandro Passera (Balzarotti lab)
How Do You Do It, 3.3.2023

What am I going to talk about

What are DNA origami?

How do we use DNA origami in the lab?

What can you do with DNA origami?

How do I get started with the technique?

Origin of DNA origami

First ideas of programmable structures composed of DNA is from the early 90s.
The first actual realisation I could find is from 2004:

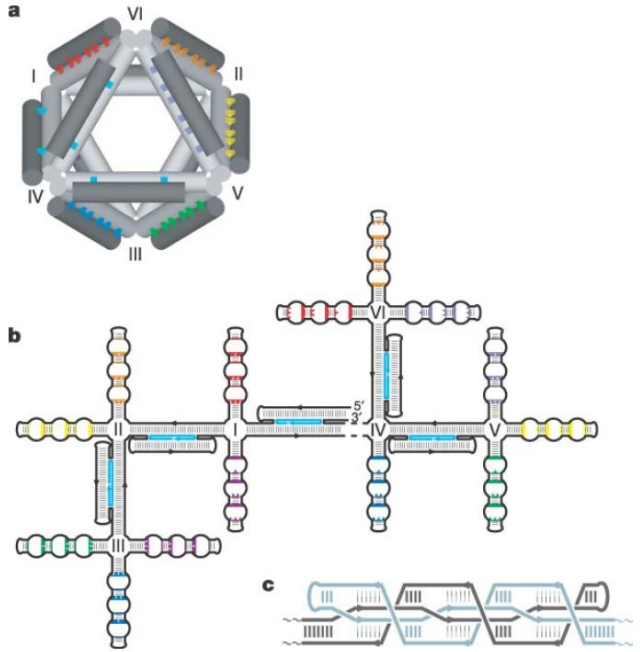
Published: 12 February 2004

A 1.7-kilobase single-stranded DNA that folds into a nanoscale octahedron

William M. Shih, Joel D. Quispe & Gerald F. Joyce

Nature **427**, 618–621 (2004) | Cite this article

8889 Accesses | **774** Citations | **23** Altmetric | Metrics



A single, *ad hoc* designed ssDNA strand (black) assembles when 5 shorter oligonucleotides (cyan) are mixed with it

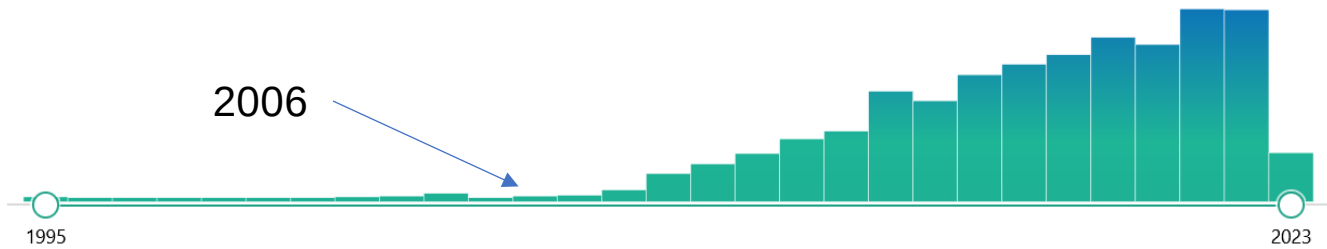
Published: 16 March 2006

Folding DNA to create nanoscale shapes and patterns

Paul W. K. Rothemund

Nature **440**, 297–302 (2006) | Cite this article

95k Accesses | **5123** Citations | **428** Altmetric | Metrics



Paul Rothemund, now professor at Caltech

Huge difference: shapes can be arbitrarily programmed, using the same framework

How does it work?

The scaffold is not a special sequence: in our case M13 bacteriophage ssDNA, circular, 7249 nt-long and very cheap.

What determines the shape are the short (~30 nt) oligos ("staples") that anneal to distant parts of the scaffold and bring them together in 3D

In our case they are 184

Scaffold and staples are mixed, the solution is heated to 80°C to melt any structure, then slowly brought to 4°C over the course of a few hours to allow annealing

Buffer is a weak Tris buffer pH 8, with the only crucial component being the Mg^{2+} ion

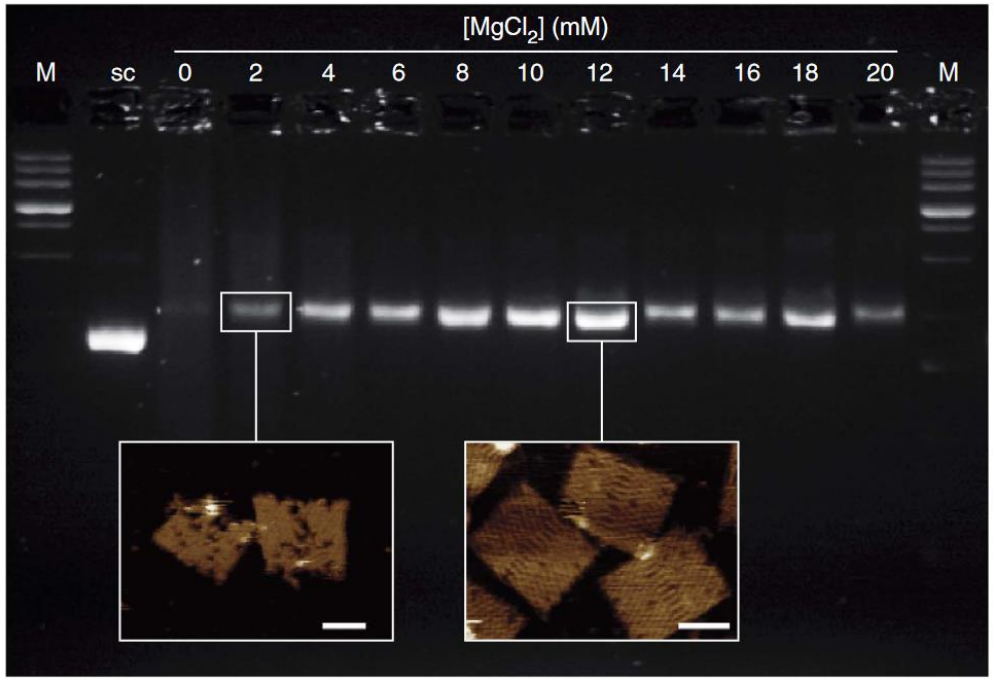
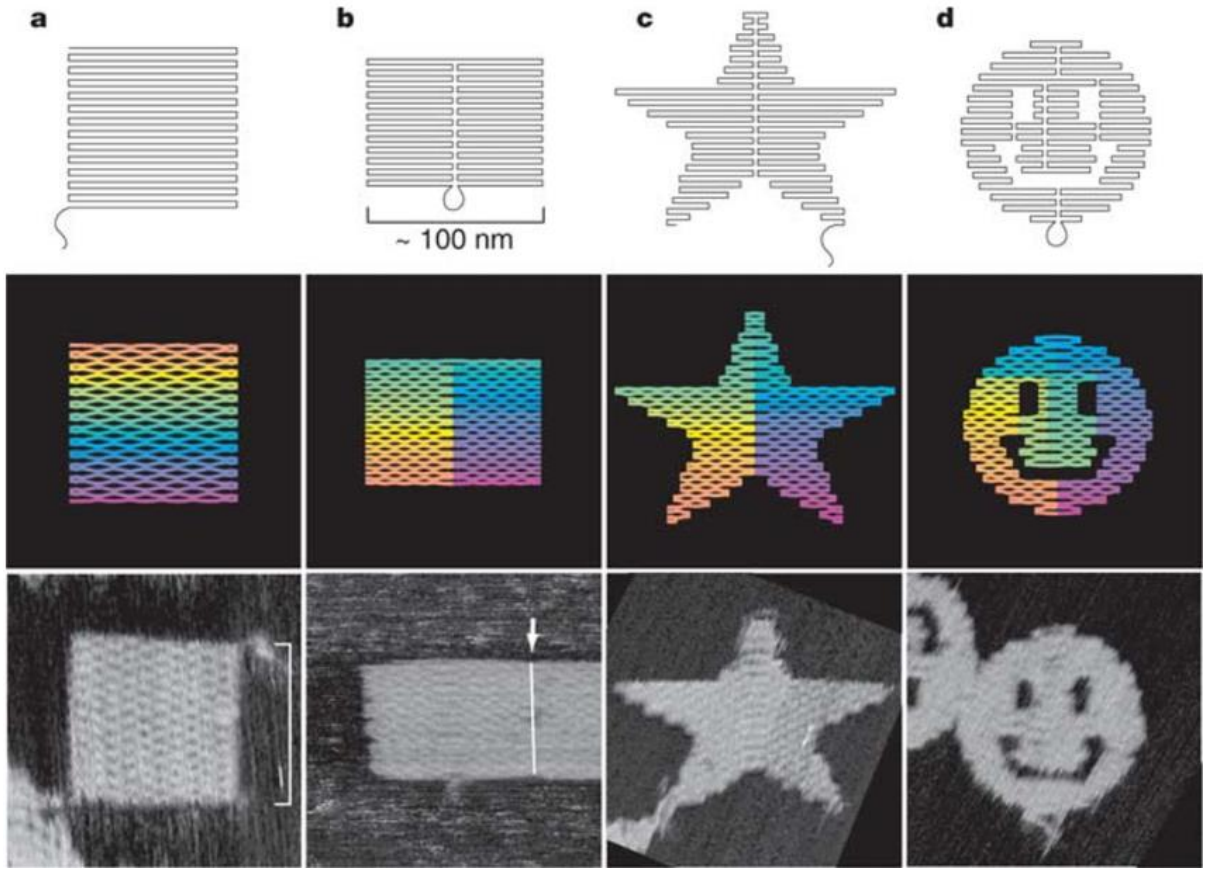


