WP2
Milestone n.: 11
Milestones name: M1.11
Delivery Date: Month 48

"Final workshop and Report "

All META partners

Milestone M1.11 Report on the final meeting

On the days 26th and 27th of May 2015, representatives of the research groups from Bratislava, Roma and Oak Ridge, met at the Center for Nanoscale Materials Science at the Oak Ridge National laboratories to analyze and discuss the results obtained in the course of the META project.

META
[*MATERIALS ENHANCEMENT FOR TECHNOLOGICAL APPLICATION*]

META Projects achievements in WP1 and WP2
FINAL MEETING
CNMS Oak Ridge National Laboratory
27th May 2015

AGENDA

9:00	Welcome address, Dr. Hans Christen, CNMS Director (ORNL)
9:30	S. Licoccia U. of Rome Tor Vergata in Videoconference
10:00	P. Morales, ENEA Casaccia and U. of Rome Tor Vergata in Videoconference
10:30	*Coffee break*
11:00	T. Hianik, CUB Bratislava
11:30	G. Balestrino, U. of Rome Tor Vergata in Videoconference in videoconference
12:00	R. Senesi U. of Rome Tor Vergata in Videoconference
12:30	A. Andreani U. of Rome Tor Vergata in Videoconference
13:00	*Lunch*
14:30	I. Ivanov, CNMS Director (ORNL)
15:00	I. S. Anderson, (ORNL)
15:30	M. Pelach, CUB Bratislava
16:00	Discussion
17:00	*End*

As for the one day workshop held in June 2014, the Director of CNMS, dr. Hans Christen participated.

The Agenda of the meeting included a first overview of the results obtained by each group and an overview of the general objectives of the project. Two main talks of one hour each were scheduled to outline in detail the achievements relative to workpackage I and II. These talks were delivered by Dr. P. Morales at CNMS (Workpackage I) and by Prof. G. Balestrino in teleconference from Roma (Workpackage II). On a more detailed analytical basis, Prof. Ian Anderson from Oak Ridge introduced the work performed at the Spallation Neutron Source on the structural investigation performed on ionic conductor materials investigated in WP I. These were further commented by Prof. C. Andreani, in teleconference from Rome Tor Vergata.

In relation to the latest activities relating to WP I, dr. I. Ivanov from CNMS and dr. M. Pelach from CUB illustrated in detail the experimental work of characterization of the successive steps of materials organization at the nanometer scale, performed by the simultaeous use of Surface Enhanced Raman Spectroscopy and Quartz Crystal Microbalance, monitoring the adsorption

kinetics of single molecules on the nanoboards. Professor Tibor Hianik from CUB also showed the analysis of the latest results obtained, with the collaboration of Dr. M. Pelach.

Relative to the activities concerning WP2, G. Balestrino from Tor Vergata, showed how the joint research work between Tor Vergata and CNSM on complex oxides has been able to demonstrate that Electrochemical Strain Microscopy (ESM) is a viable technique to investigate the local electrochemical activity in terms of both surface activity and bulk ionic mobility. In the framework of the project, the applicability of the ESM technique was extended to high temperatures (up to 400 °C) and controlled atmosphere (both oxidizing and reducing). In this framework, it is opinion of the researchers, that the ESM approach can be extended to disentangle different transport mechanisms (i.e. ionic or protonic) on a local scale with a substantial advantage relative to standard transport characterizations. Along this direction, further analysis of the huge wealth of experimental data collected at CNSM is going ahead at Tor Vergata and further interesting results are expected at brief.
Finally, G. Balestrino underlined that the joint work has resulted in about 10 papers, with mixed CNMS/Tor Vergata authorship, published on high IP journals.

The second day of the meeting was devoted to the analysis of future perspectives of the research. It was agreed that given the extremely positive results obtained within META further collaborative research should be carried out along the tracks established by this project. P. Morales illustrated the project newly submitted for approval of the European Comission within the Future and Emerging Technologies framwork of H2020. The new project (CONORI, CONnecting ORIgami) has an extended research consortium now including the cDNA Center of the University of Aarhus (DK) with the coordination of Professor Kurt Gothelf, the Hebrew University of Jerusalem, under the responsibility of Prof. Danny Porath, and the Technical University of Kobnhavn under the coordiatinof Prof. Anja Boisen. This new project aims at the application of the META project results to assemble and test molecular electronic devices assembled on the DNA origami breadboards and internally connected via single conducting polymer chains. This FET project cannot include laboratories in the USA but it was agreed that the consortium will find all possible ways to subsidize secondment of european researchers to the CNMS facility for further user projects on the subject.
Dr Ivanov also illustrated the new instrumental and infrastructural acquisition of the CNMS facility which would be perfectly suitable and promising for further collaborative research.
At the end of the second day of meeting, Dr. Morales also had a short meeting with dr. B. Sumpter, head of the computational unit of CNMS, for a resume of the results obtained in the course of META. Dr. Sumpter was also extremely pleased with the simulation work performed on the organic-inorganic interface and agreed on finding new subsidies for CNMS collaboration to new European projects.

