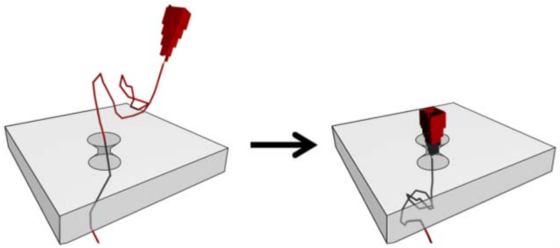
UNIVERSITY OF CAMBRIDGE



DNA Origami Nanopores

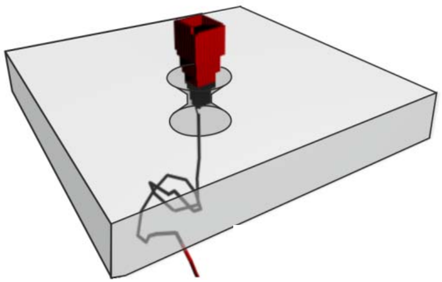
Ulrich F. Keyser

ufk20@cam.ac.uk

Cavendish Laboratory, University of Cambridge, UK

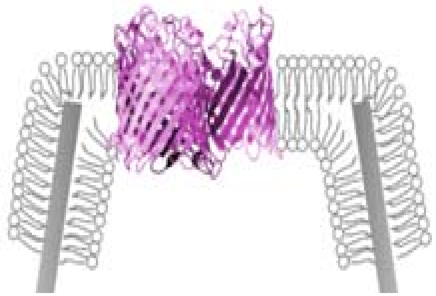
Physical principles governing membrane transport

DNA origami nanopores



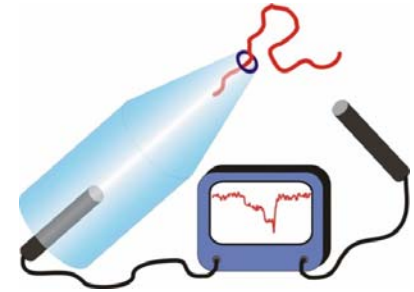
Bell *et al*. **Nano Lett**. 2012
Bell *et al*., **Lab on Chip** 2013
Hernandez-Ainsa *et al*. **ACS nano**, 2013

Protein nanopores



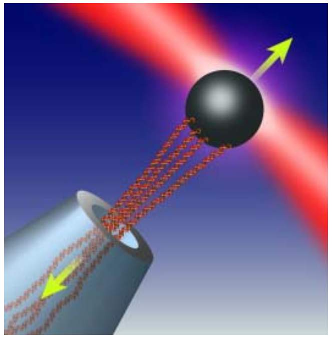
Gornall *et al.* **Nano Lett.**, 2011
Pagliara *et al*. **Lab Chip** 2011
Goepfrich *et al*., **Langmuir** 2013

Glass Nanopores



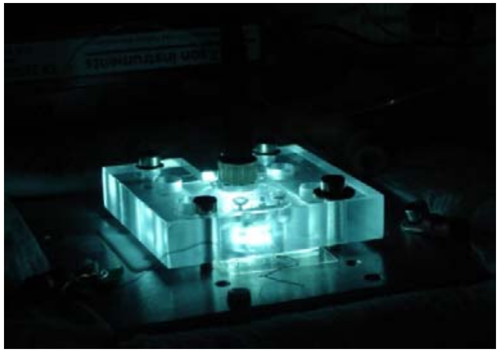
Steinbock *et al*. **Nano Lett.** 2010
Steinbock *et al.* **J. Phys. Cond.Mat.** 2011
Steinbock *et al.* **Electrophoresis**, 2012
Hernandez-Ainsa *et al*. **Analyst**, 2013

Optical tweezers & nanopores



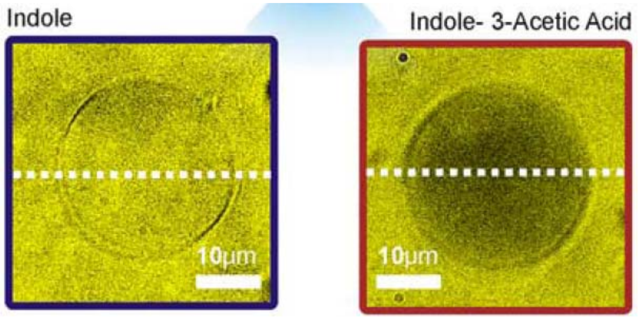
Keyser, **J. R. Soc. Interface**, 2011
Sturm&Otto *et al*., **Nature Comm.** 2013
Laohakunakorn *et al*., Nano Letters 2013

Fast particle tracking



Otto *et al*. **Rev. Sci. Instr**. 2008
Otto *et al*. **Optics Express** 2010
Otto *et al*. **J. Optics** 2011
Otto *et al*. **Rev. Sci. Instr**. 2011

Transport through lipid membranes



Wunderlich *et al*. **Biophys. J**. 2009
Pinero et *al*. **J. Bacteriology** 2011
Chimerel *et al*., **BBA Biomembranes** 2012
Chimerel *et al*., **ChemPhysChem**, 2013

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Foundation for Basic Research in Medicine

EPSRC
Engineering and Physical Sciences Research Council

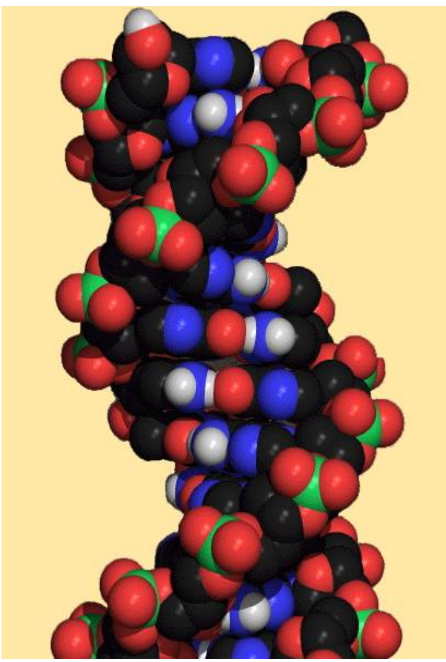
Nano Doctoral Training Centre Cambridge

Nanoscience E+ ERA-Net
Cambridge European Trust

Oxford NANOPORE Technologies

Single molecules: Length scales

- Typical diameter of DNA: 2 nm
- Typical dimension of a protein : ~10 nm
- Typical wavelength of visible light : 400 – 800 nm



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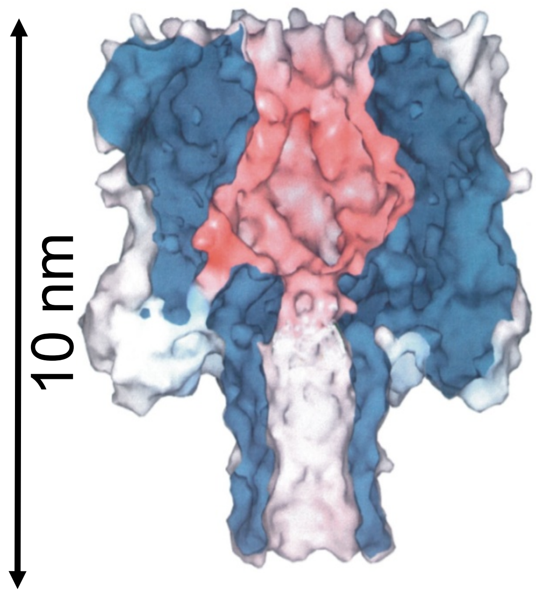
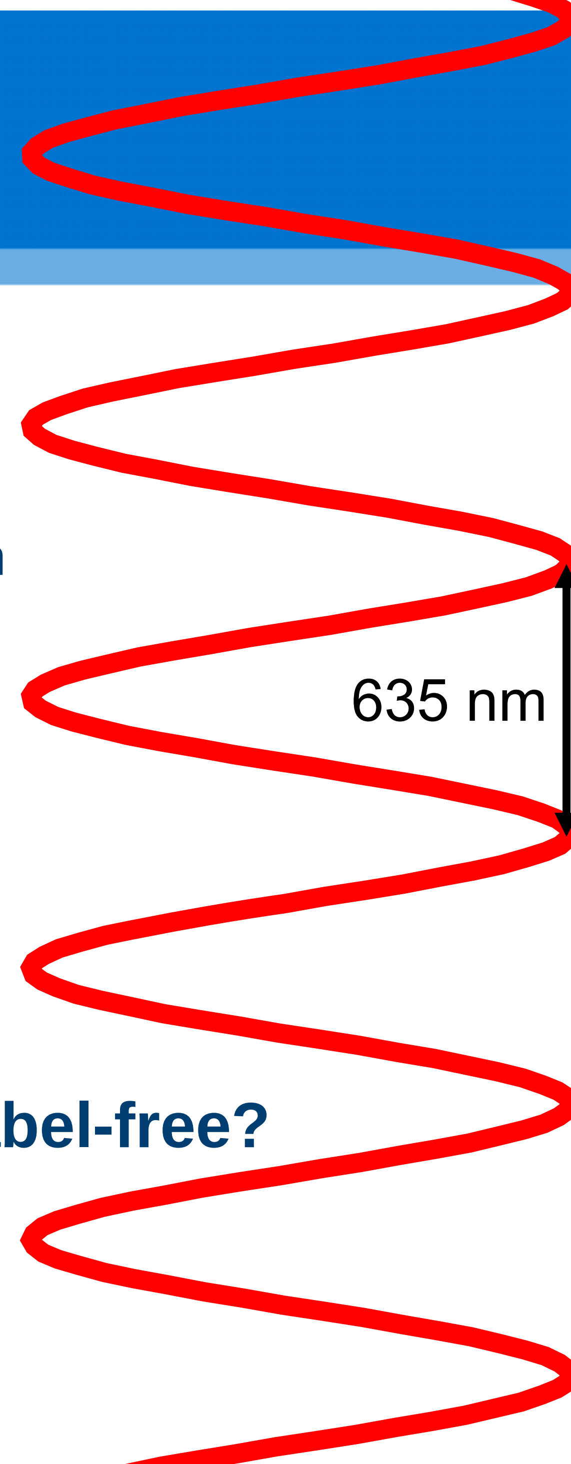
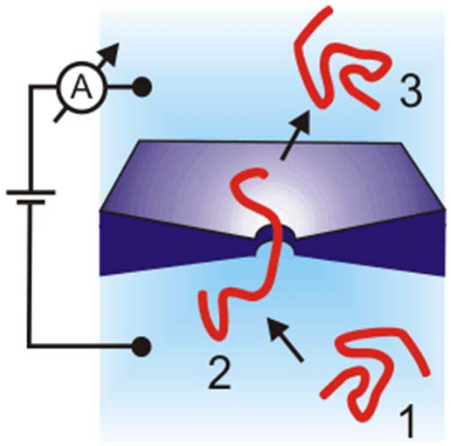


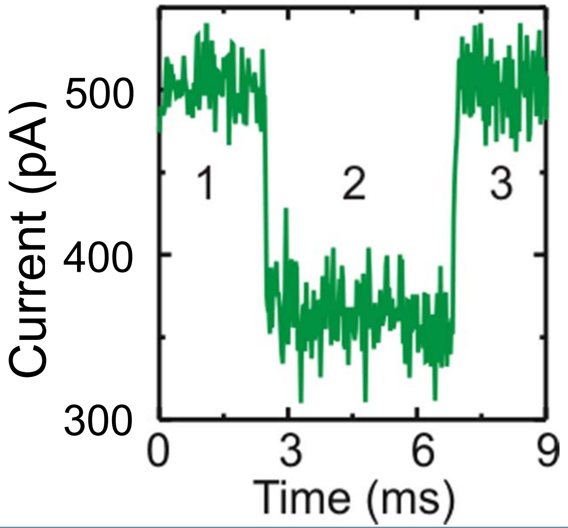
- label-free?



Molecular Coulter counters: nanopores

- A nanopore is a small hole with diameter <100 nm
- Electrical field in salt solutions is confined ⇔ nanopore is a spatial filter
- Possible applications for nanopores:
  Single molecule detectors
  Label-free detection
  Analysis of biopolymers
  Lab-on-a-chip
  Model systems for biological pores
  DNA Sequencing



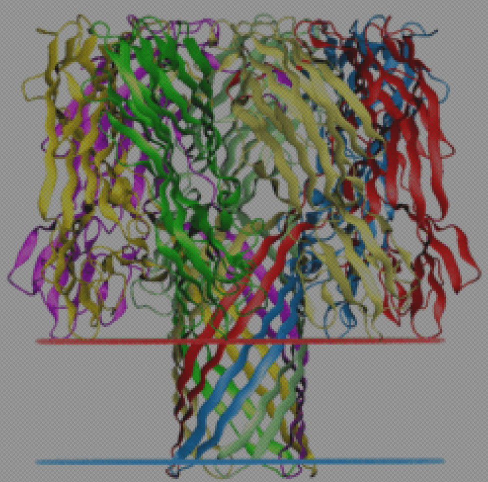


Since 1994 Bezrukov, Kasianowicz, Branton, Bayley, Deamer, Akeson, Meller…

Nanopore systems under active development

Biological nanopores

Membrane proteins reconstituted into artificial lipid bilayers e.g. α-Haemolysin from *Staphlococcus Aureus*

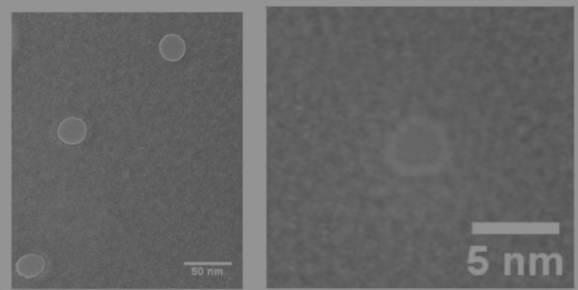


Deamer, Church, Bayley, Bezrukov, Branton, Akeson, Meller, …

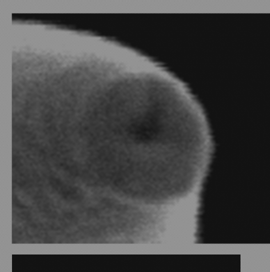
DNA sensing since 1996

Solid state nanopores

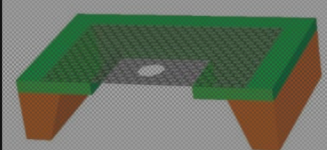
Use TEM to sputter away atoms from a SiN or graphene membrane, glass nanopores



Glass



Graphene



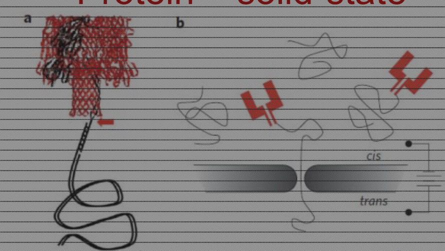
Golovchenko, Dekker, Timp, Klenerman, White, Drndic, Keyser, …