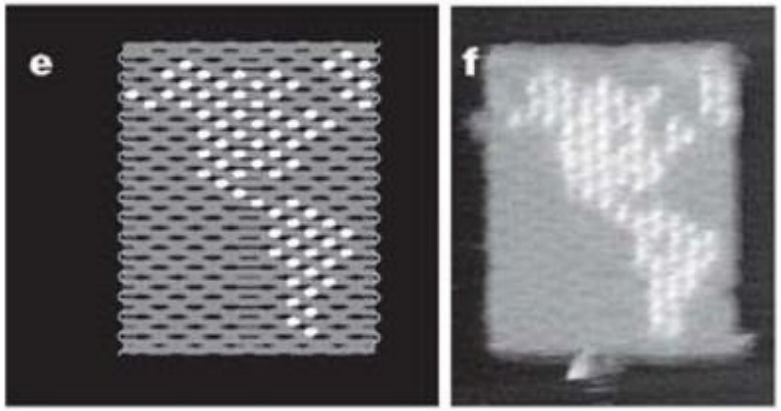
Image from Schmied, J. J., P. Tinnefeld. et al. "DNA Origami–Based Standards for Quantitative Fluorescence Microscopy." *Nature Protocols* (2014)

A very robust technique

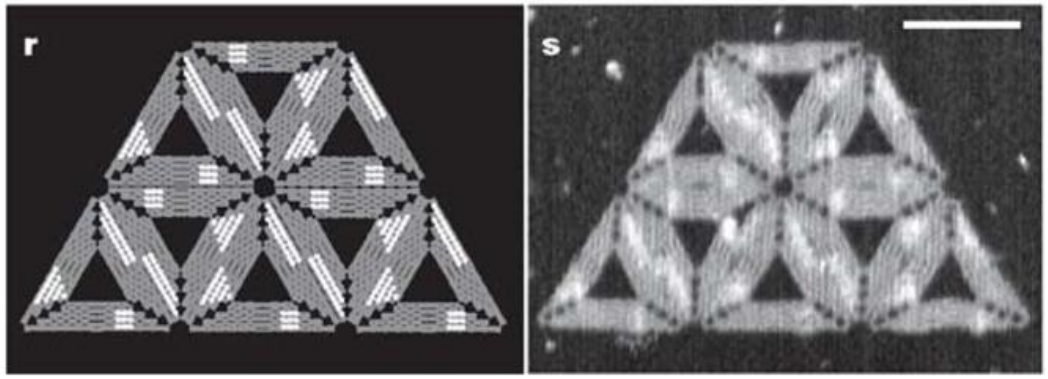
Diverse structures can be programmed with the same sequences



Origamis are breadboards

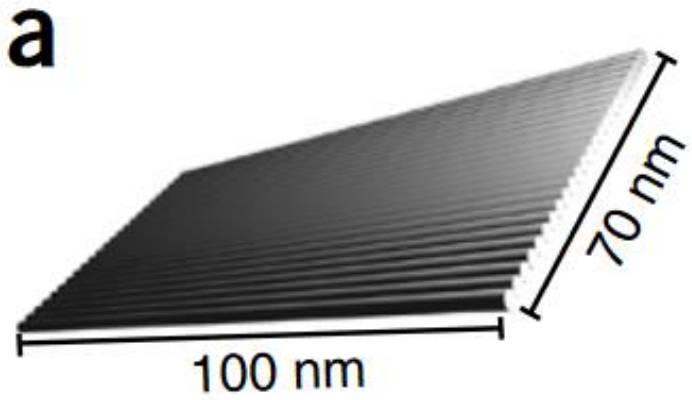


Origamis can combine into larger structures

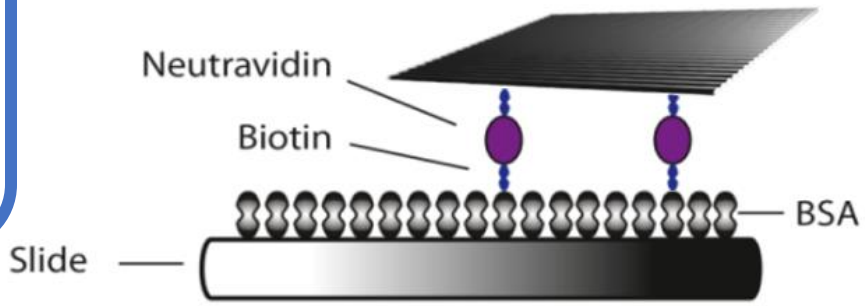


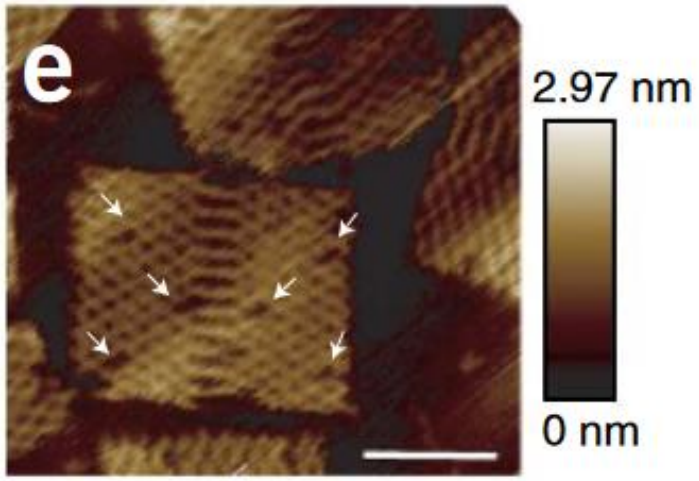
Images from Rothemund, Paul W. K. "Folding DNA to Create Nanoscale Shapes and Patterns." *Nature* (2006)