ANNEX I hereafter is the presentation given at the meeting on the result of WPI (P. Morales)

The META project concluding workshop: WP I

DNA origami "breadboards" for molecular and bio-electronics

Piero Morales

ENEA, Centro Ricerche della Casaccia

Centro NAST, Università degli Studi di Roma Tor Vergata

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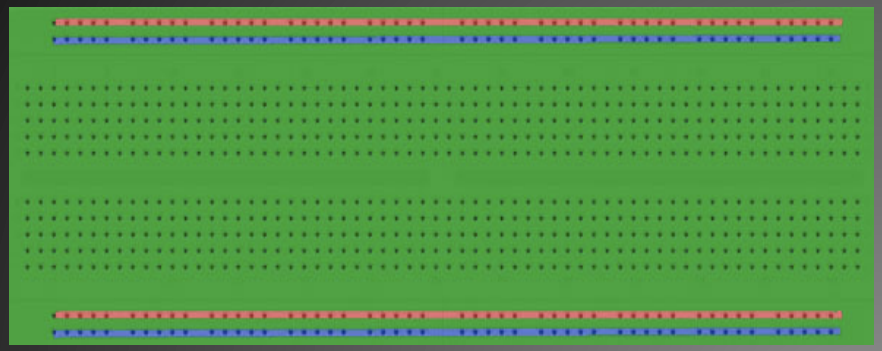
Our group and partners

- Liqian Wang, Katia Spinella, Wei-hua Han (NAST Center Università di Tor Vergata, Roma, I)
- Claudia Dalmastri, Lucia Mosiello, Bruno Rapone Massimo Celino, Francesco Buonocore, Caterina Arcangeli (ENEA, Roma, I)
- Bobby Sumpter, Scott Retterer, Ilia Ivanov (CNMS, Oak Ridge National Laboratory, USA)
- Kurt Gothelf, Mattia De Stefano, Abhichart Krissanaprasit, Jesper Vinther (cDNA, Aarhus Univ., DK)
- Tibor Hianik, Ivana Karpisova, Michal Pelach

  (Comenius University, Bratislava, SK)

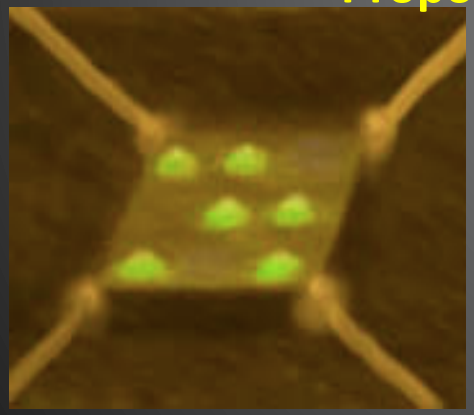
The "DNA breadboards" concept
(full selfassemblage on artificially patterned substrates)



A fiberglass breadboard

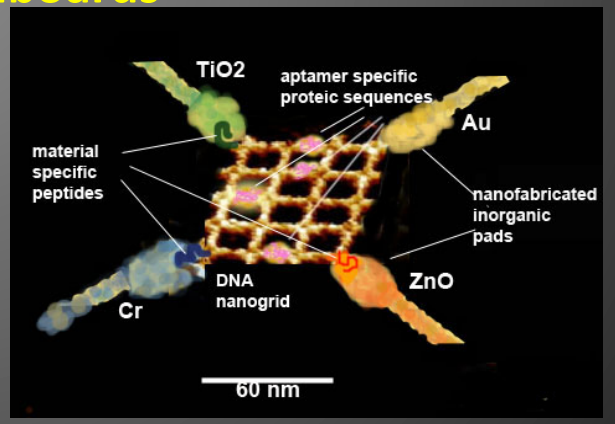
An electronic device on a fiberglass circuit board

Proposals for DNA "breadboards"



With DNA origami and gold connections (thiols)

Proteins can be very smart "components", and DNA aptamers good connectors



With nanogrids and conducting/ semiconducting connections (material selective peptides)

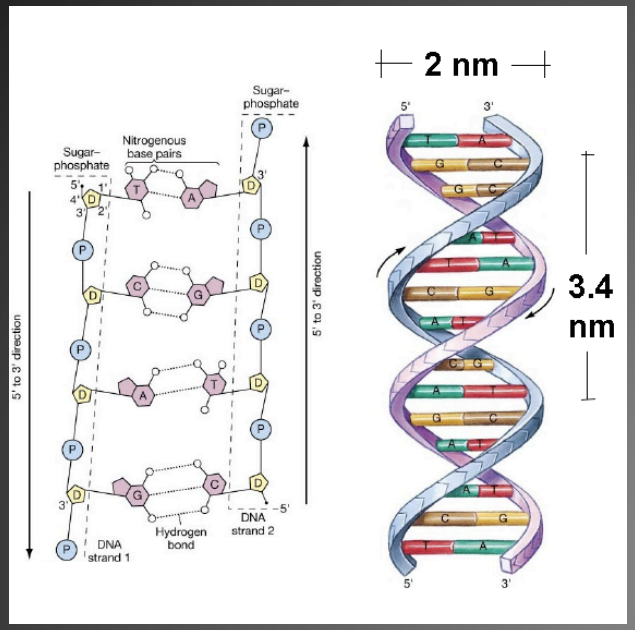
Related studies

- Simulation of organic-organic and organic-inorganic interactions
- Measurements of affinities, docking rates, efficiency etc (FRET, SERS, QCM, AFM…)