DNA sensing since 2001

Hybrid nanopores

Combinations of protein or DNA origami nanopores with solid-state nanopores

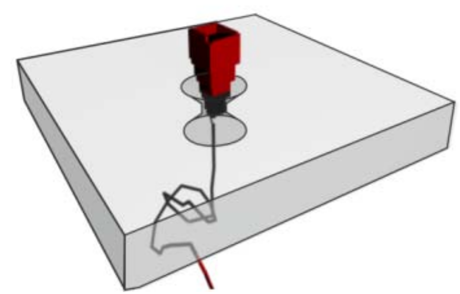
Protein + solid-state



Dekker & Bayley, *et al.*, …

DNA sensing since 2010

DNA origami + solid-state



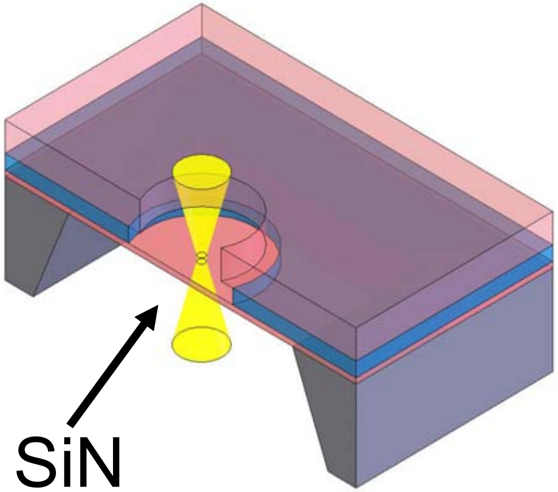
Keyser & Liedl, *et al.*, …

Rant & Dietz, *et al.*, …

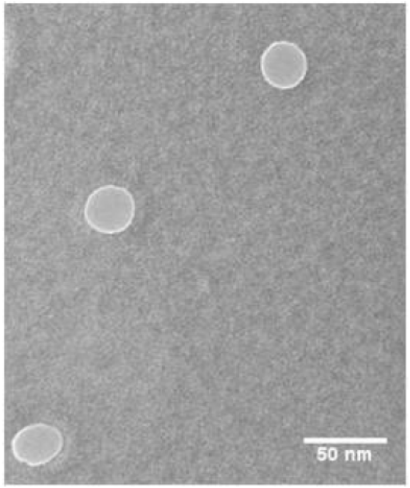
DNA sensing since 2011

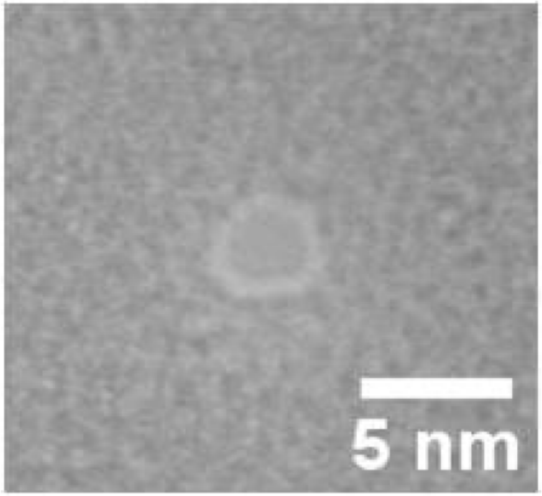
Solid-State Nanopores

Drilling & sculpting nanopores with an electron beam



N. Bell & C. Ducati, Cambridge

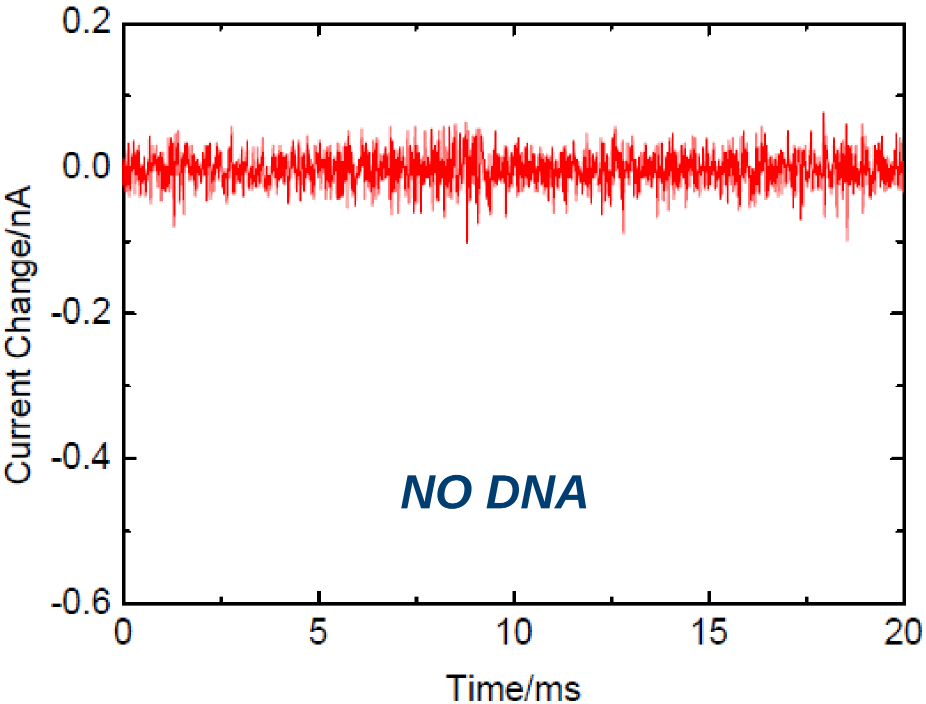


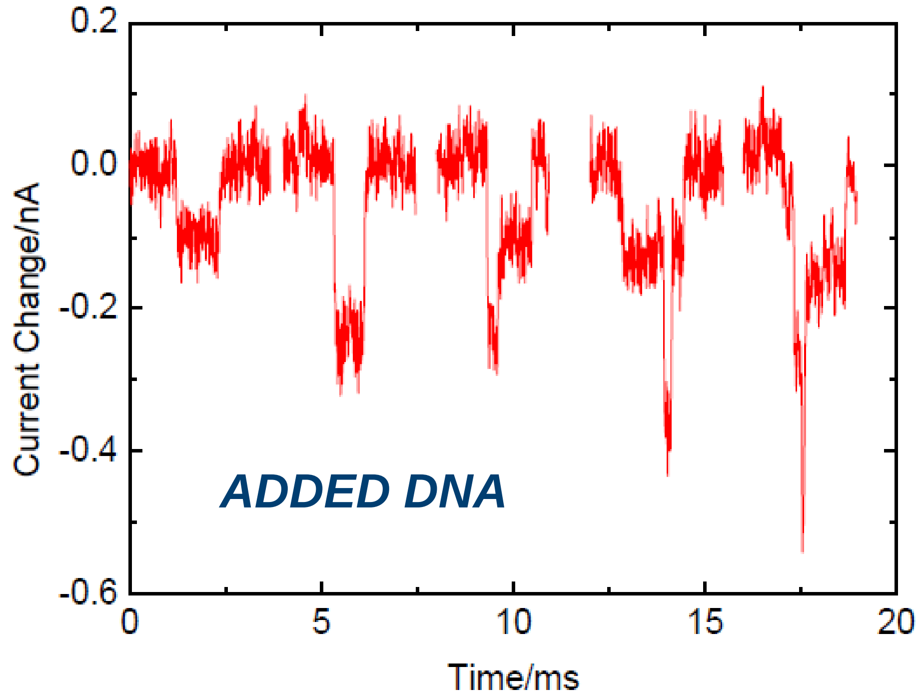


- diameter: variable
- very robust, pH, solvents, …
- Problem: no control on atomic level
- ***OUR SOLUTION: DNA origami***

Golovchenko Group (2001) Dekker Group (2003) Timp Group (2004) ... and many more now

DNA folding can be analysed





- Analysing DNA structure is possible
- Folding is indicated by ionic current levels

Objective for nanopore fabrication

- Single molecule sensing with solid-state nanopores works for:
  DNA, DNA-protein complexes, RNA, proteins etc.

BUT:

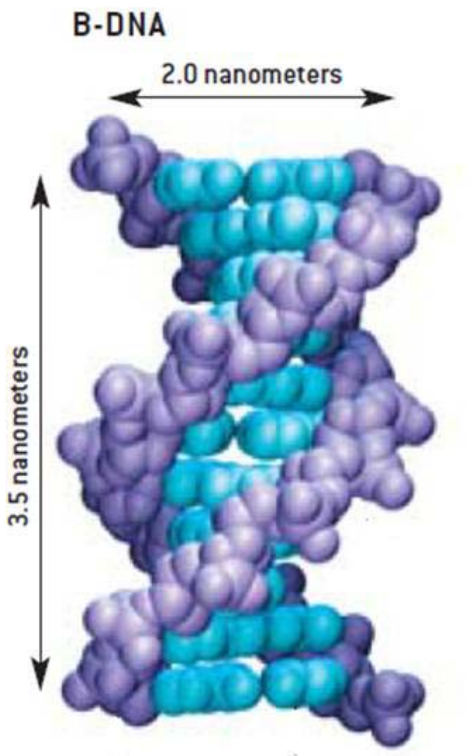
- Ideally we would like to control the

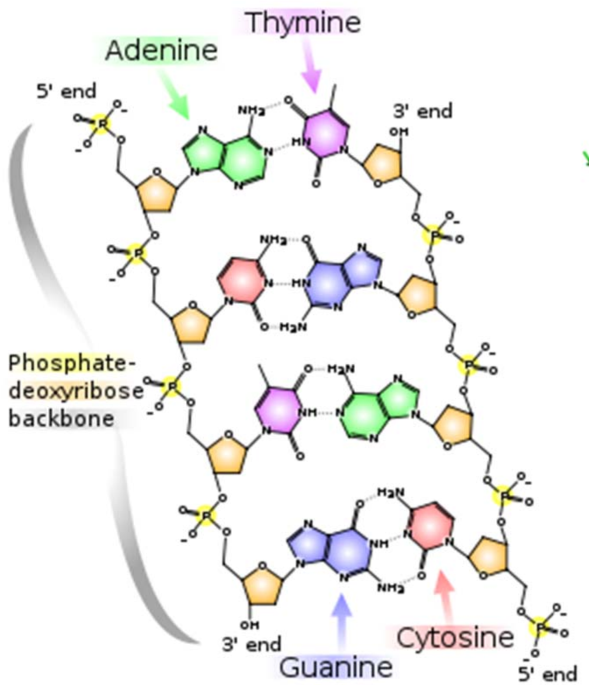
surface properties and shape

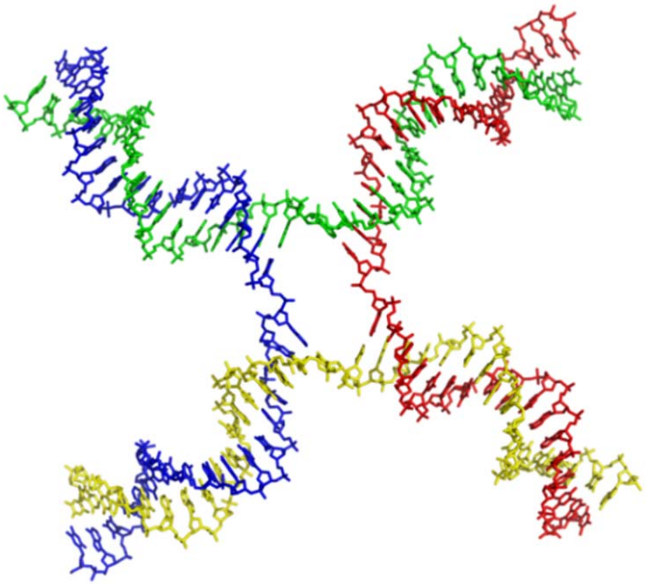
on molecular (atomic) level

to increase the specificity and sensitivity

Structural DNA nanotechnology



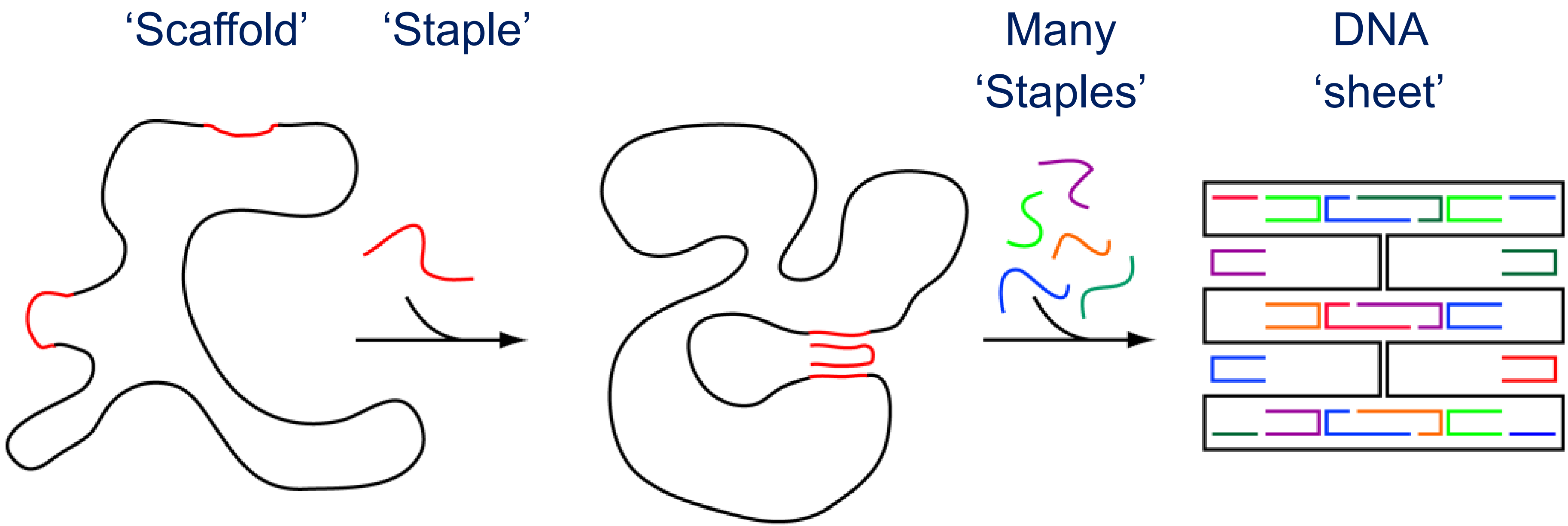




- DNA can be arranged into diverse structures by harnessing basepairing

Seeman, N.C. *Scientific American* **290**, 64-75 (2004).