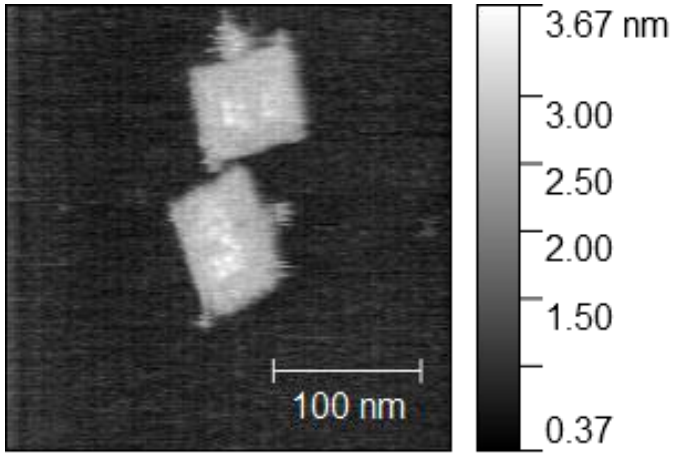
What do we do with them in the lab?



Fantastic samples for super-resolution (breadboards with a ~5 nm pixel size)







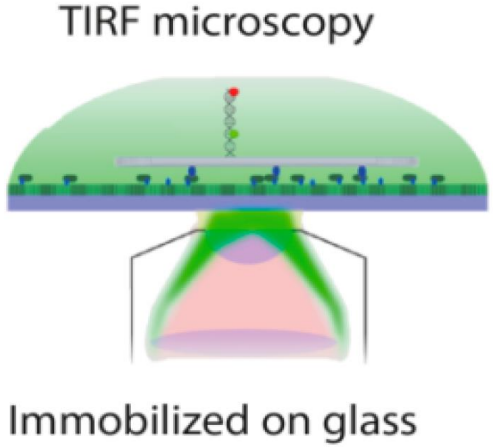
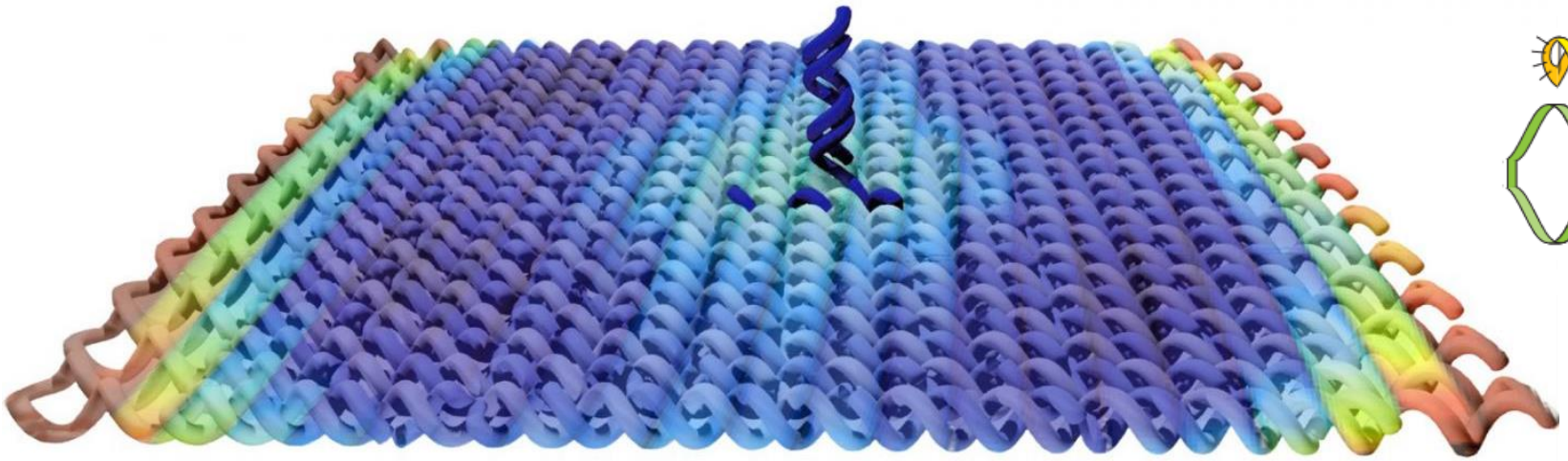


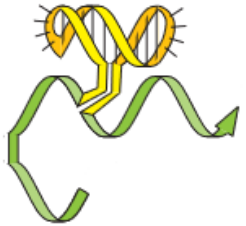
Image from Schmied, J. J, *Nature Protocols* (2014)

High-speed AFM with Sabrina Horn (Peters)

Modified from Gust et al., *Molecules*, 2014

Modifying DNA origamis





Extending a staple strand at its 5’ or 3’ will have the extension stick outside the structure

For example, one can buy biotinylated oligos, and program it so that all the biotins stick out from the same side

Then, one can extend other oligos with excess DNA that sticks on the other side, and use these as **handles**