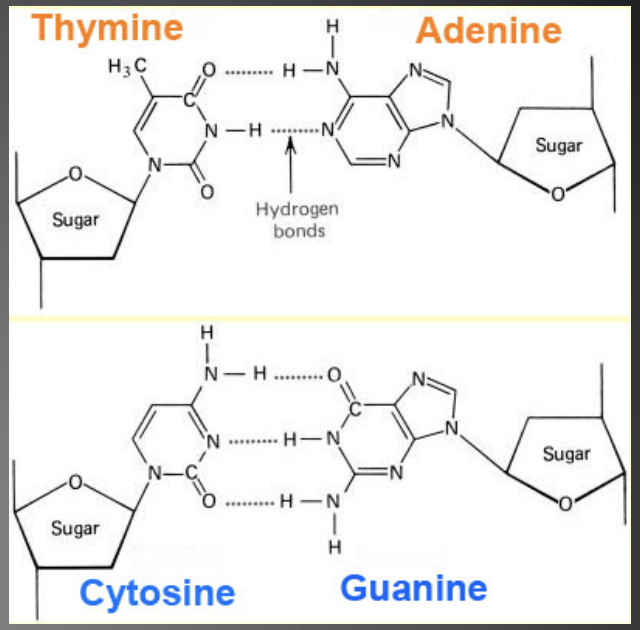
Why use DNA to build nanoscaffolds

- Its typical sizes are truly nanometric (2-3.5 nm)
- It is programmable: each nm of the chain can be made different and can bind selectively different sequences
- It selfassembles into smart 2D and 3D architectures
- Easier to control with respect to proteins

Basic DNA information:



The DNA helical double strand



The 4 nucleotidic bases

Rule: Thymine only binds Adenine and viceversa A---T, T---A
Cytosine only binds Guanine and viceversa. G---C, C---G

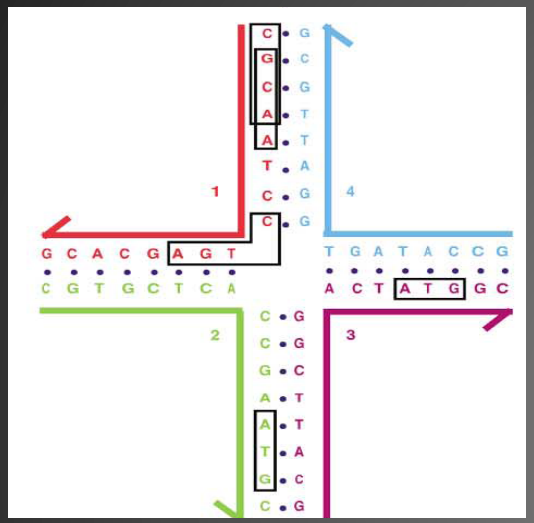
For example:

AGT AGT GGG CTC AGT CGG ATG AGC

AC TCG CTA CAT GGT GAG ATA

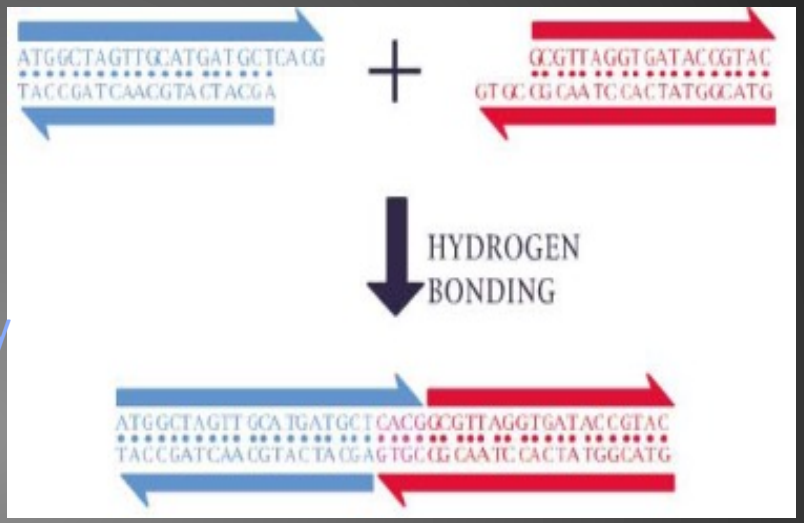
These two yellow ends are "sticky"