DNA origami self-assembly



- Fold long single strand DNA using short 'staple' strands into any shape
- Molecular self-assembly: One pot mixture heated to 80°C and cooled to room temperature over several days

Rothemund, P.W.K. *Nature* **440**, 297-302(2006).
Animation – Shawn Douglas, Wyss Institute

DNA origami self-assembly

- Fold single stranded DNA using short ‘staple’ strands into any shape
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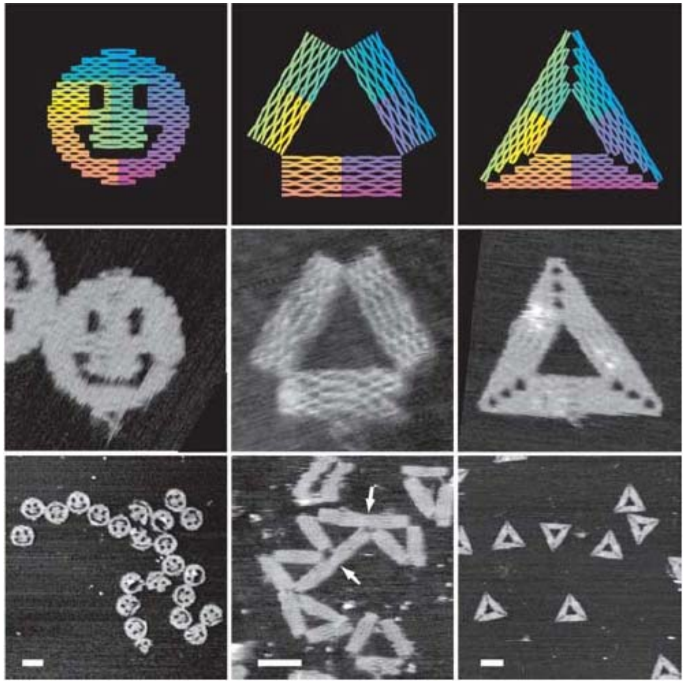
Rothemund, P.W.K. *Nature* **440**, 297-302(2006).
Animation – Shawn Douglas, Wyss Institute

DNA origami self-assembly



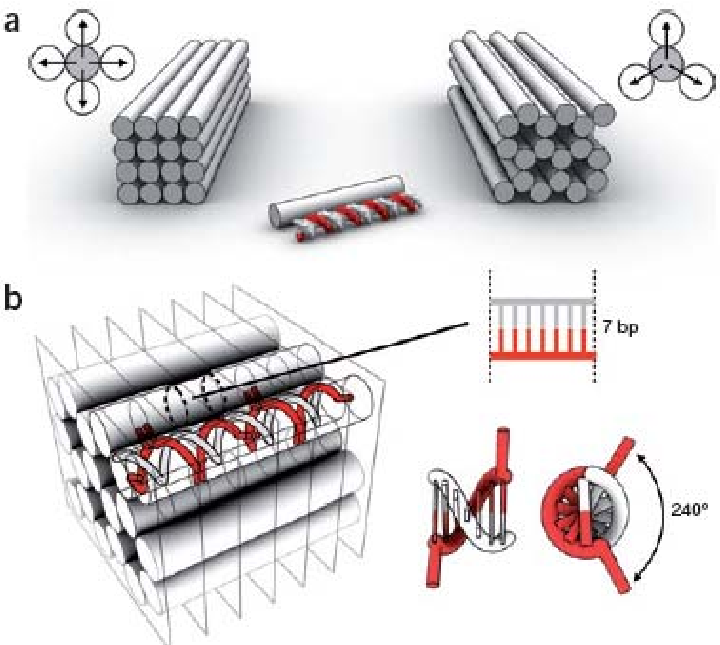
Scale bars = 100nm

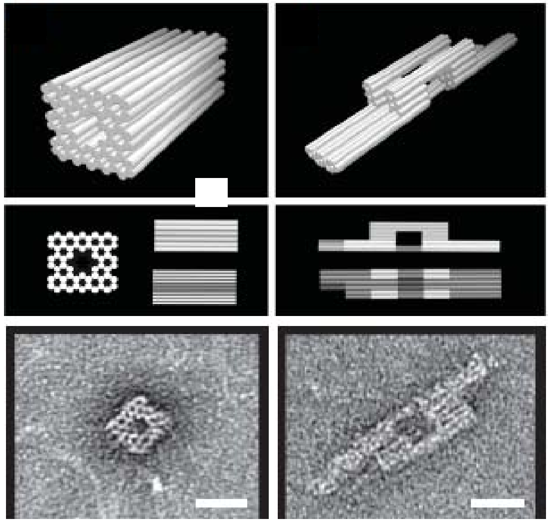
- Fold single stranded DNA using short ‘staple’ strands into any shape

- Molecular self-assembly: One pot mixture heated to 80°C and cooled to room temperature over several days

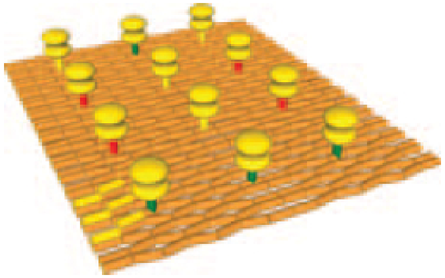
Rothemund, P.W.K. *Nature* **440**, 297-302(2006).

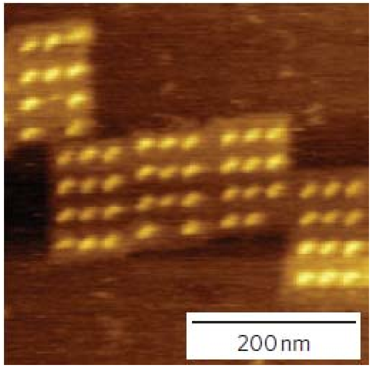
DNA origami in three dimensions





Scale bars = 20nm





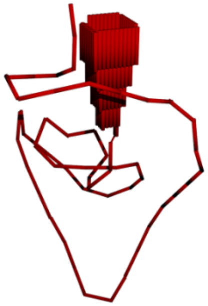
- Three dimensional structures can be made by extending the scaffold through hexagonal or square lattices

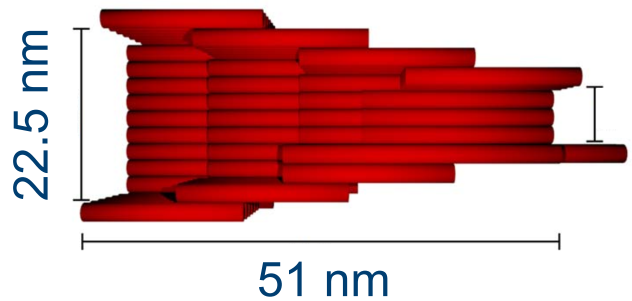
- Staple strands can be modified for site specific attachments

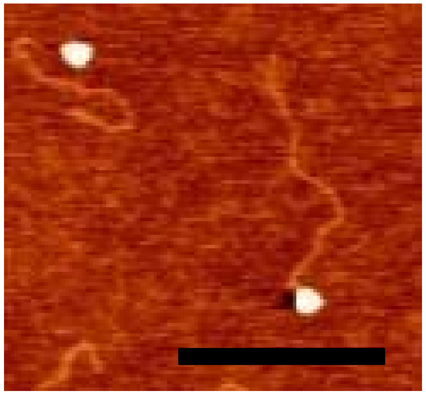
Voigt, N.V. *et al. Nature Nanotechnology* **5**, 200-3 (2010).
Castro, C., *et al. Nature Methods* **8**, 221-229 (2011).

First DNA origami nanopore

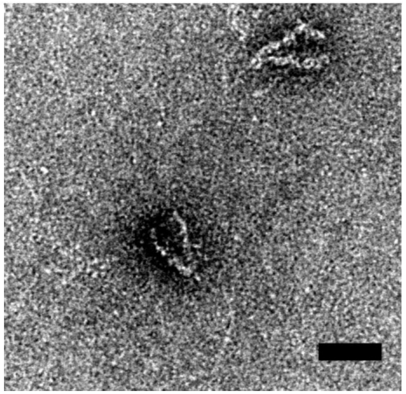
- 3D DNA origami nanopore with a 7.5nm central constriction designed to fit into a solid state nanopore with diameters 10-20nm



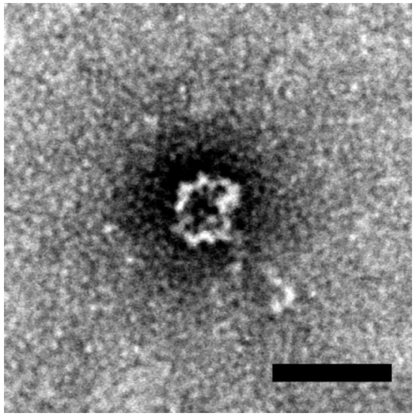




Scale bar=500 nm



Scale bar=50 nm

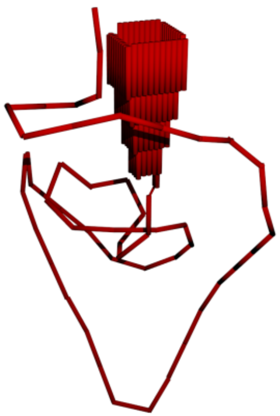


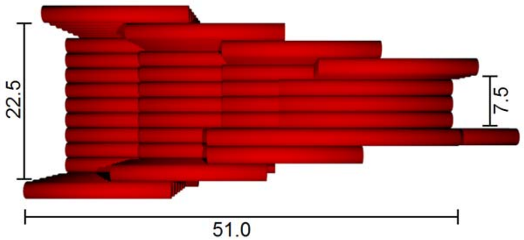
Scale bar=50 nm

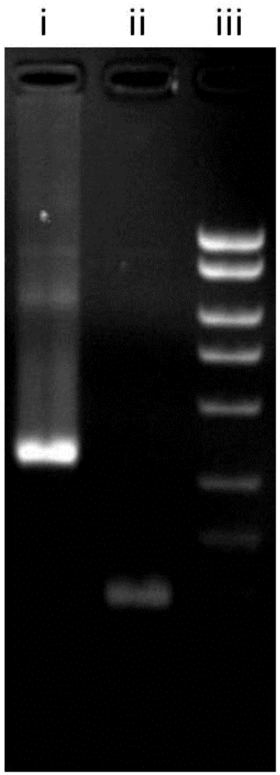
N. Bell *et al.*, Nano Letters (2012)

DNA origami nanopore

- We have designed a 3D DNA origami nanopore with a narrowest constriction of 7.5nm
- Agarose gel electrophoresis shows a well defined band containing the correctly folded structures at 14mM $MgCl_2$
- DNA construct is stable at 1M KCl



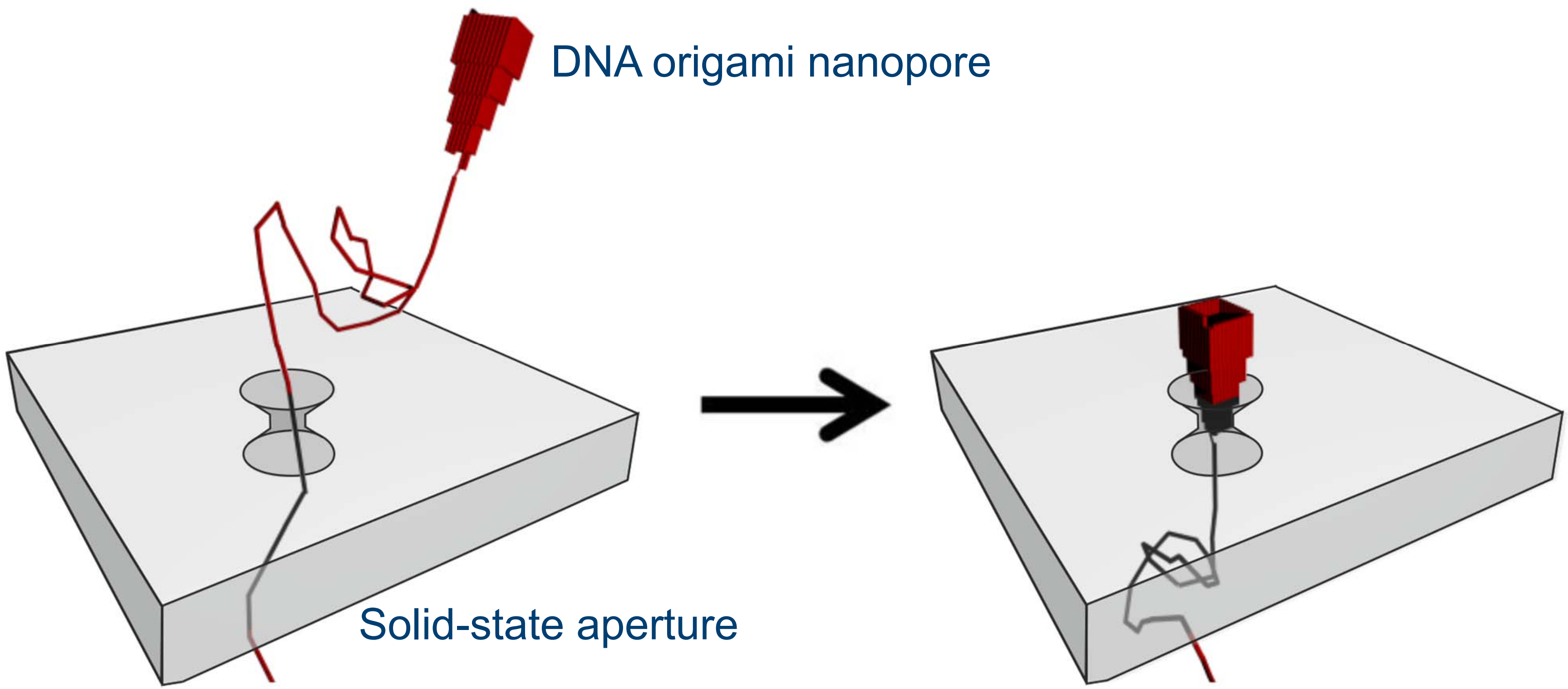




Lane i = DNA origami nanopore
Lane ii = M13 ssDNA
Lane iii = DNA ladder

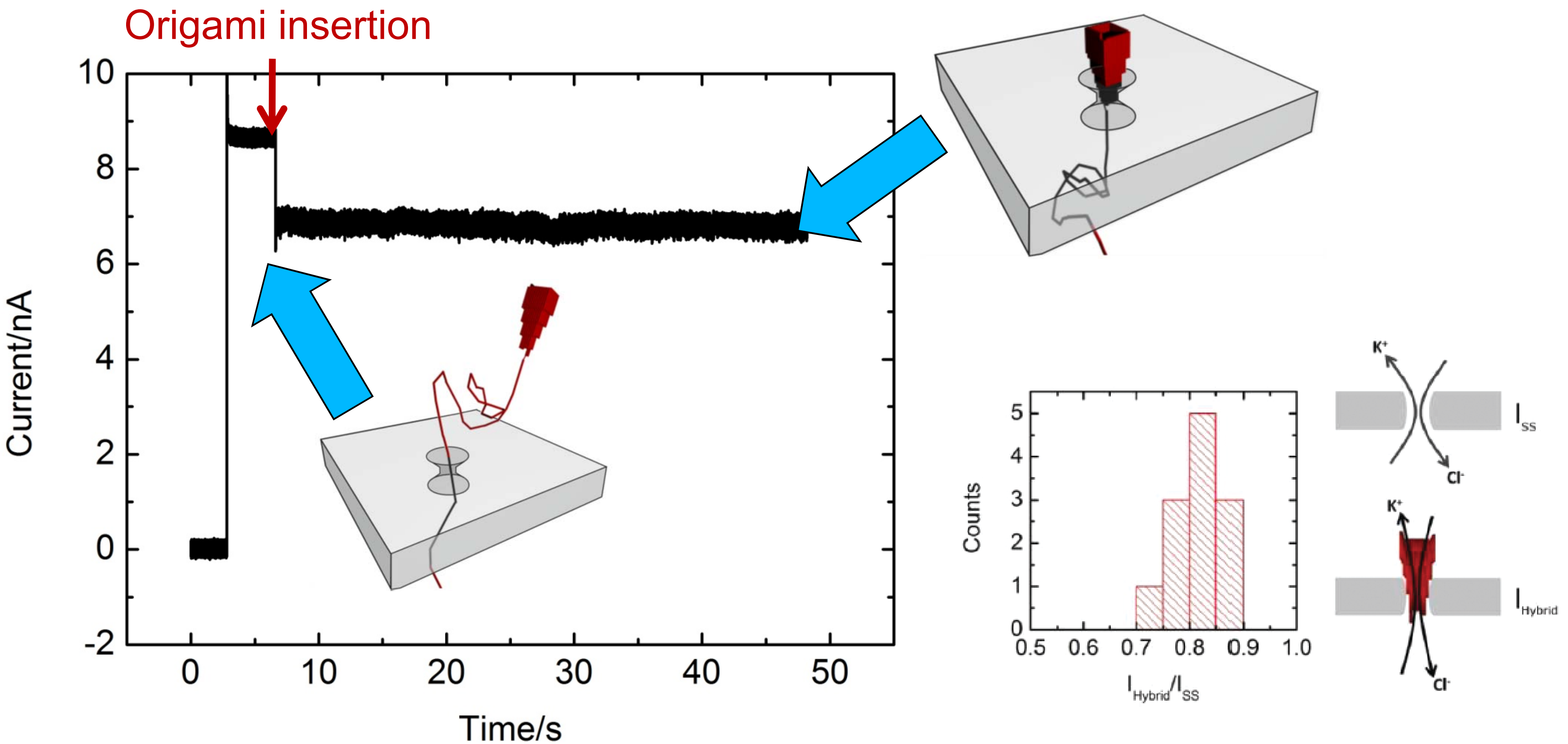
N. Bell *et al.*, Nano Letters (2012) (published online 23/12/2011)