Origamis for DNA-PAINT

Points Accumulation In Nanoscale Topography

Objective: extend staples to have DNA-PAINT binding sites

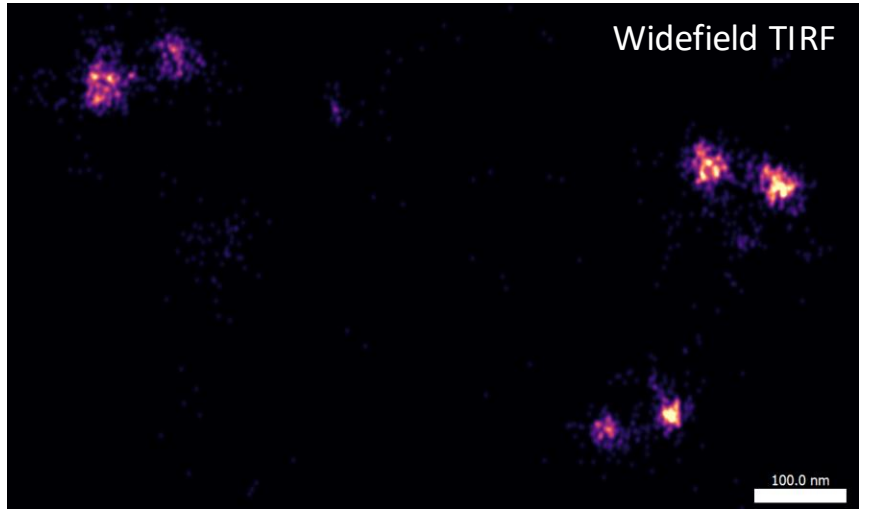
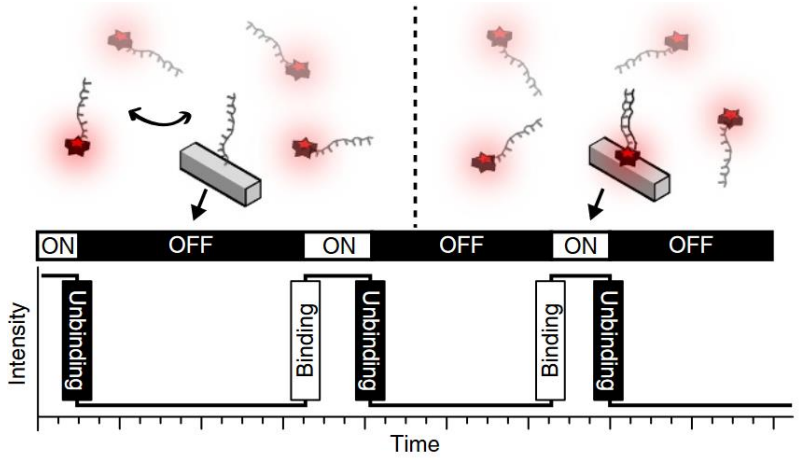
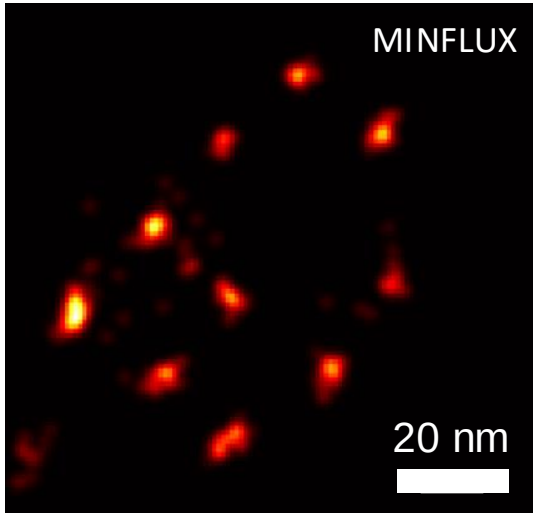


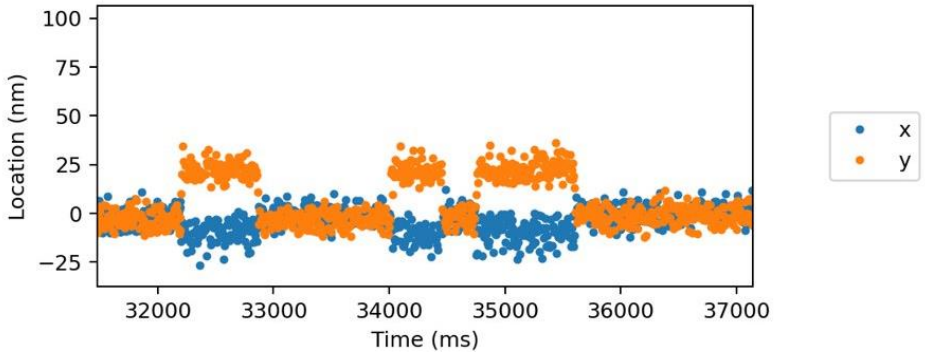
Image by Claudio Polisseni (Tanaka)

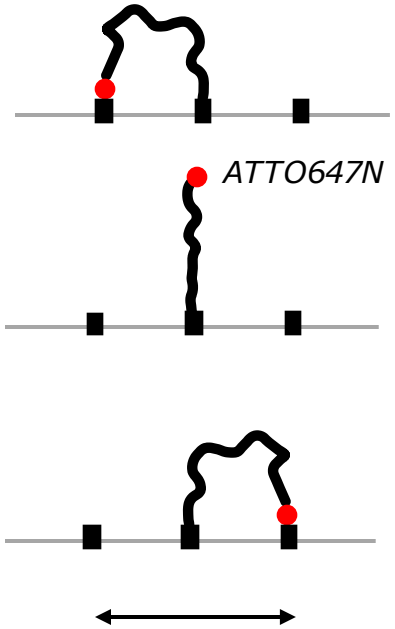


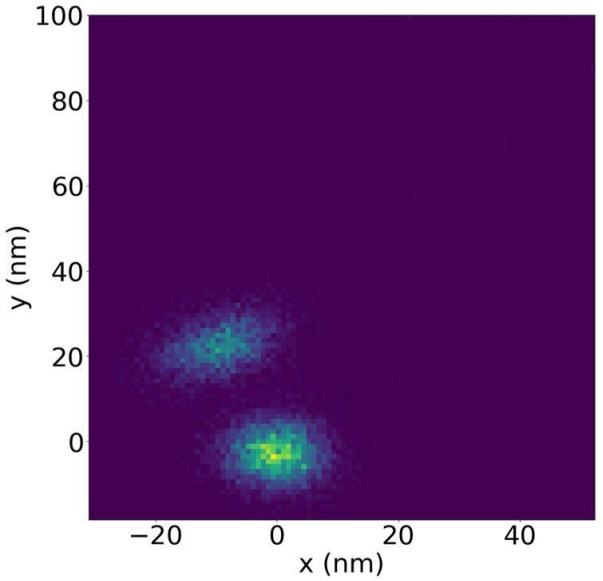


“Dynamic” origamis

2D MINFLUX tracking

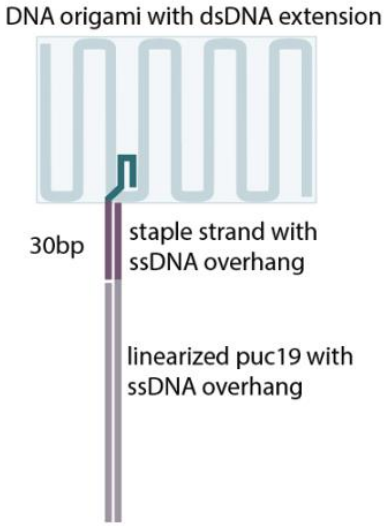






Proxy for protein complex movements, etc.

Origamis for AFM and single molecule fluorescence



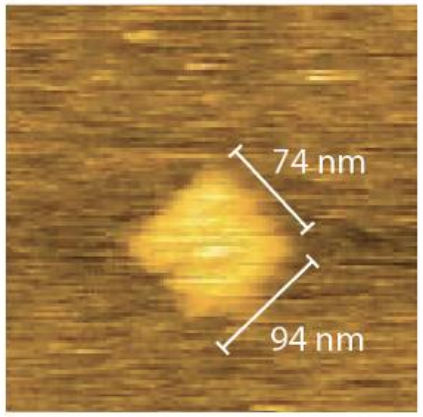
Attaches well to mica surface for AFM

Easily resolvable

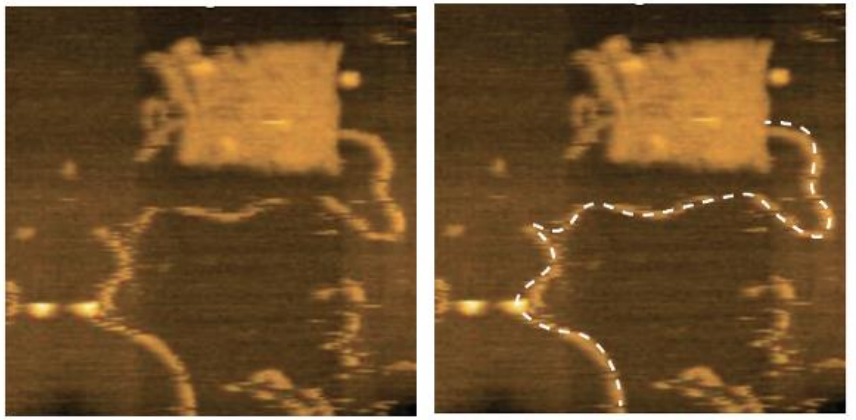
Long pieces of DNA can be attached specifically to the origami