Elementary DNA nanotechnology based on sticky ends

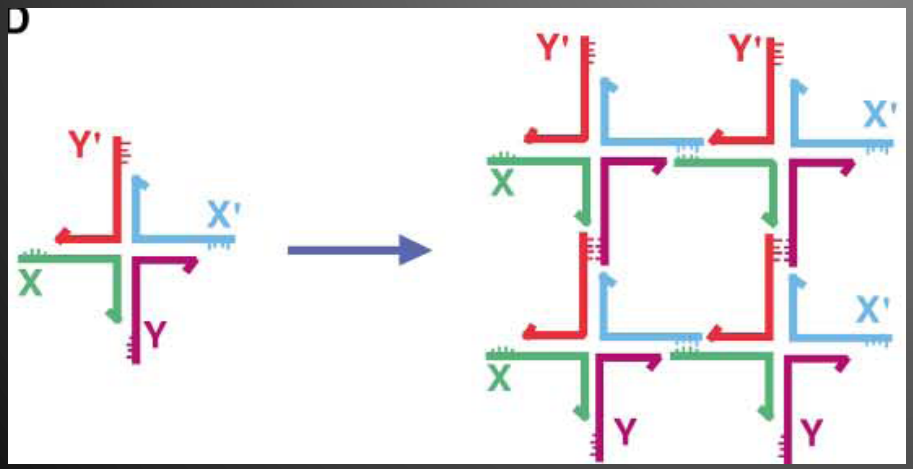


A "tile" made of 4 single strands

Two d.s. joined by their sticky ends

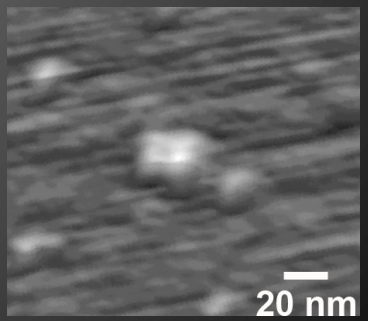


Four "tiles" make a square

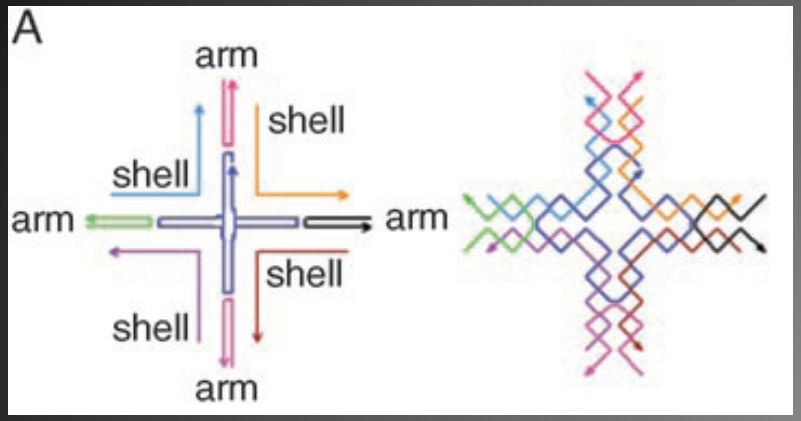


Base complementarity and sticky ends allow construction of DNA nanostructures

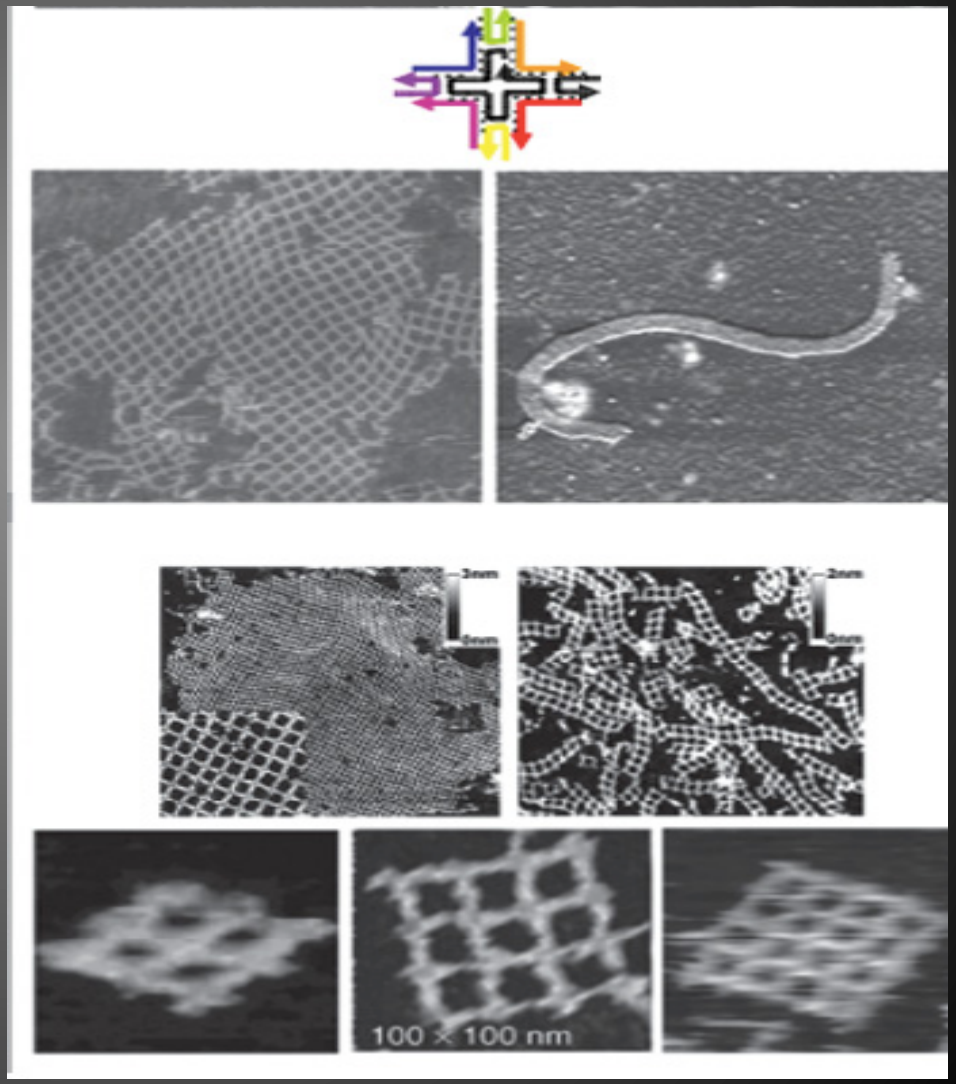
AFM of a 20 nm side square



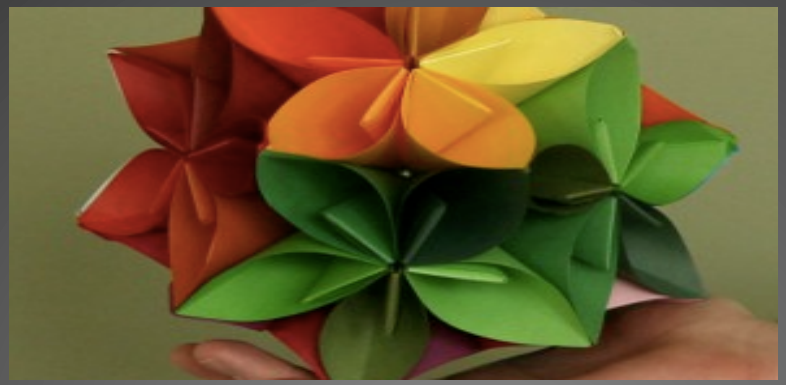
More complex and rigid “tiles” make more complex structures