Voltage-driven assembly of a DNA origami nanopore



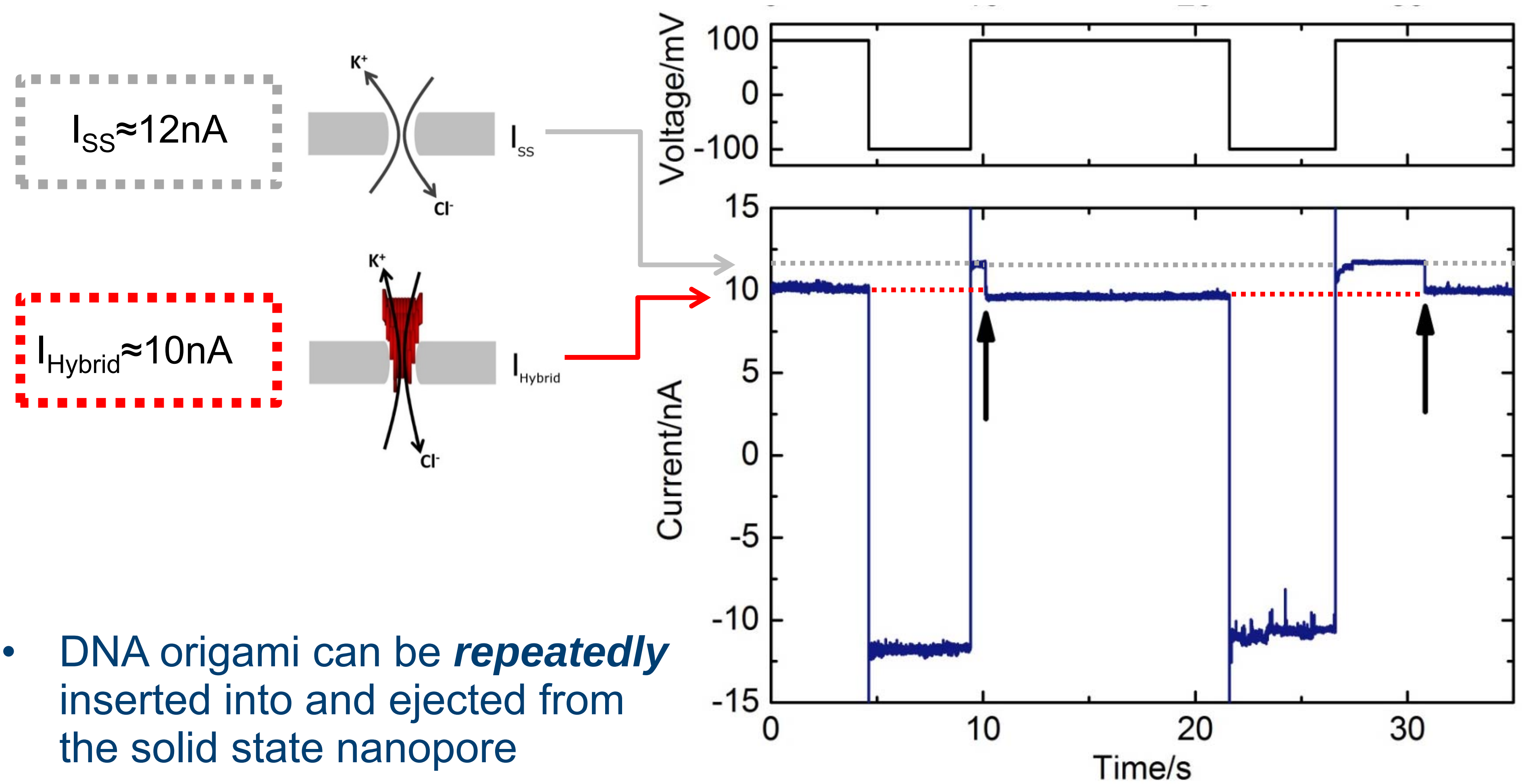
N. Bell *et al.*, Nano Letters (2012) (published online 23/12/2011)

Insertion of DNA Origami into a solid-state hole



- For each run add 5μL of origami solution (from gel extraction) to 5μL 2M KCl, 0.5xTBE. Final solution of 1M KCl, 0.5xTBE, 5.5mM $MgCl_2$, pH 8.0.

Repeated Assembly of DNA origami hybrid pore

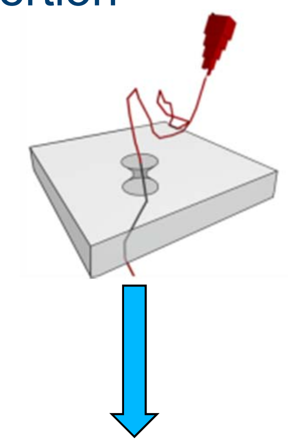


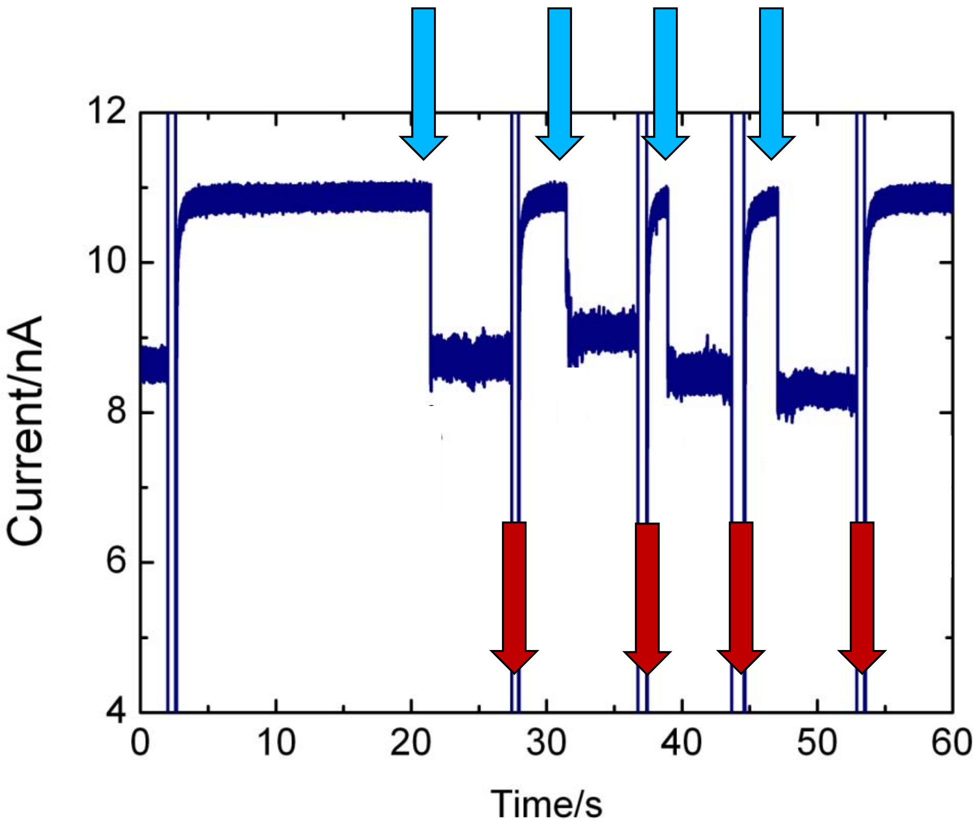
- DNA origami can be ***repeatedly*** inserted into and ejected from the solid state nanopore

N. Bell *et al.*, Nano Letters (2012) (published online 20/12/2011)

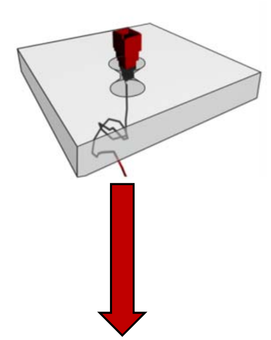
Fast cycling of DNA origami nanopores

Insertion





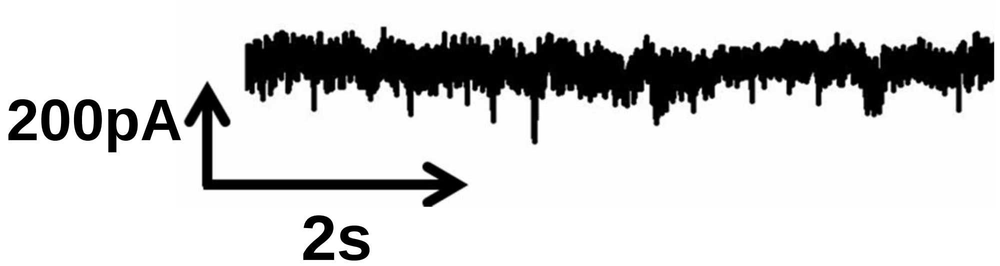
Pull through

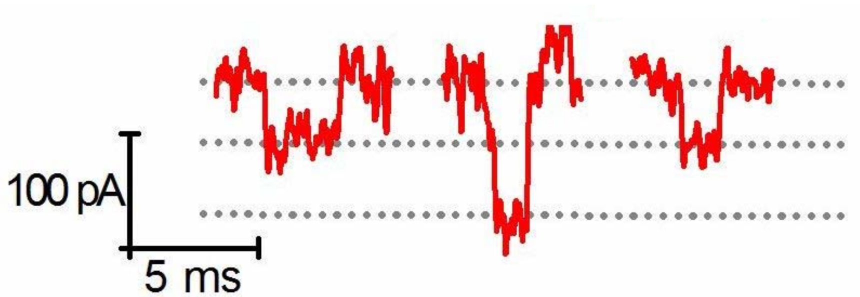


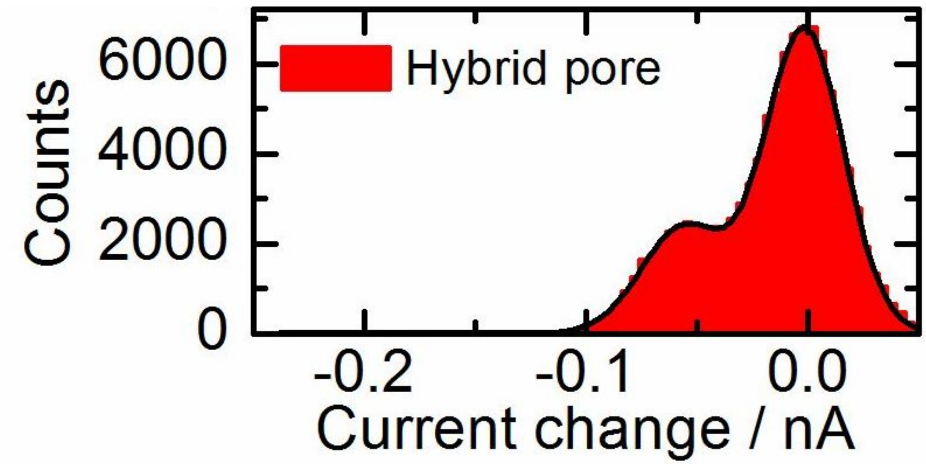
- Many pores can be cycled in a few seconds through the solid state pore by applying >1V and pulling the DNA origami through the nanopore

DNA detection with DNA origami nanopore

- λ-DNA translocations after formation of hybrid nanopore







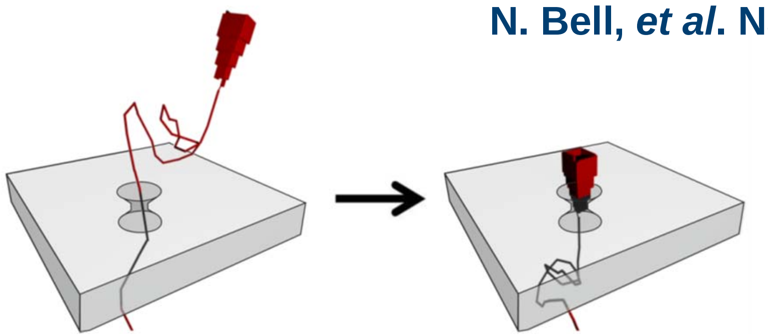
N. Bell *et al.*, Nano Letters (2012) (published online 20/12/2011)

DNA Origami Nanopores

with

N. Bell, M. Ablay, C. Engst, G. Divitini, C. Ducati, T Liedl

N. Bell, *et al*. Nano Letters 12, 512 (2012)



Highlighted in ***Nature Materials*** Feb. 2012

nature materials

Designer nanopores

Christian Martin

Nature Materials **11**, 95 (2012) | doi:10.1038/nmat3243
Published online 24 January 2012

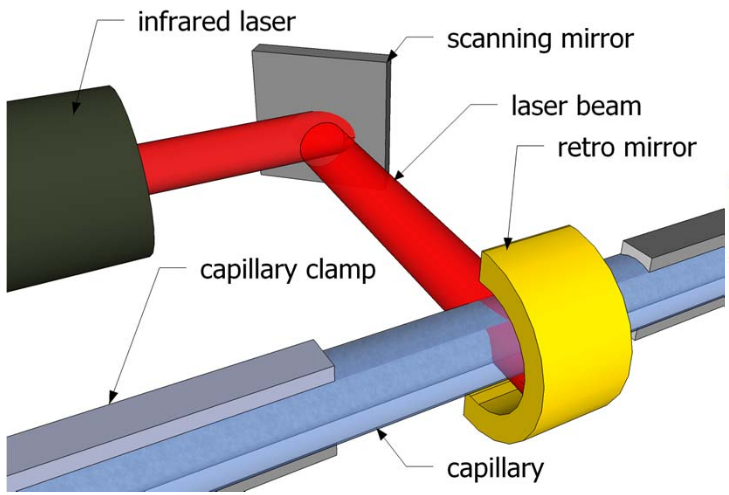
Nano Lett. **12**, 512–517 (2012)

nature nanotechnology

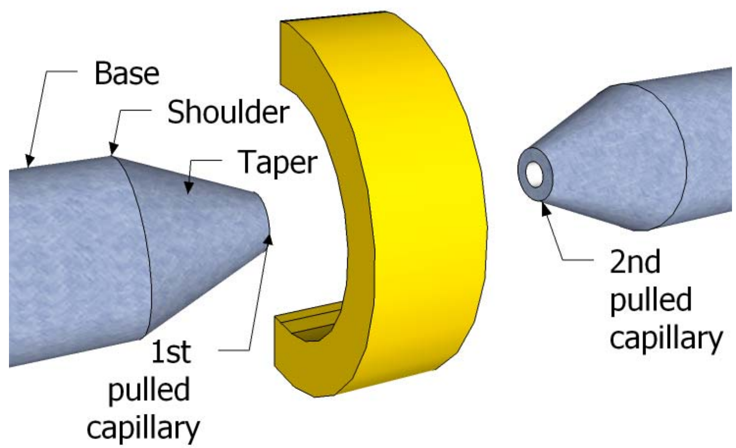
Highlighted in ***Nature Nanotechnology*** Feb. 2012
O. Vaughn, *Nanopores: Built with Origami*

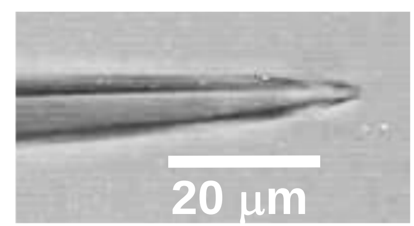
Fabrication of Glass Nanocapillaries

1. Glass capillary placed in puller
2. Laser heats up capillary and force applied to both sides: glass softens and shrinks
3. Strong pull separates glass in two parts