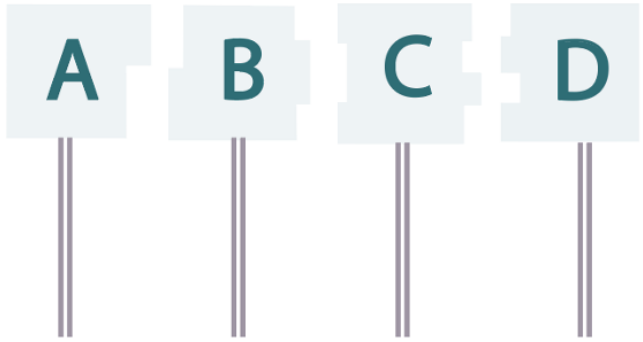
The sample can then be used for AFM or single-molecule fluorescence

B



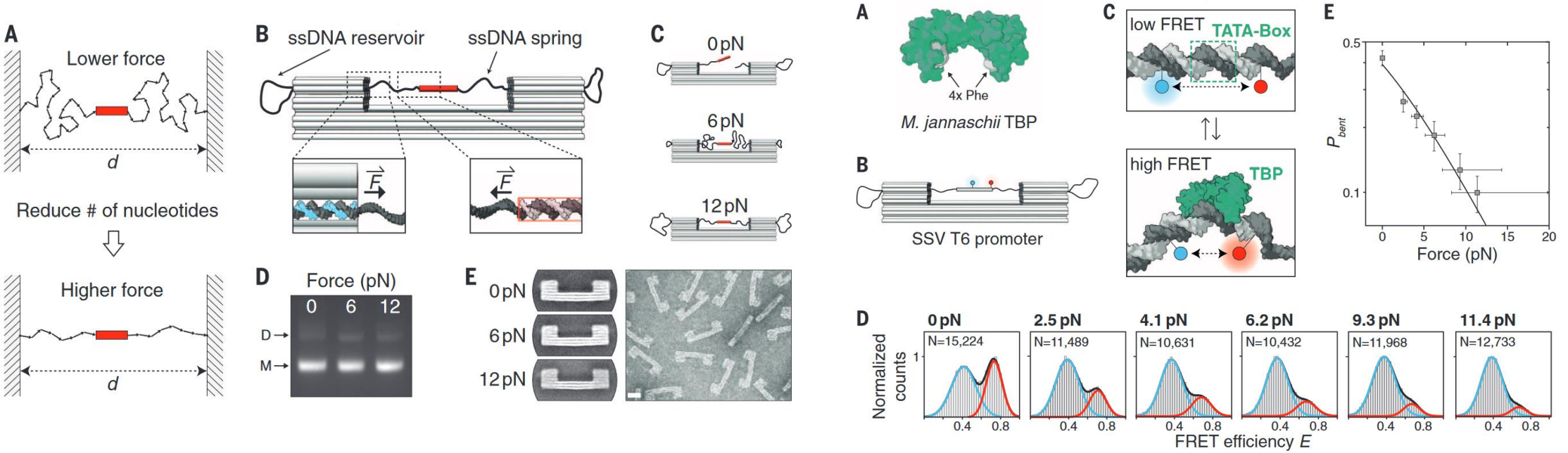
C





Images by Sabrina Horn (Peters)

Force sensors or springs



Goal: create an alternative to AFM or optical tweezers (costly, low throughput) for the application of controlled forces

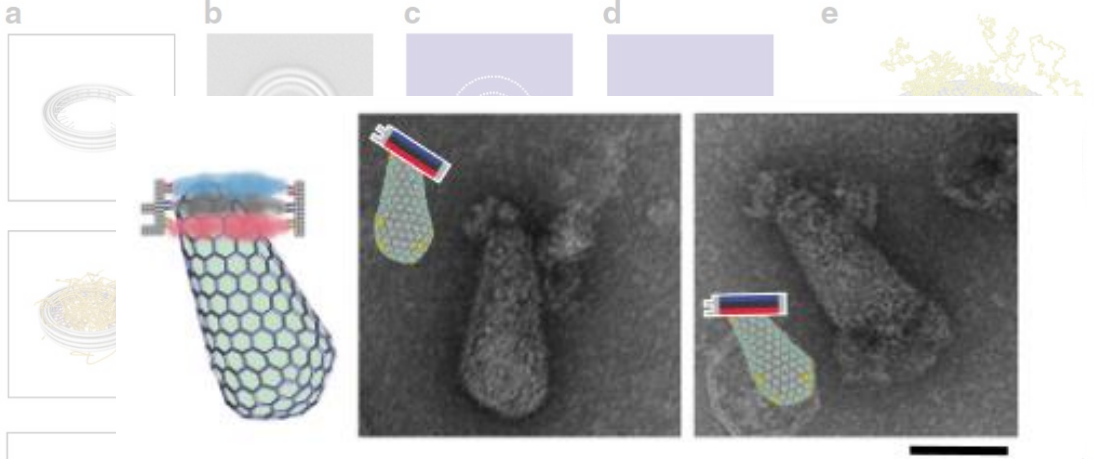
The DNA origami can apply forces from 1.2 to 50 pN to the ends of a DNA molecule

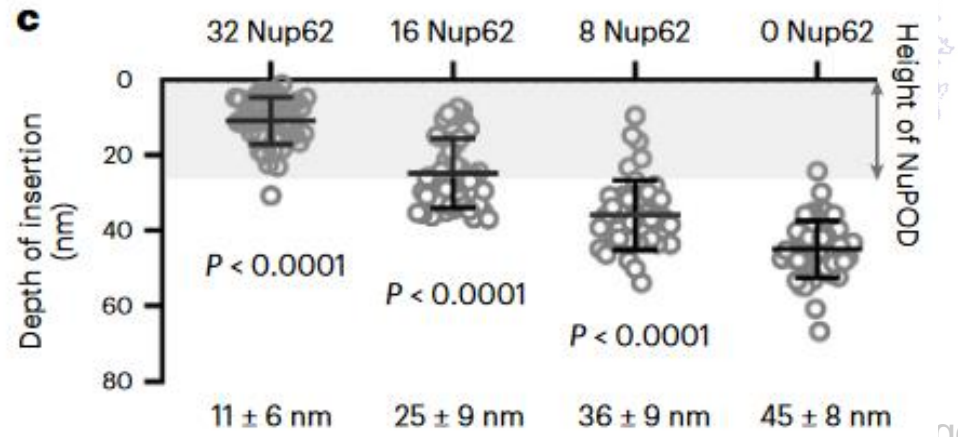
Useful for studying DNA binding proteins, or any target as long as the two pieces to pull can be functionalized with DNA handles