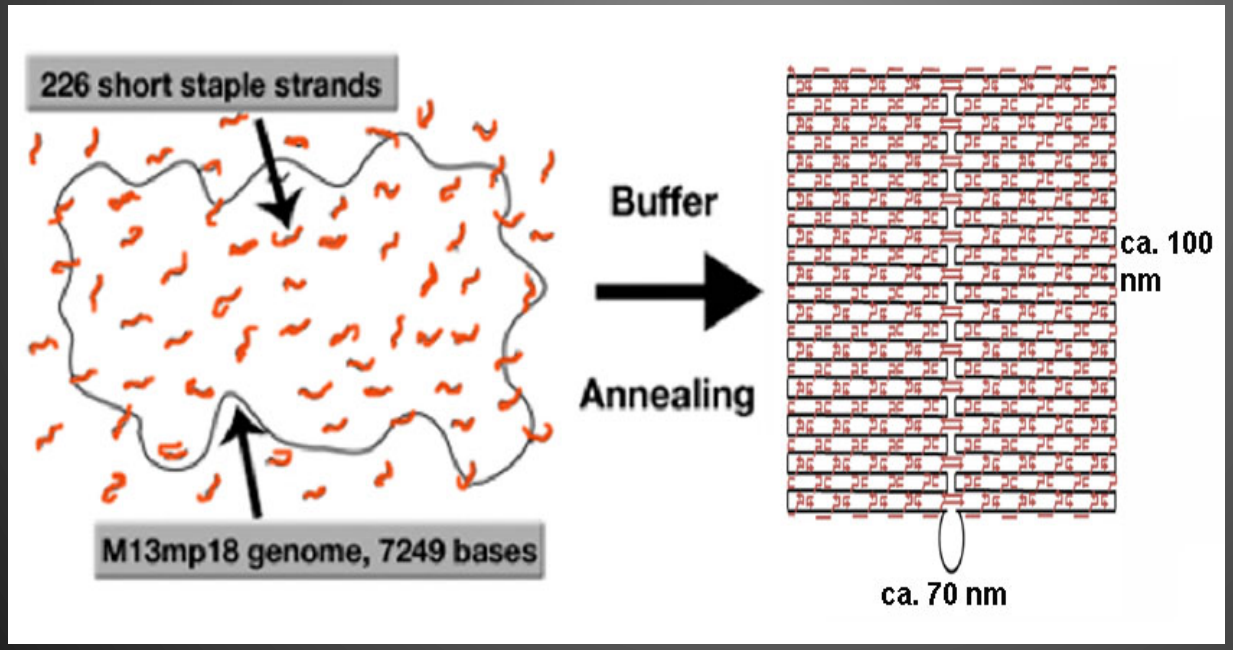
This type of tile has two double helices joined by “crossovers”. It is made of 9 single strands rather than 4. It is thus much more stable and rigid



A different approach: the DNA “origami”



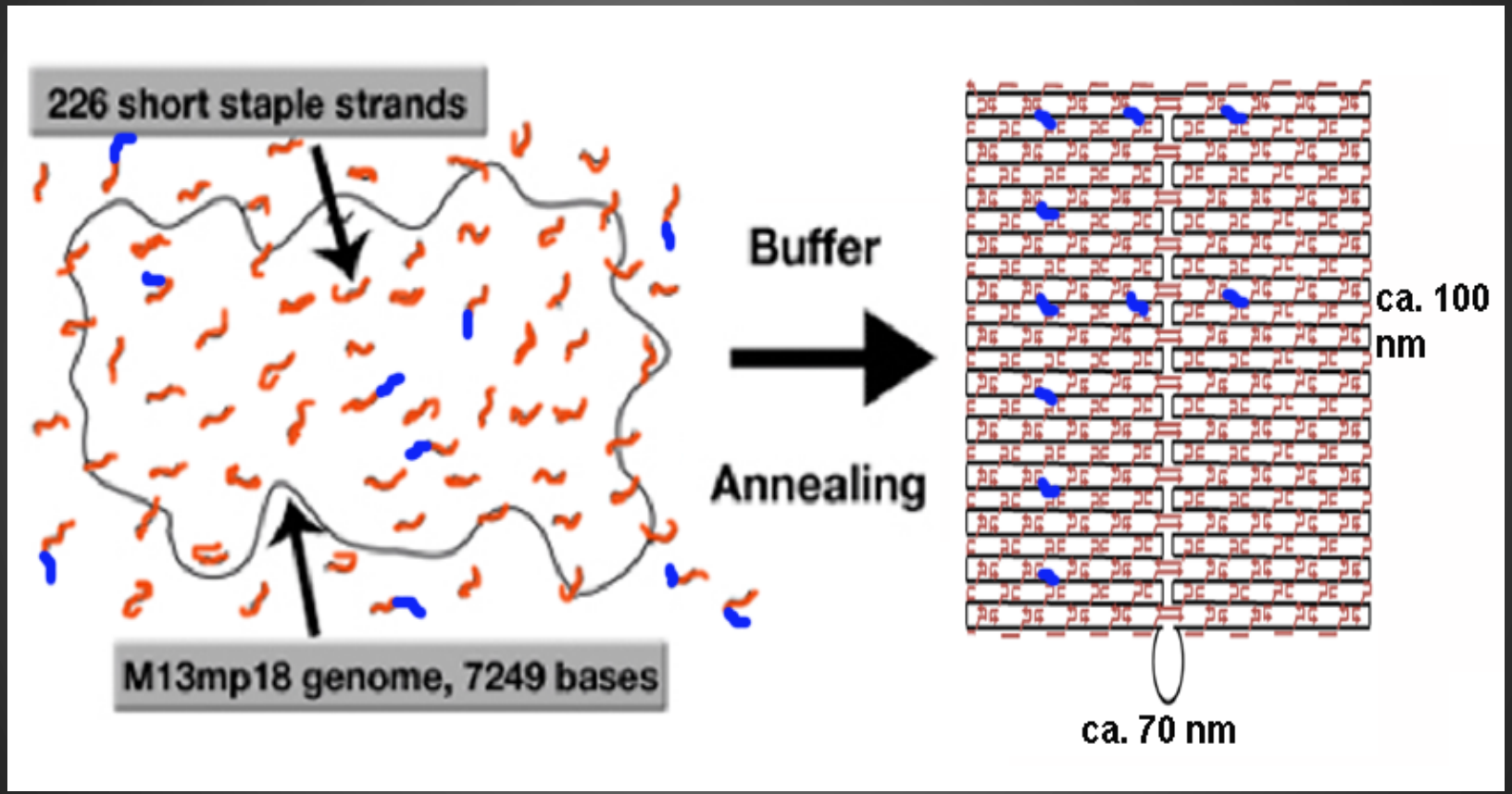
Folding a single sheet of paper into appealing shapes and sometimes stapling it in shape