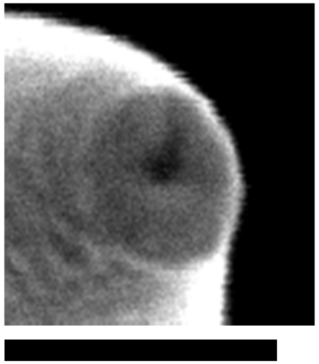
Sutter P-2000

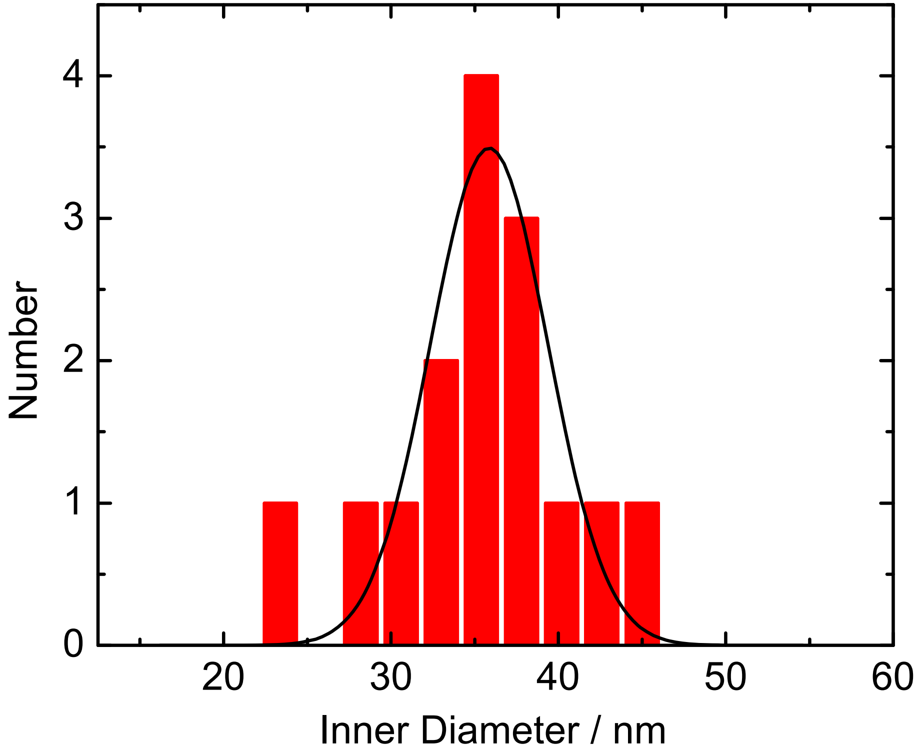


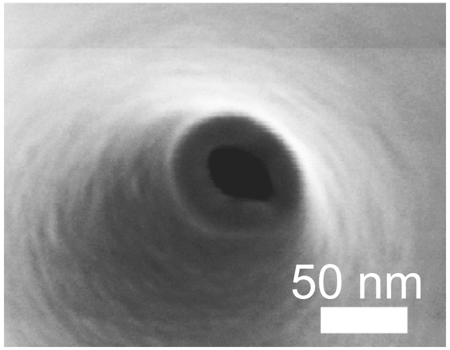


Diameters of Nanocapillaries

~20 nm diameter

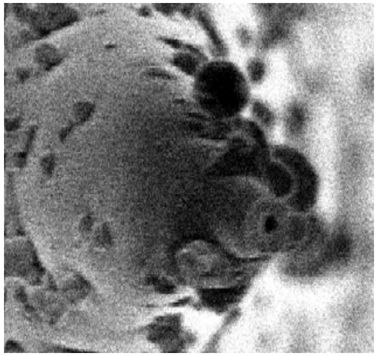






- Optimization of pulling parameters for nanocapillary diameters down to ~20 nm

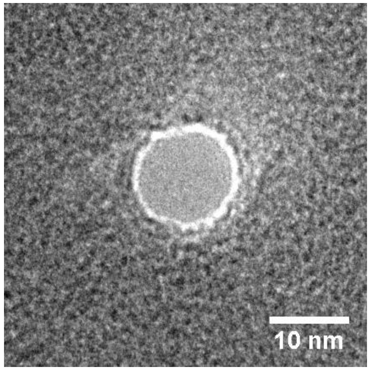
~40 nm diameter



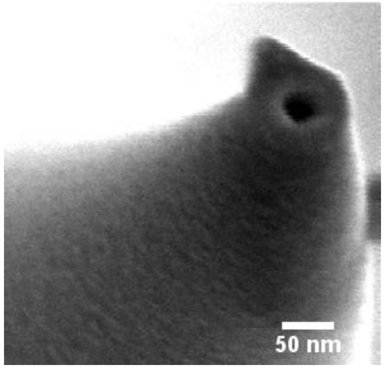
e.g.: Klenerman *et al*. Biophys. J. (2004), PRL(2007), White *et al.* JACS(2008), Steinbock, *et al.* Nano Lett.(2010)

Adapting DNA origami nanopores

SiN nanopores



Glass nanopores

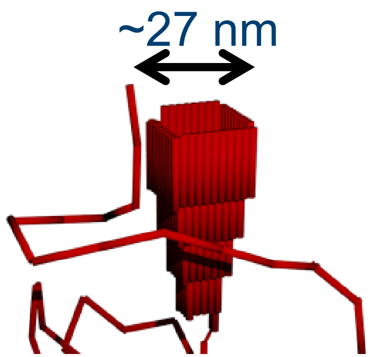


However…

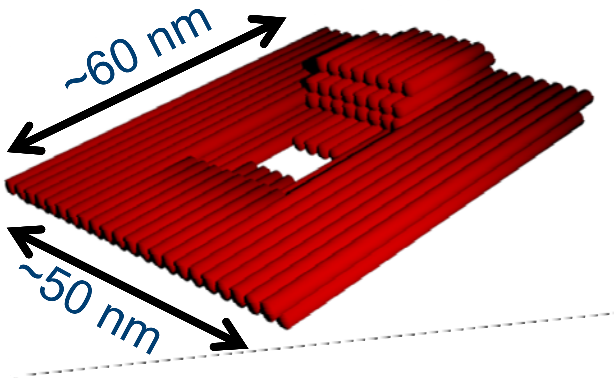
Fabrication of SiN nanopores is challenging and requires use of TEM to ablate the surface

Nanopores from pulled glass capillaries represent a good alternative due to their lower cost and fast preparation time

'3D' DNA origami nanopore



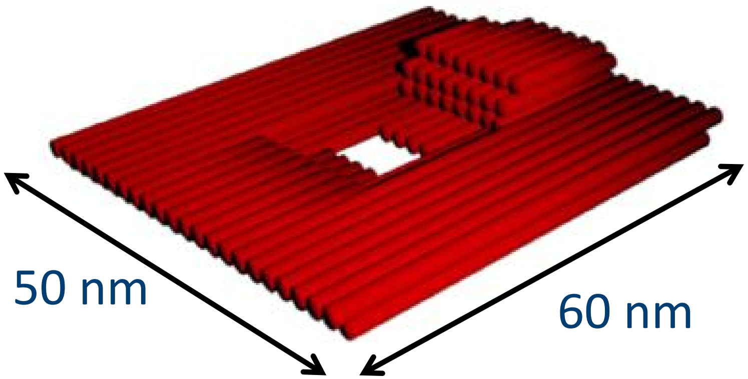
'2D' DNA origami nanopore



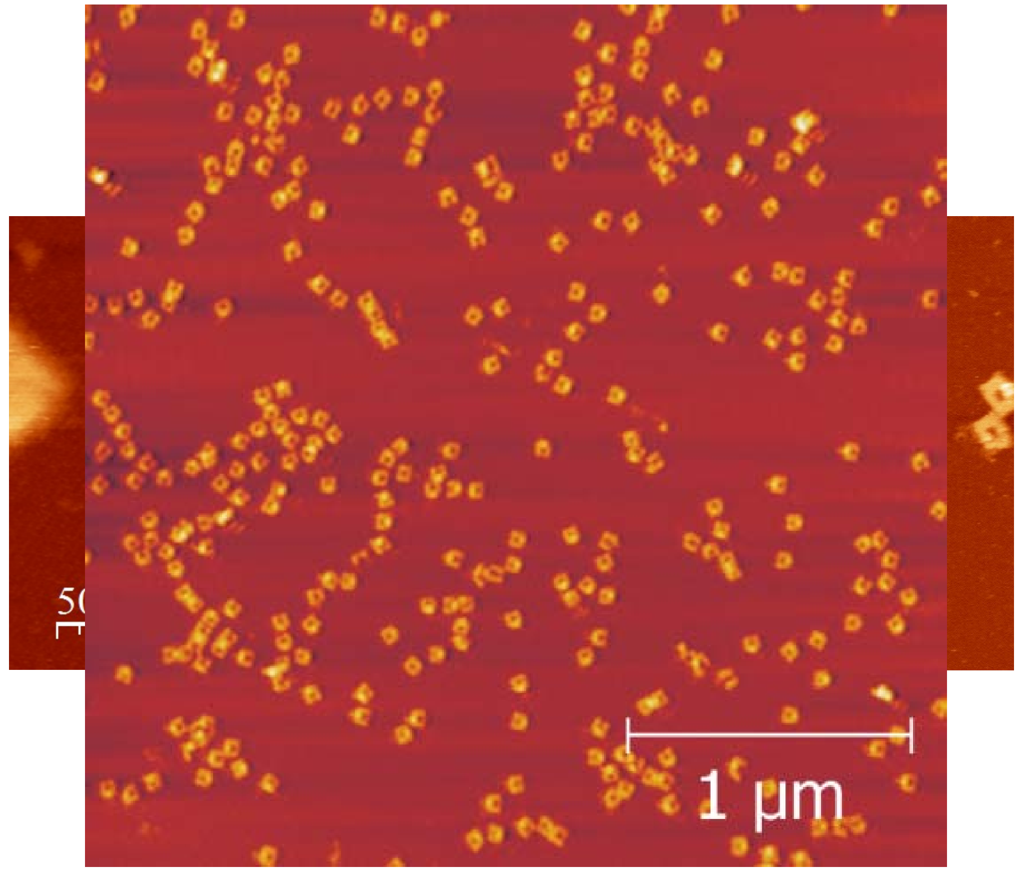
L. J. Steinbock et al. *Nano Lett* .**2010**, *10*, 2493

Flat origami design for nanocapillaries

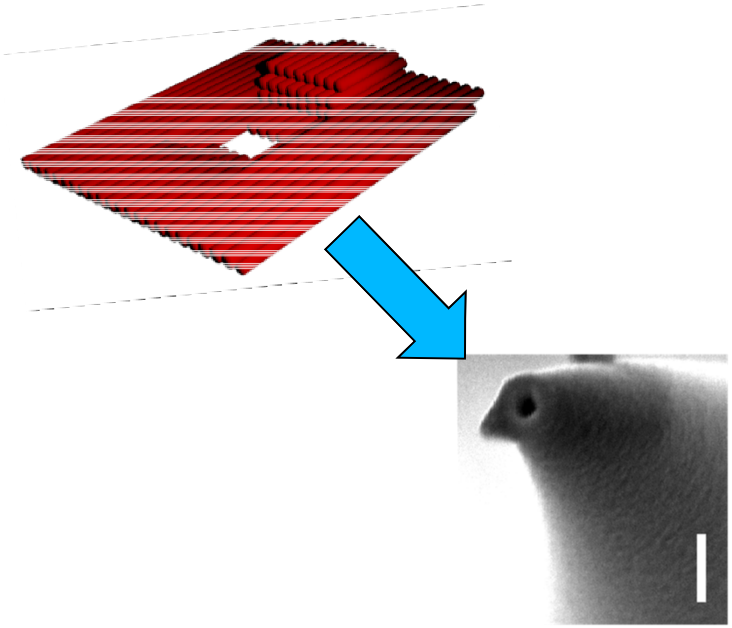
Flat design

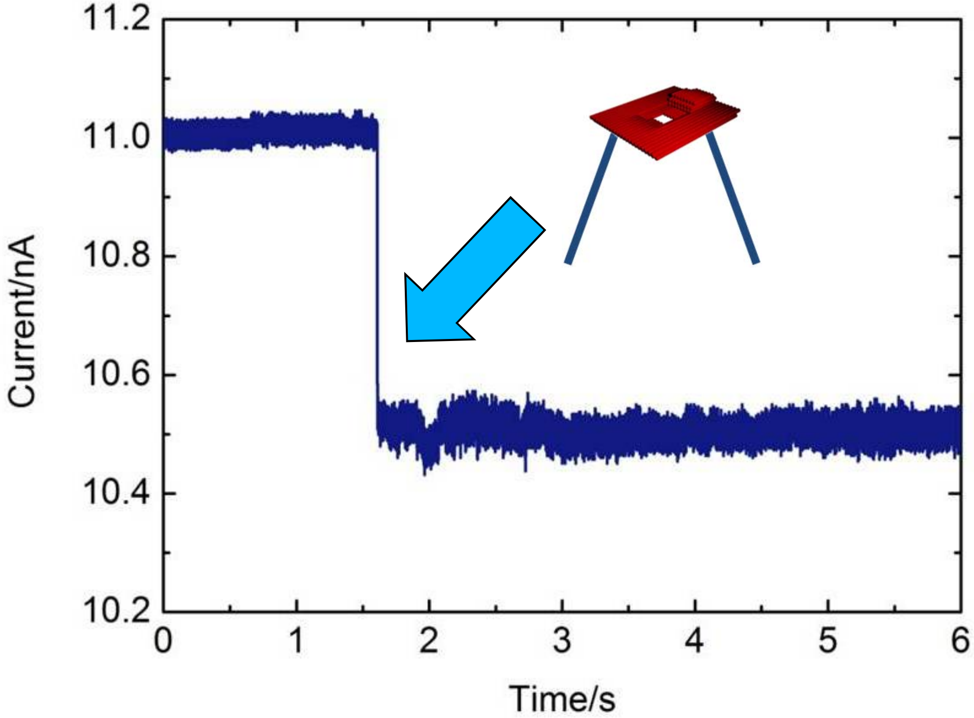


AFM



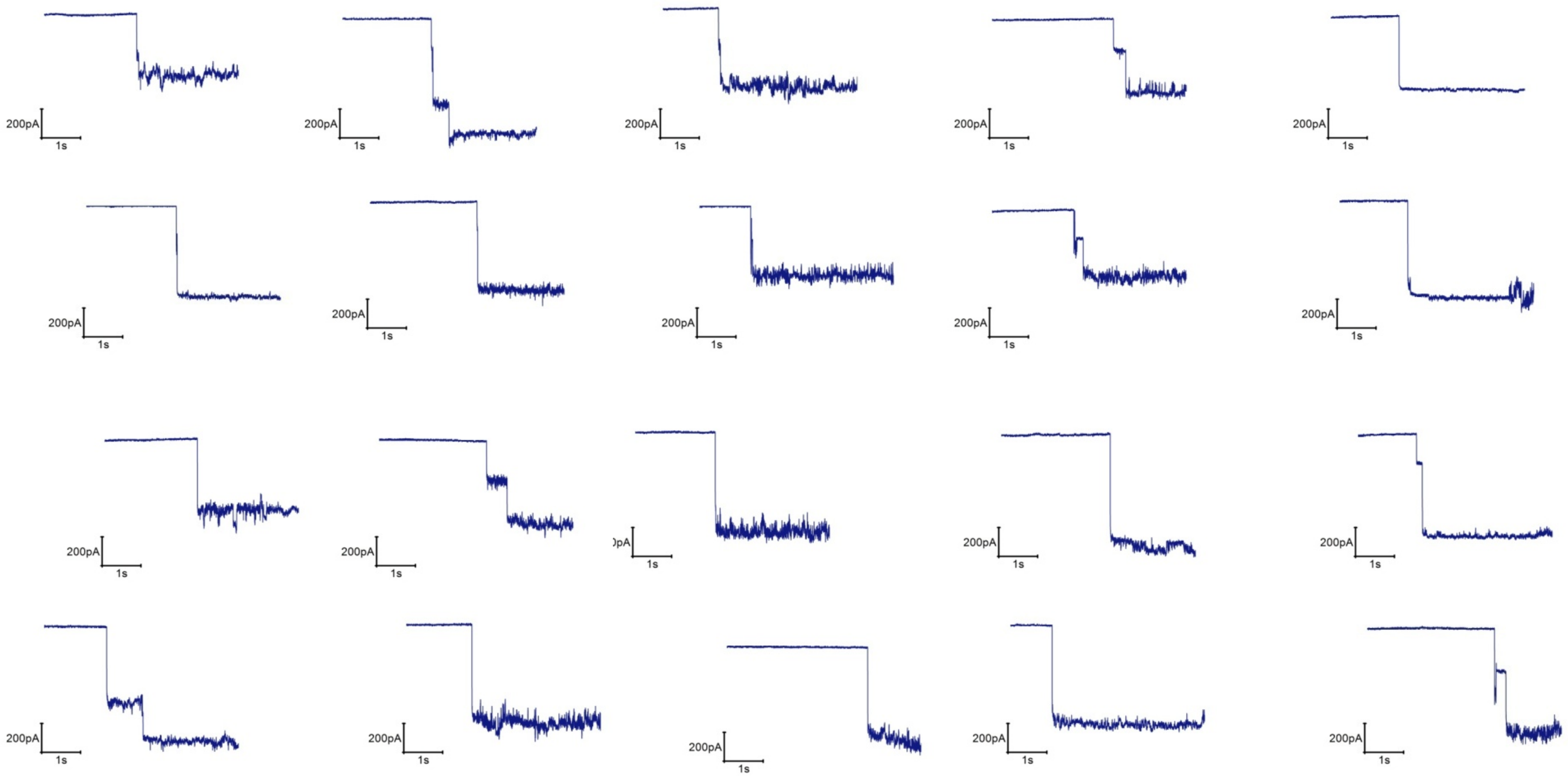
DNA origami combined with nanocapillaries