Nickels, P. C., P. Tinnefeld et al. "Molecular Force Spectroscopy with a DNA Origami–Based Nanoscopic Force Clamp." *Science* (2016)

Platforms for protein attachment

Shen, Q., Shi, J., et al. "Modeling HIV-1 Nuclear Entry with Nucleoporin-Gated DNA-Origami Channels." *Nature Structural & Molecular Biology* (2023)

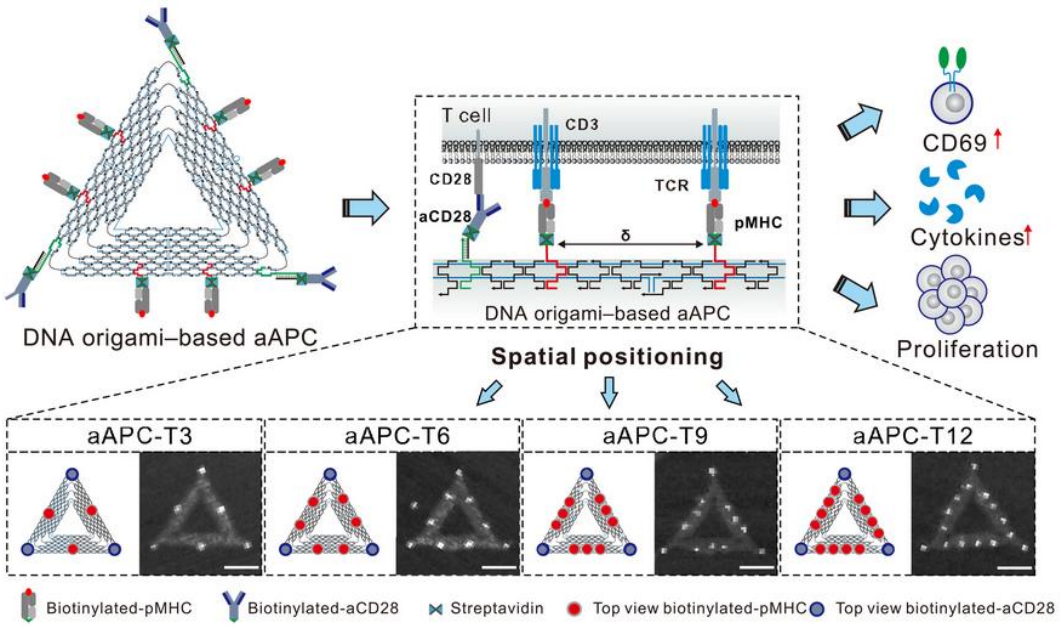


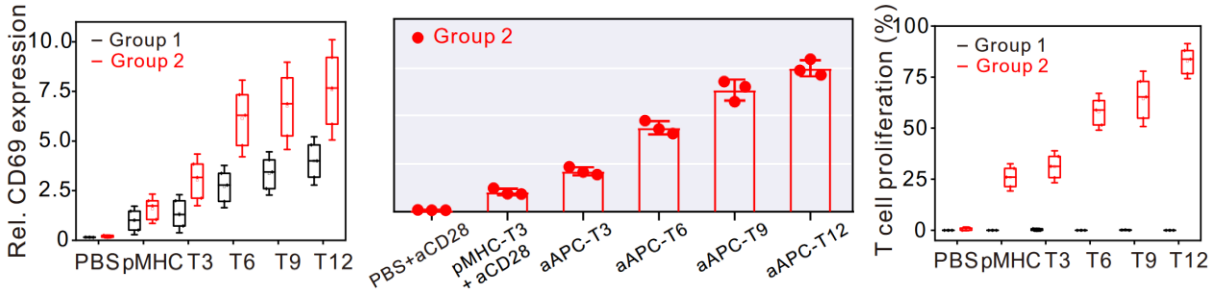


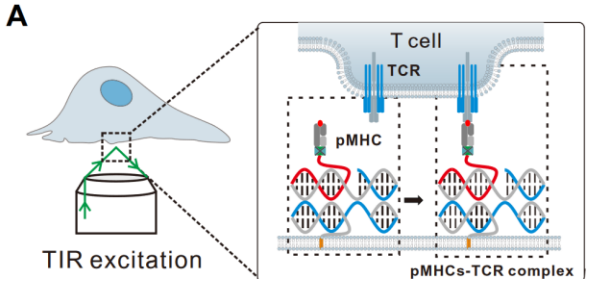
arbitrarily in xyz

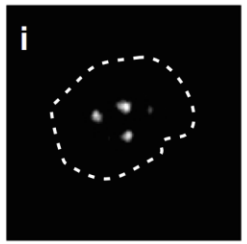
Ketterer, P., Dekker C. et al. "DNA Origami Scaffold for Studying Intrinsically Disordered Proteins of the Nuclear Pore Complex." *Nature Communications* (2018)

Artificial antigen-presenting cells



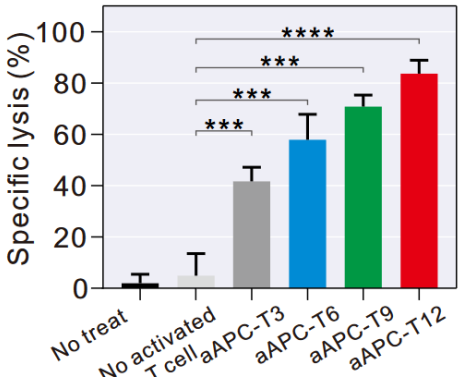




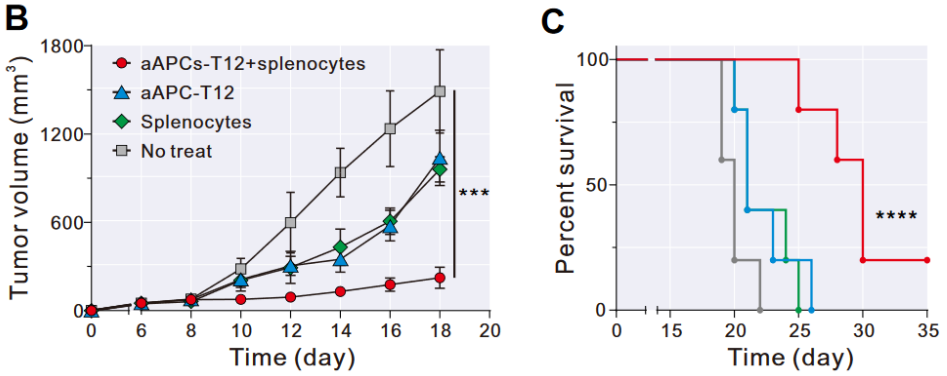


Single particle tracking on supported lipid bilayers: average binding time increases by ~30%