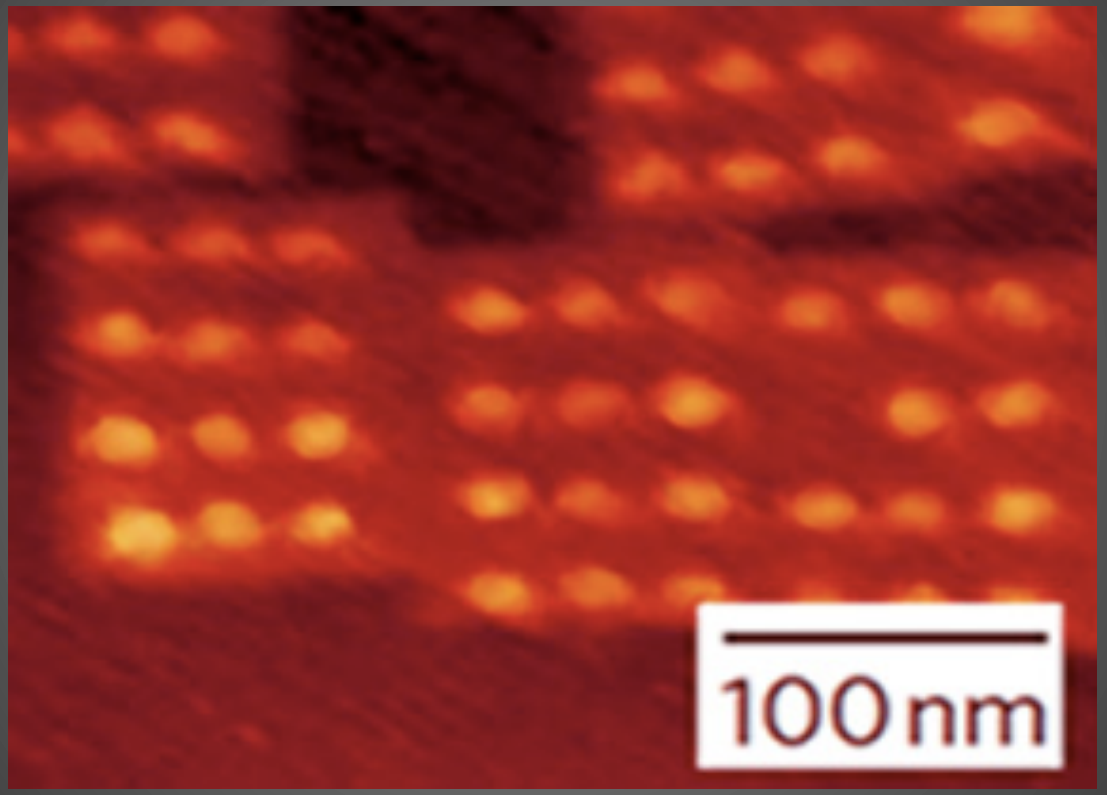
Folding a long single strand of DNA into a square by stapling it with many 35 base-long specifically sticky nucleotide sequences

Extremely assemblage high yield (>85%)