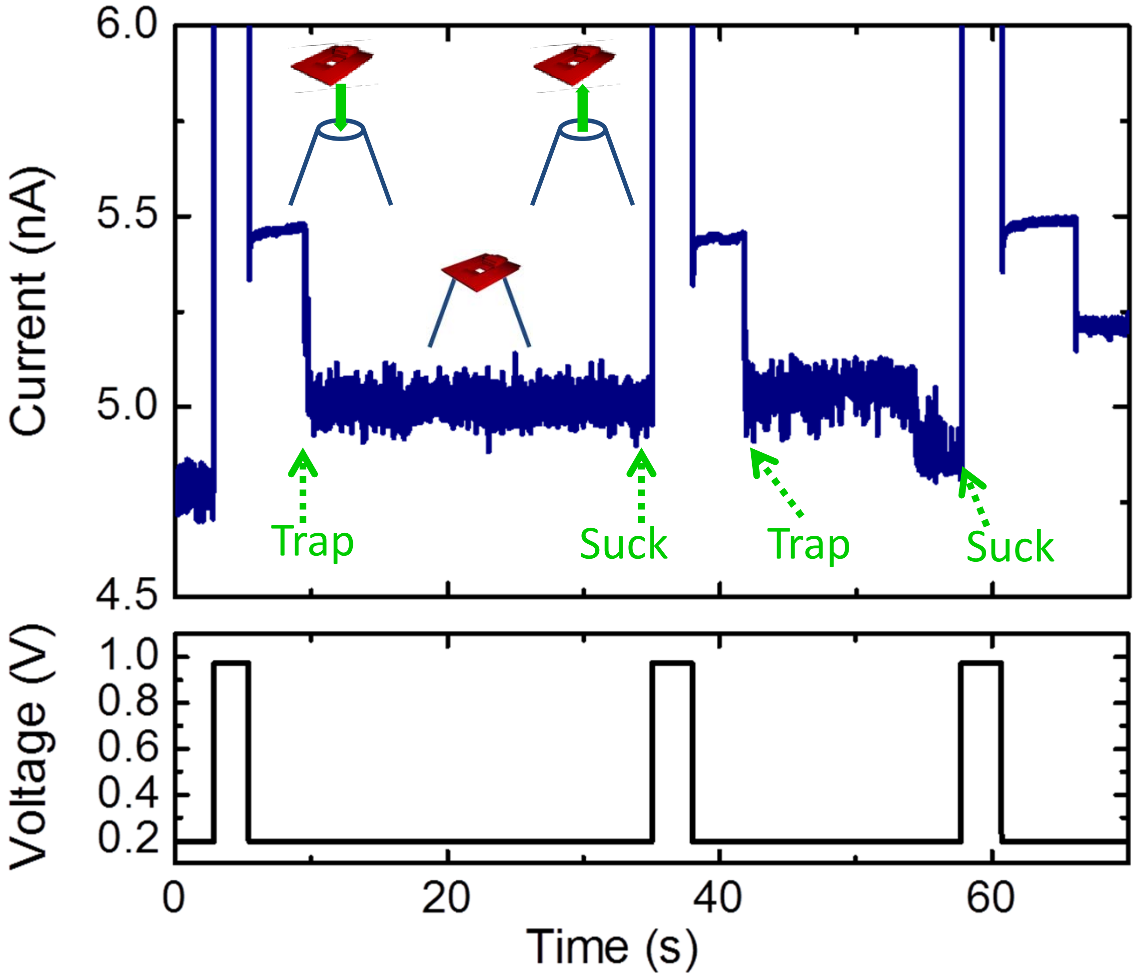
- Successful assembly of DNA origami nanopores on nanocapillaries
- Greatly simplified approach to fabrication and measurement process

Hybrid nanocapillary-origami nanopores



- Successful assembly of DNA origami nanopores on nanocapillaries, **again, ... and again, ... and again**

Flat origami trapping on nanocapillaries



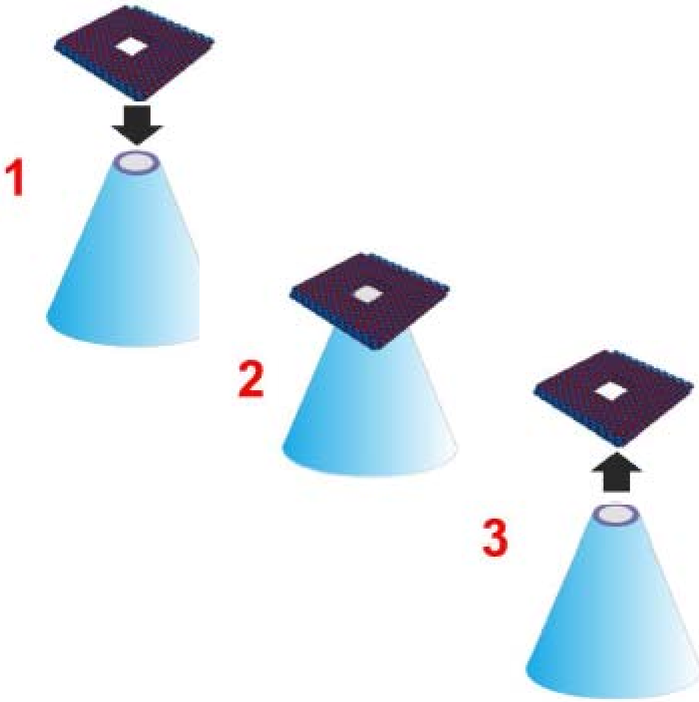
Flat origami is trapped upon applying 0.2 V

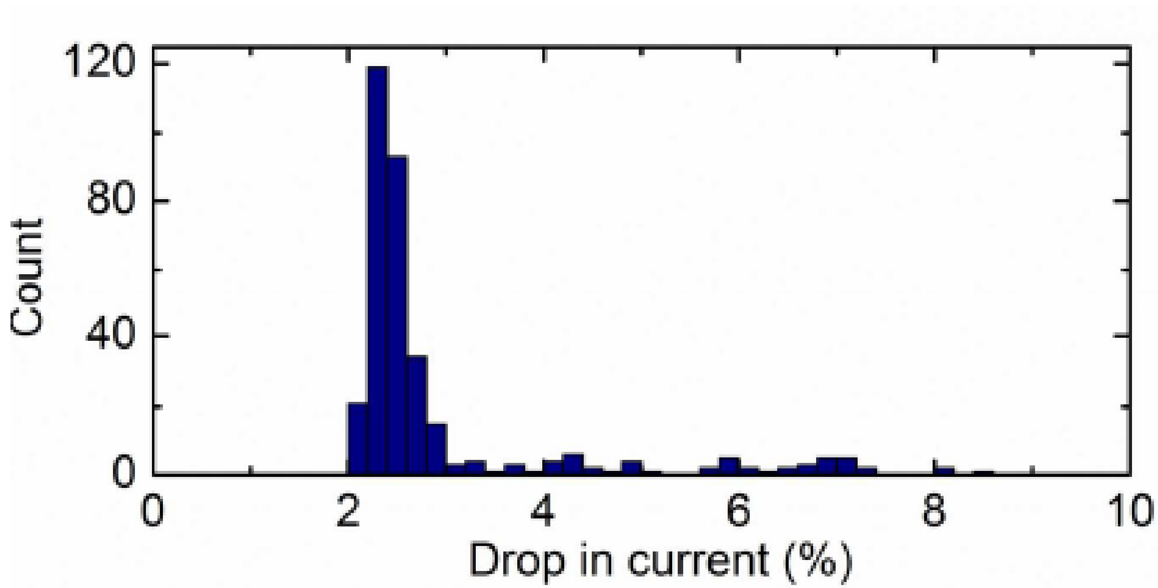
Current noise increases when the origami is attached

Flat origami can be also sucked by applying 1V

Trap and suction can be reversibly performed more than hundred times

Hybrid nanocapillary-origami nanopores

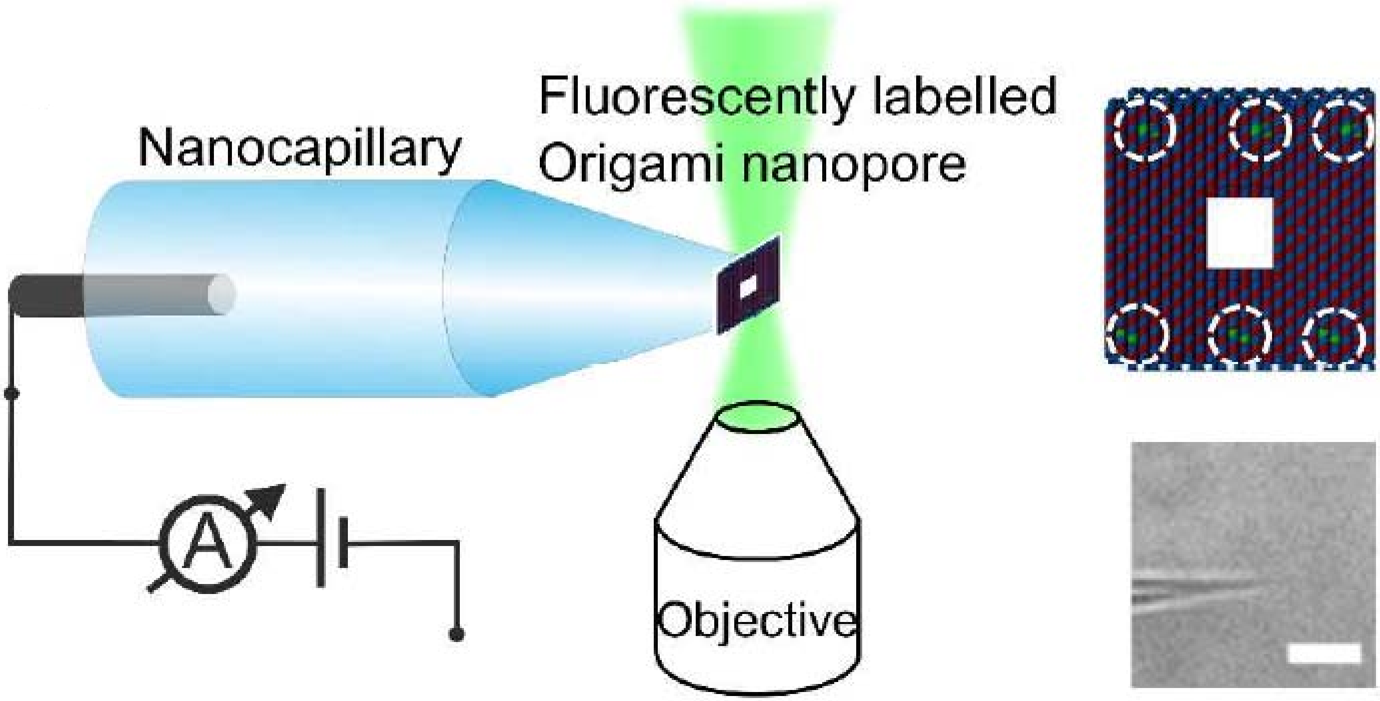




- Repeating the experiments 100s of times allows to resolve details like multiple insertions

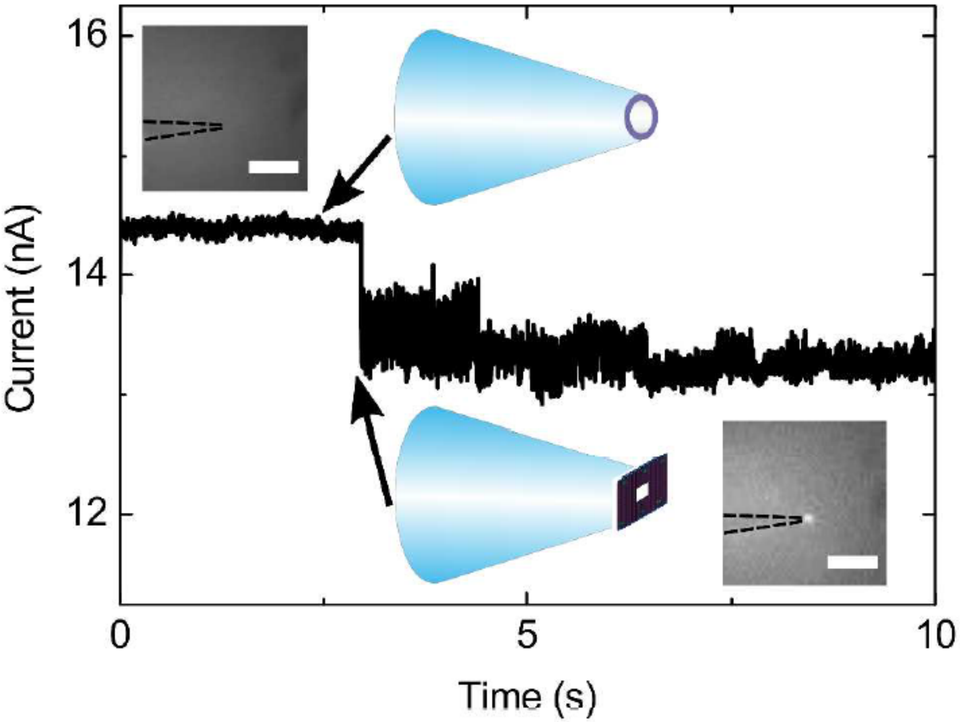
Hernandez Ainsa, et al., ACS nano.**2013**, to appear

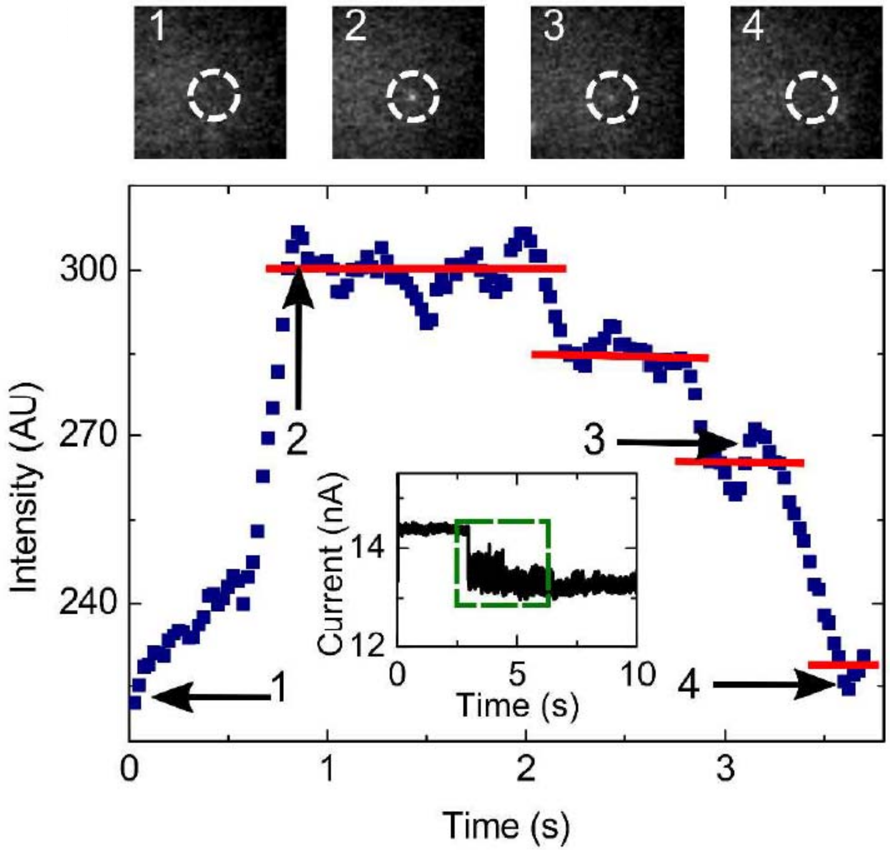
Simultaneous current and fluorescence measurements