In vitro: treatment of melanoma cells with origami pre-incubated T cells

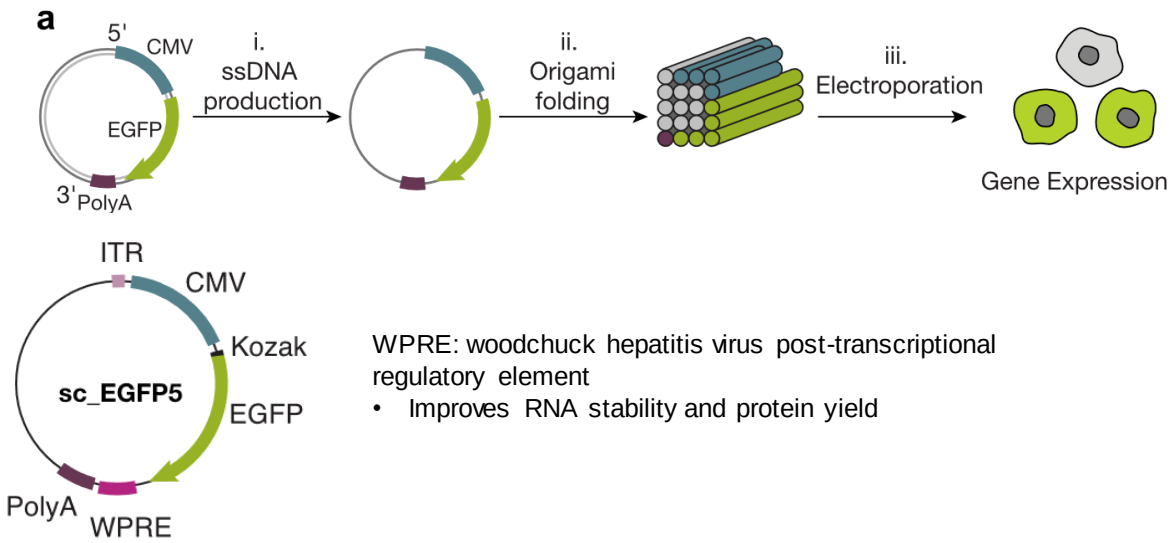


In vivo: (co-)injection of origami and/or T cells in mice



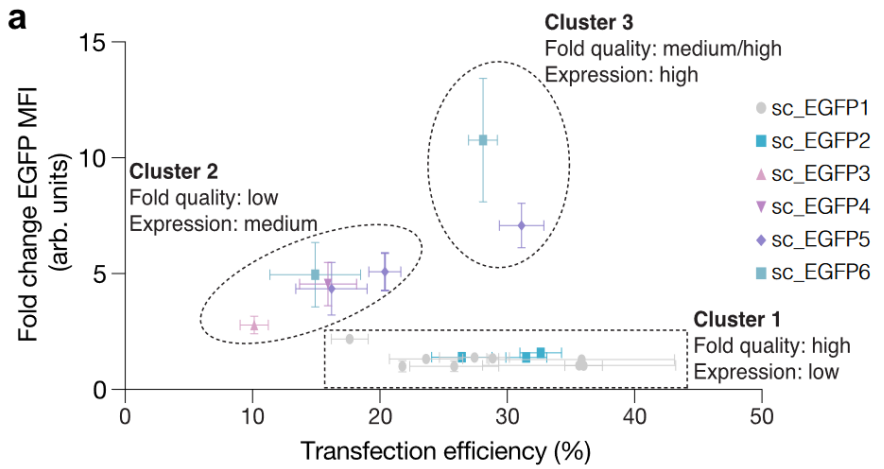
Sun, Y., Pei, H. et al. "DNA Origami–Based Artificial Antigen-Presenting Cells for Adoptive T Cell Therapy." *Science Advances* (2022)

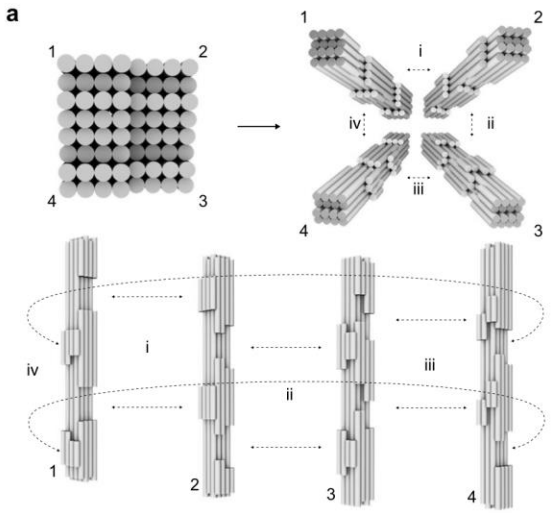
Gene delivery cassettes

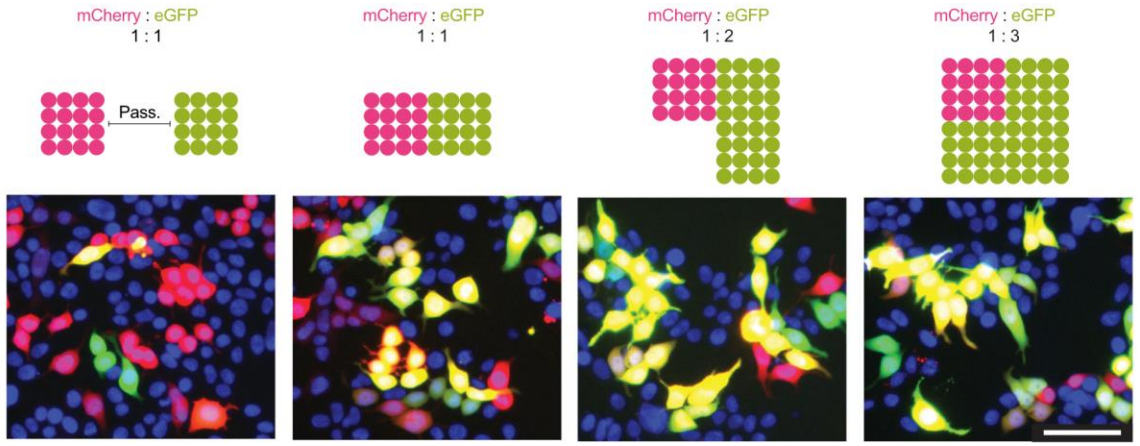


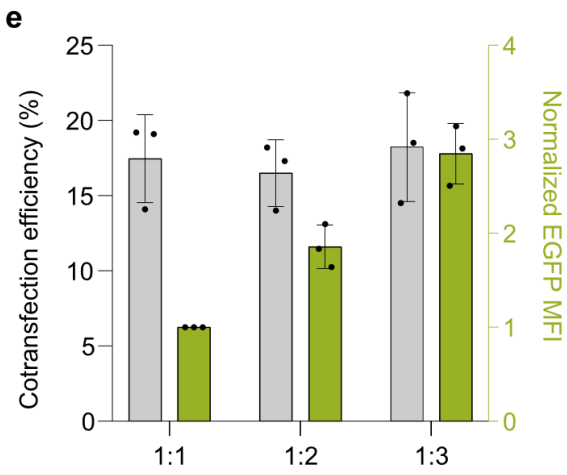
WPRE: woodchuck hepatitis virus post-transcriptional regulatory element