Selfassemblage: origami architectures with "sticky" extensions



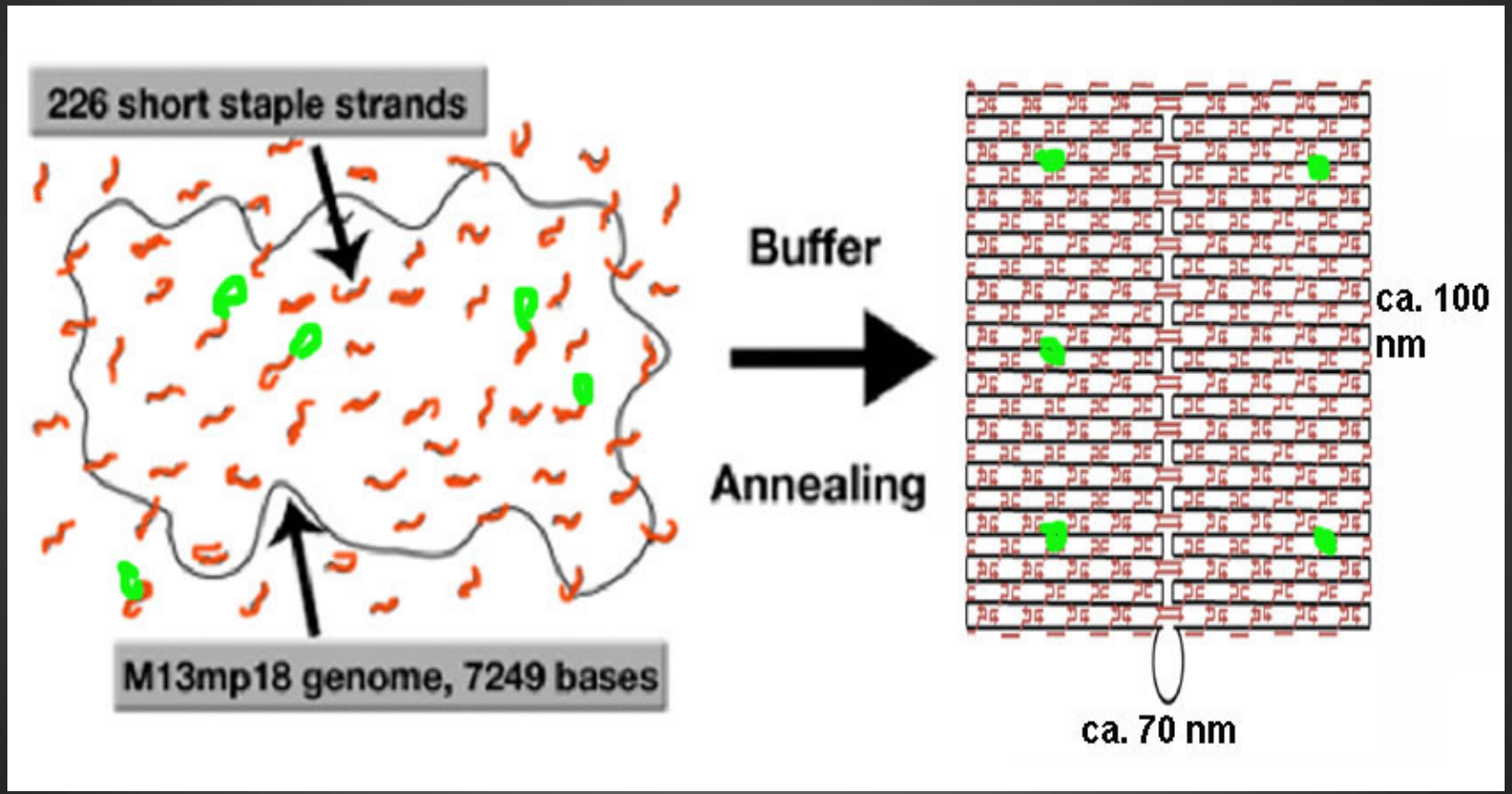
Regular arrays of biotin bonded streptavidine proteins