- Prove DNA origami formation with fluorescence microscopy and simultaneous current measurements link

Simultaneous current and fluorescence measurements



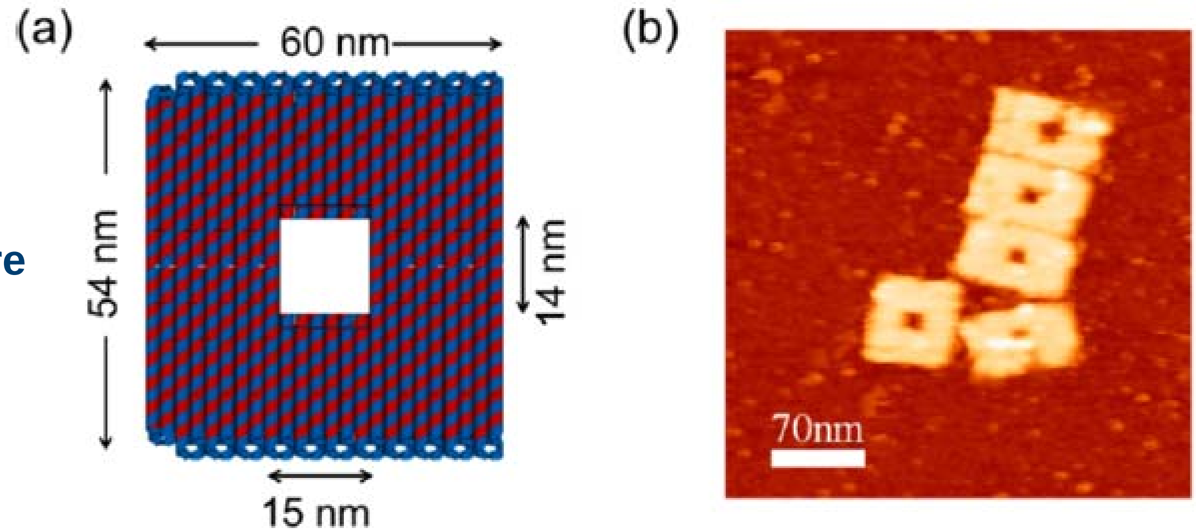


- Step-wise bleaching provides strong indication for single DNA origami

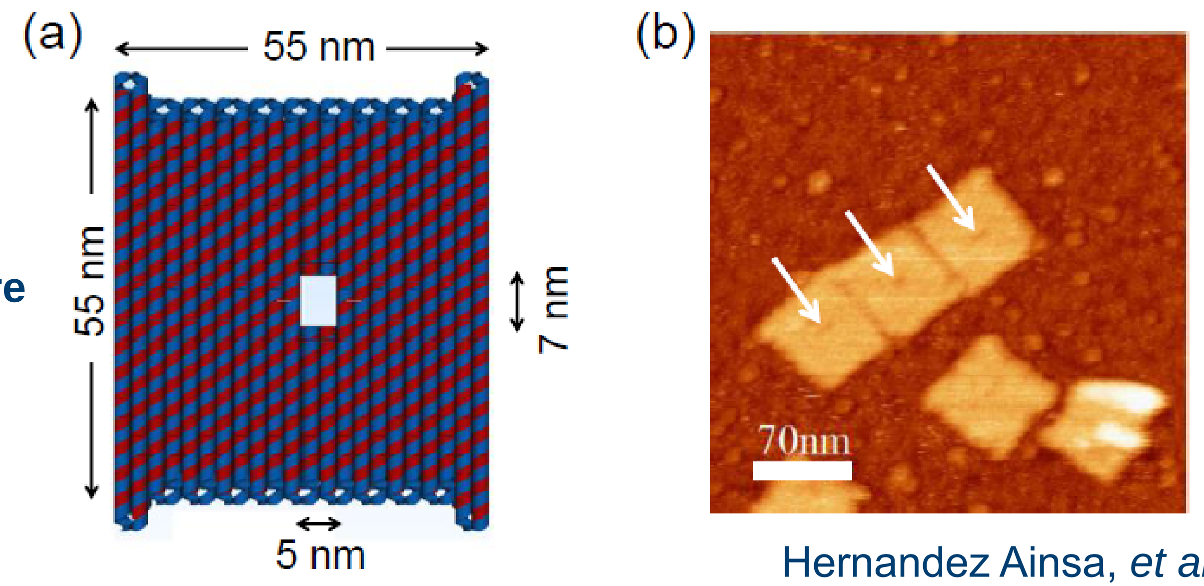
Hernandez Ainsa, *et al.*, ACS Nano **2013**

Physical control of translocation with DNA origami nanopores

14 nm pore



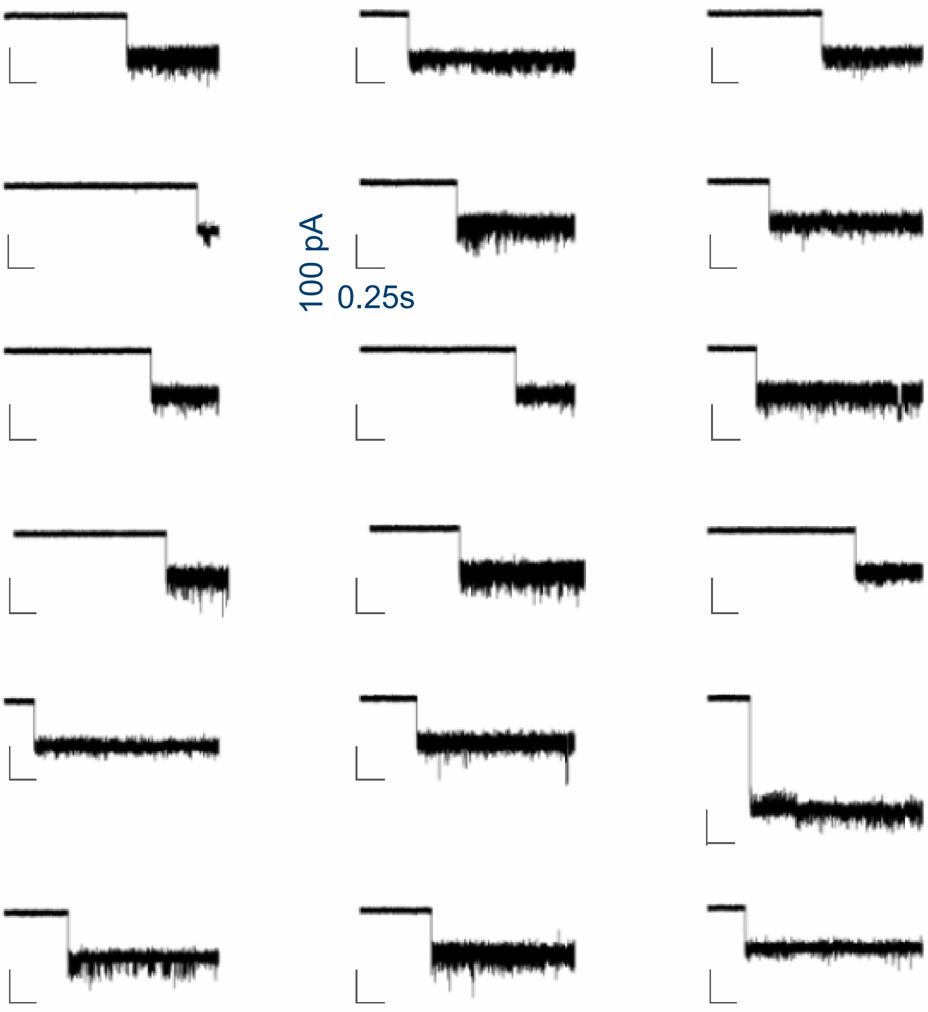
5 nm pore



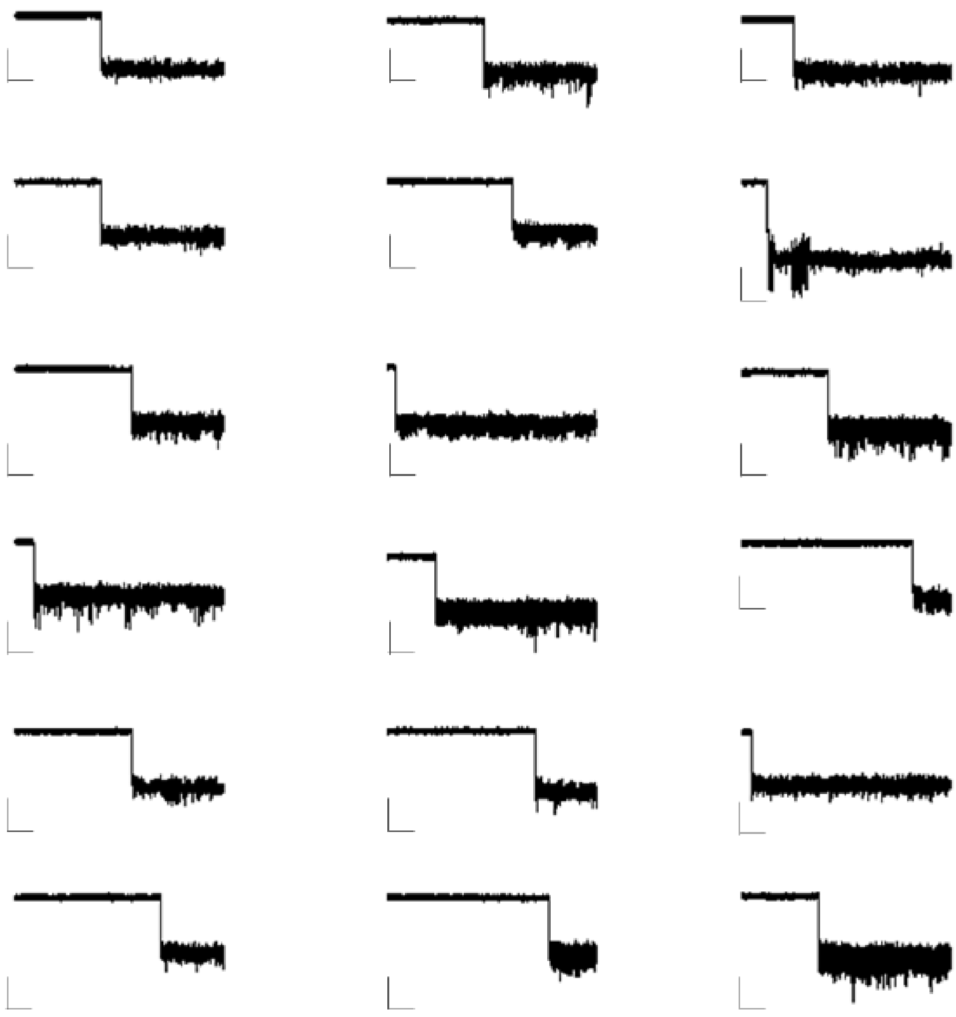
Hernandez Ainsa, *et al.*, ACS Nano **2013**