- Improves RNA stability and protein yield



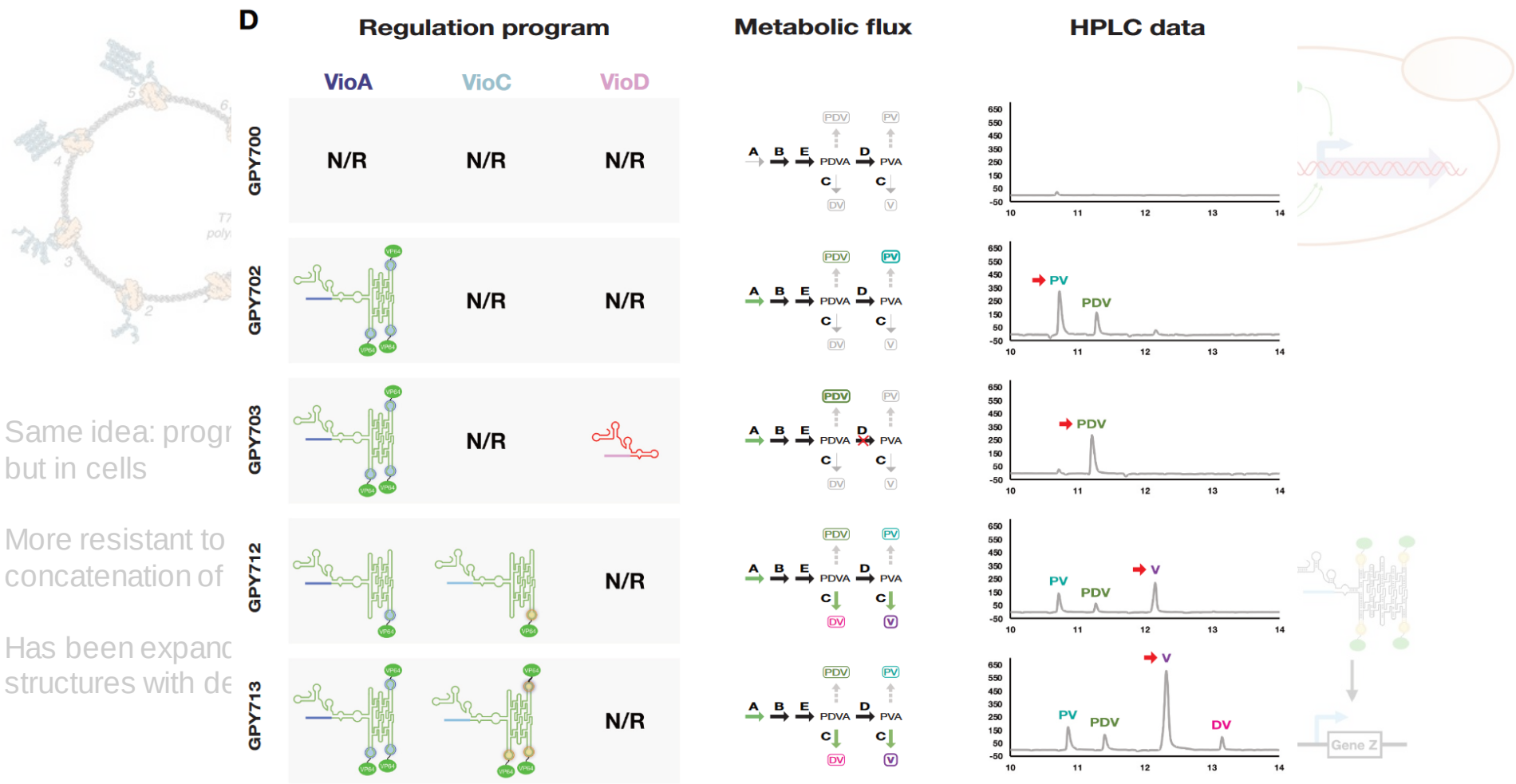






Kretzmann, Jessica A., Dietz, H. et al. "Gene-Encoding DNA Origami for Mammalian Cell Expression." *Nature Communications* (2023)

RNA origamis



Same idea: progr
but in cells

More resistant to
concatenation of

Has been expanc
structures with de

Geary, C, Andersen, E. S. "A Single-Stranded Architecture for Cotranscriptional Folding of RNA Nanostructures." *Science* (2014).

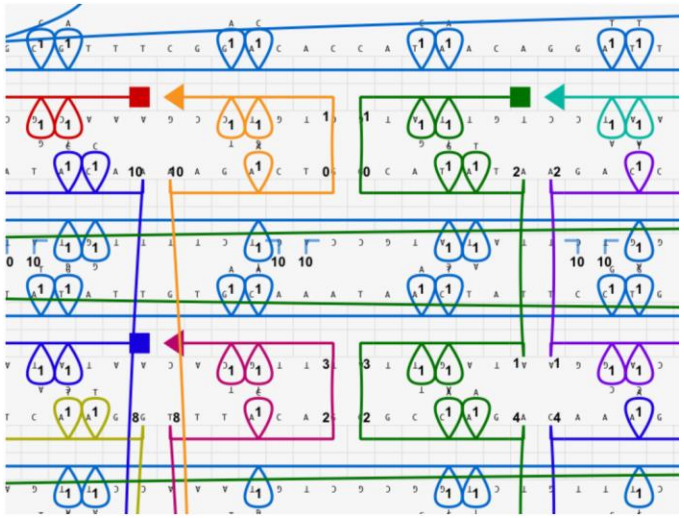
Pothoulakis, G., Andersen, E. S. et al. "Utilizing RNA Origami Scaffolds in Saccharomyces Cerevisiae for DCas9-Mediated Transcriptional Control." *Nucleic Acids Research* (2022).

How do I get started with them?

Schmied, J. J., Tinnefeld, P. et al. "DNA Origami–Based Standards for Quantitative Fluorescence Microscopy." *Nature Protocols* 9, no. 6 (June 2014): 1367–91

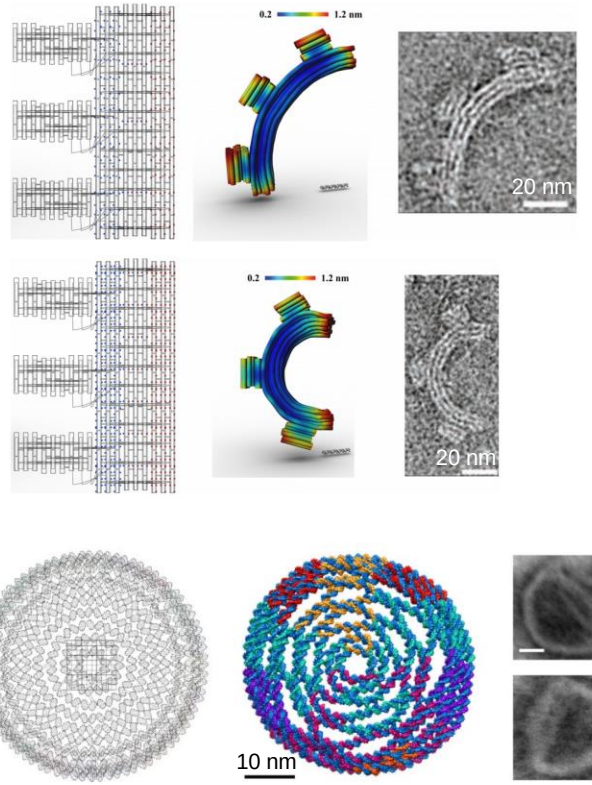
Contains recipes for rectangles and 6/12-helix bundles

Guide to the use of cadnano



CanDo

3D structure prediction tool from cadnano files



Costs and timing

For our DNA origami rectangle:

Scaffold ≈ 5 € per reaction
Staples = 184 x ~5 euros each ≈ 900 €, never bought again
Biotinilated staples = 8 x 30 € = 240 €, never bought again
No costly buffer component (comes all from the media kitchen)

~ 15 minutes handling time to assemble the reaction pot

~ 4 hours in thermocycler

~ 90 minutes purification (PEG precipitation/gel extraction)

~ 1 pmol per reaction, which for us is almost infinite