Typical traces for 14nm and 5nm designs

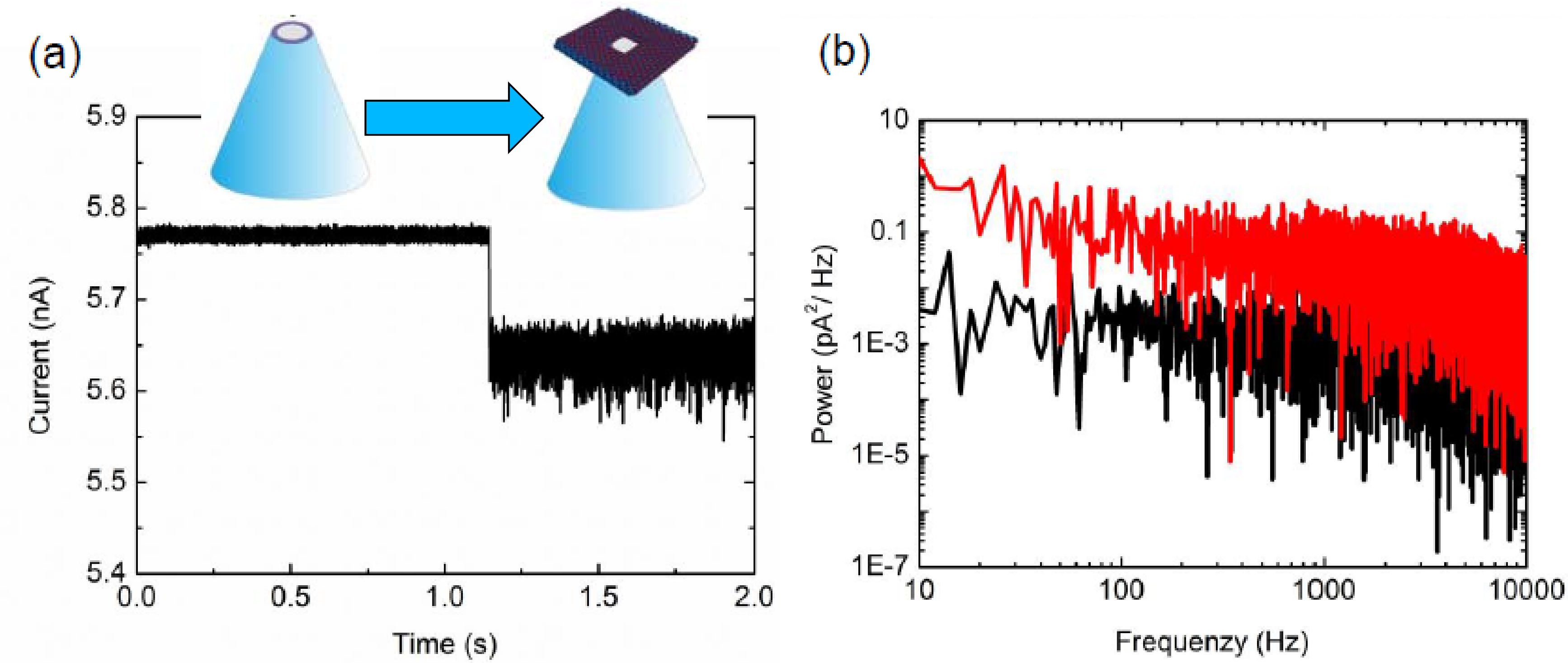
14 nm pore



5 nm pore



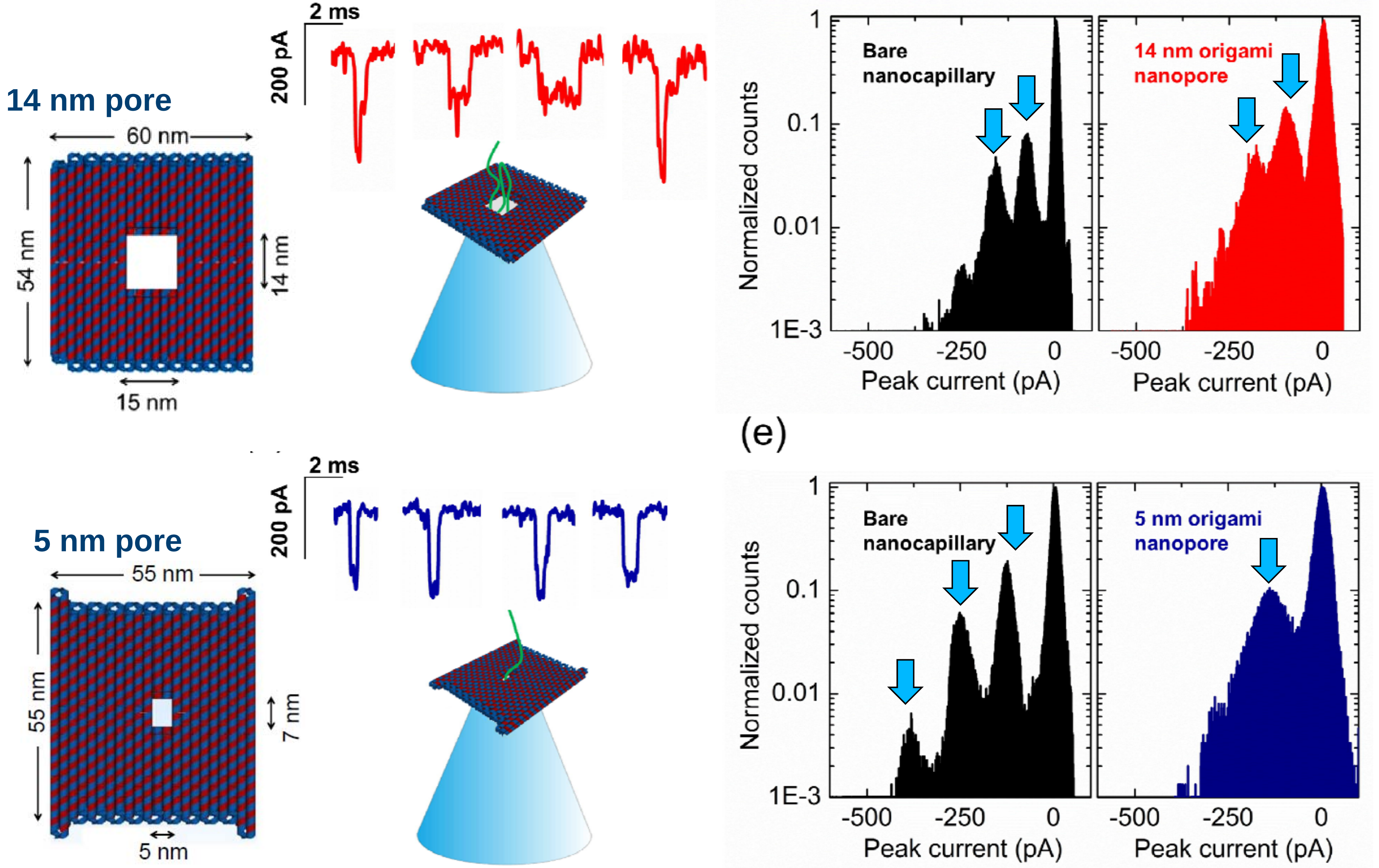
Noise analysis – what do the variations mean?



- What doe the variations in the fluctuations tell us about :
  (i) position on the solid-state nanopore ?
  (ii) integrity of the DNA origami structure?

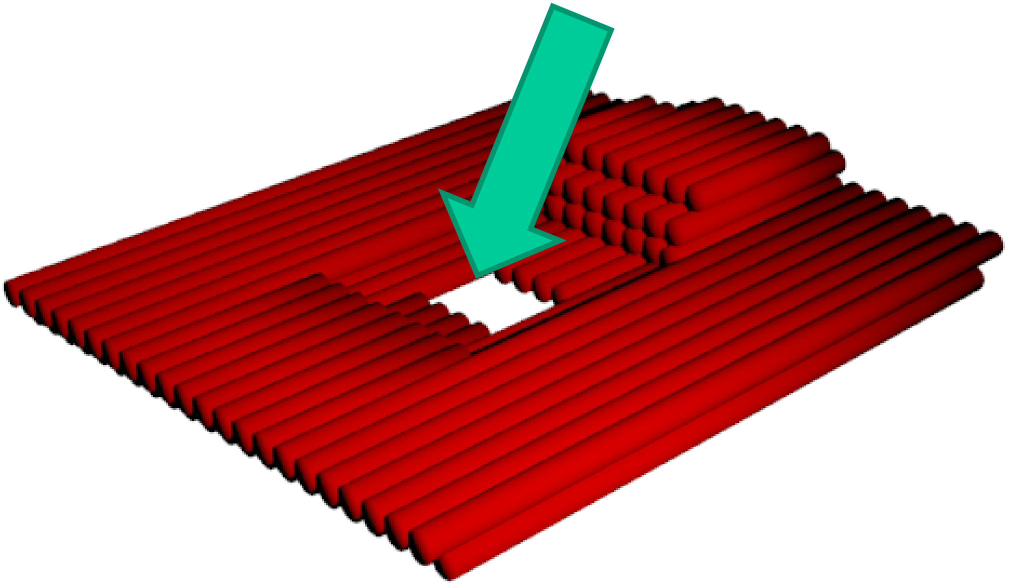
Hernandez Ainsa, *et al.*, ACS Nano **2013**

Physical control of translocation with DNA origami nanopores



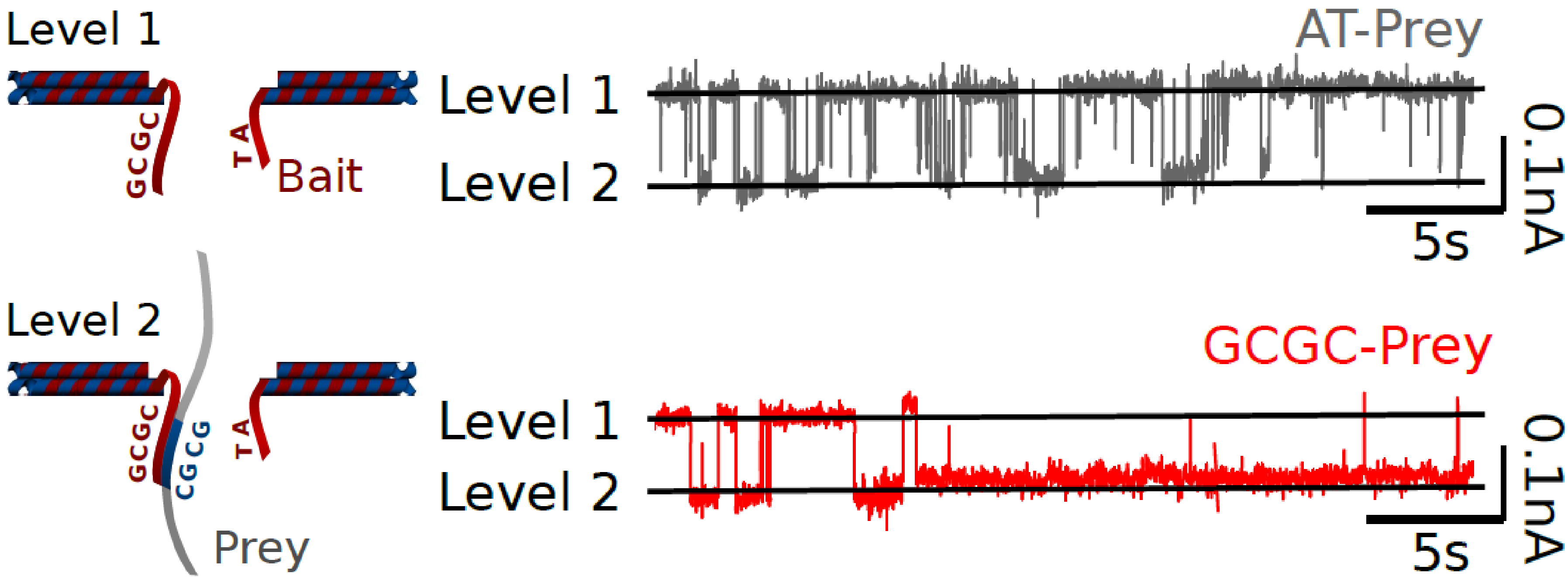
Chemical control of DNA translocation

Add binding site for protein or other molecules
Add binding sites for short DNA molecules



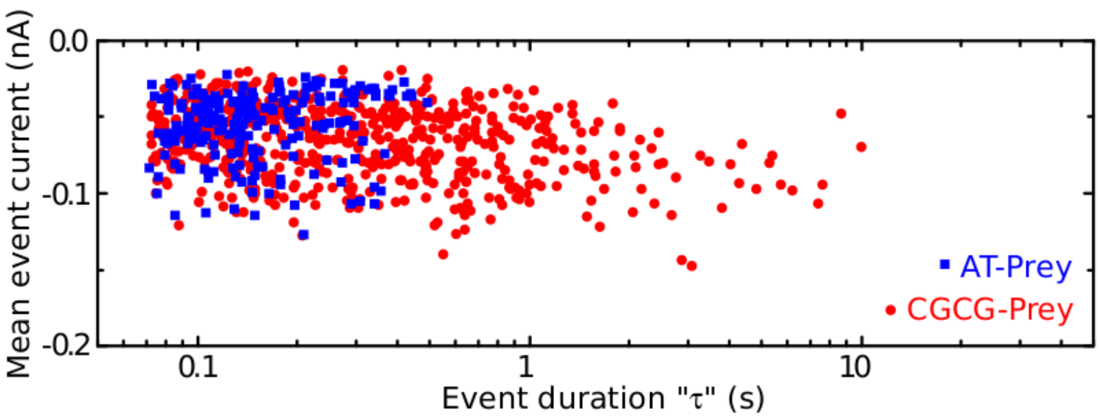
Hernandez Ainsa, *et al.*, ACS Nano **2013**

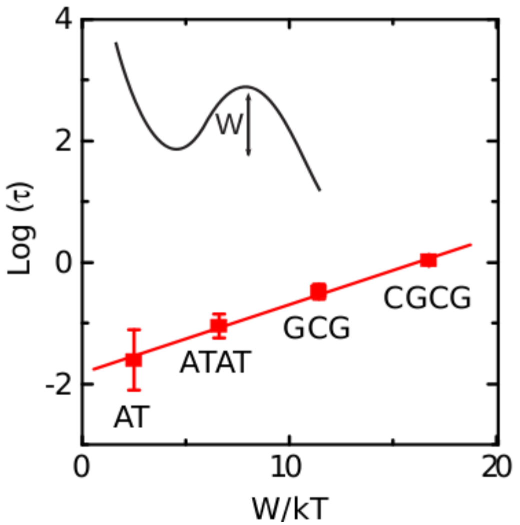
Chemical control of translocation with DNA origami nanopores (specific binding)

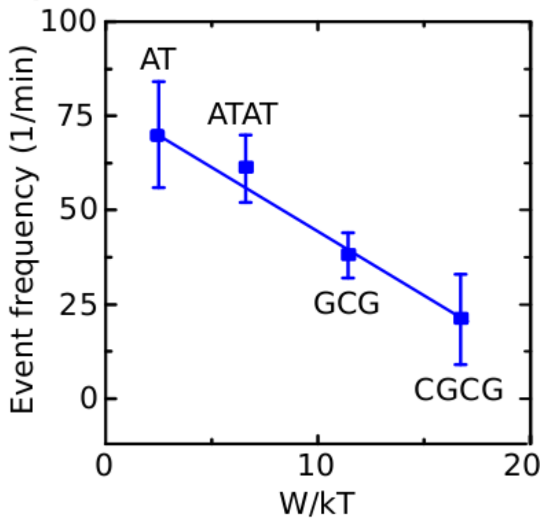


- Introduce weak binding site to briefly immobilize molecule in DNA origami nanopore allows to detect 50 bases long single stranded DNA
- Detection possible ⇔ ***same strategy for protein sensing possible*** (?)

Detection of 50 base long DNA molecules







- Two binding sites bind molecules with different affinity
- Characterization of molecules possible
- Life time can be described my simple Kramer's rate

$$\log \frac{1}{\tau} = -\frac{W}{kT} + \log \frac{\omega_a}{2\pi}$$

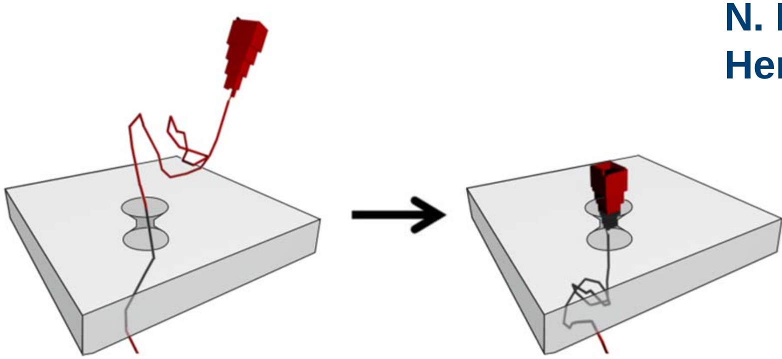
- Binding sites allow for detection of short DNA molecules

Hernandez Ainsa, *et al.*, ACS Nano **2013**

Conclusion

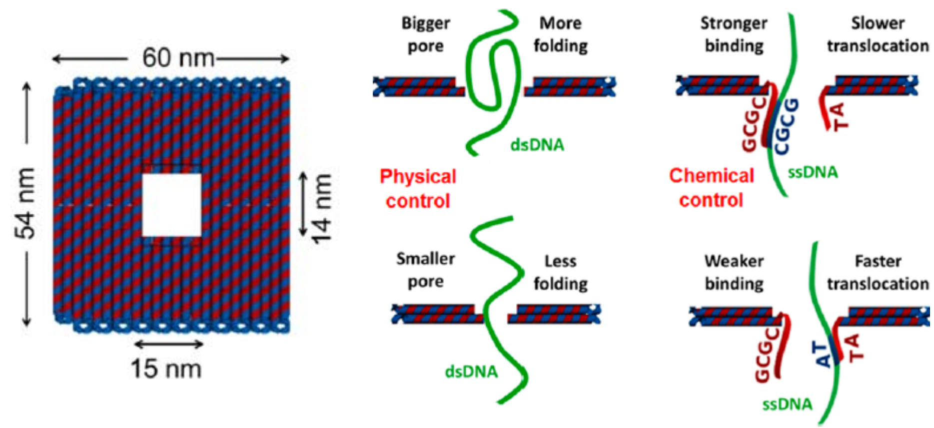
- New hybrid pores: DNA origami nanopores
- ***Designer nanopores***:
  towards atomistic control of shape and chemical composition

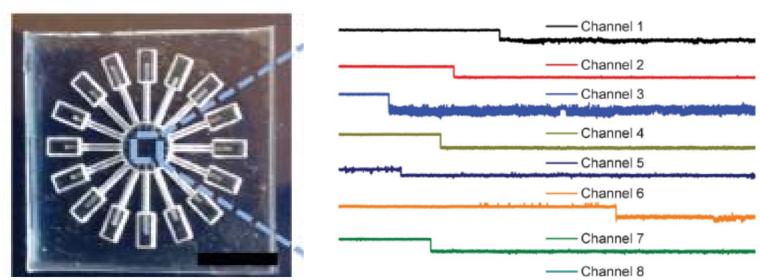
N. Bell, *et al*. Nano Letters 12, 512 (2012) (online 23/12/2011)
N. Bell et al. Lab Chip 13, 1859 (2013)
Hernandez Ainsa, *et al.*, ACS Nano (2013)



Highlighted in ***Nature Materials*** Feb. 2012

Highlighted in ***Nature Nanotechnology*** Feb. 2012
O. Vaughn, *Nanopores: Built with Origami*





Acknowledgements