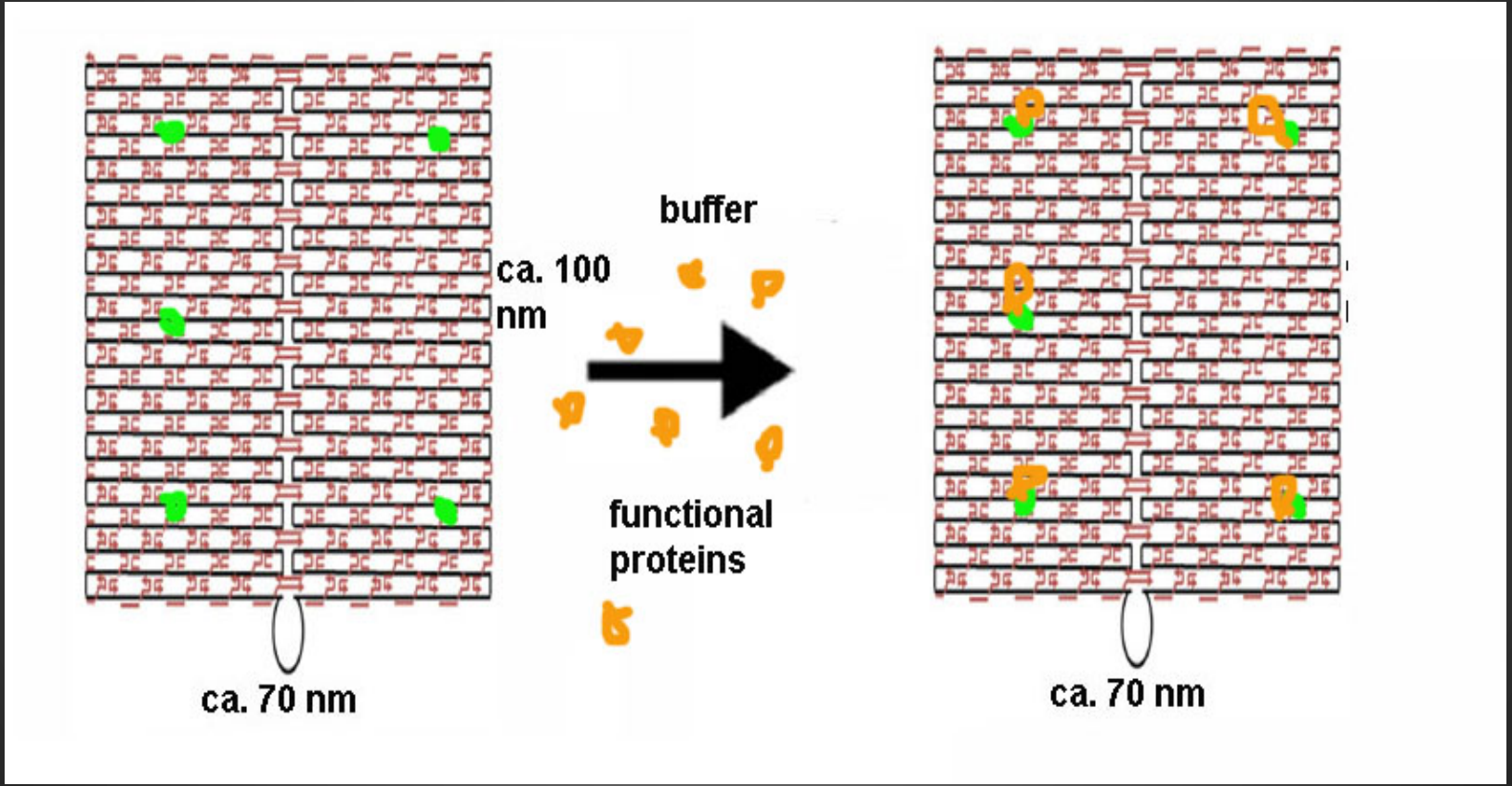
AFM in solution

Inter-protein spacing is here 15 nm x 25 nm approx.
(Gothelf and cowork. - Nature Nanotech. 2010)

Selfassemblage: origami architectures with protein specific aptamers

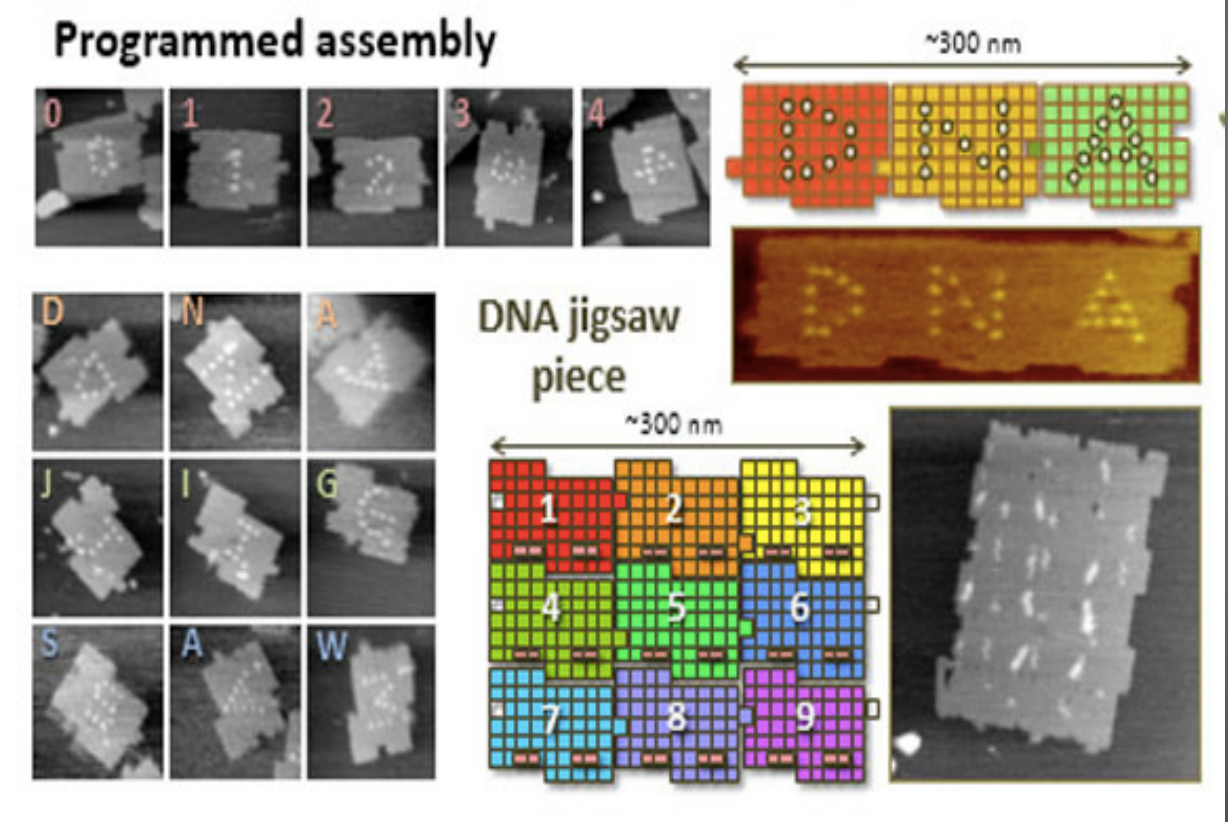


Selfassemblage: origami architectures with aptamer bound functional proteins



There is one specific, selectively addressable, sticky location every 6 nm approximately, 220 locations per 7 000 nm^2, about 30 000 per square micron

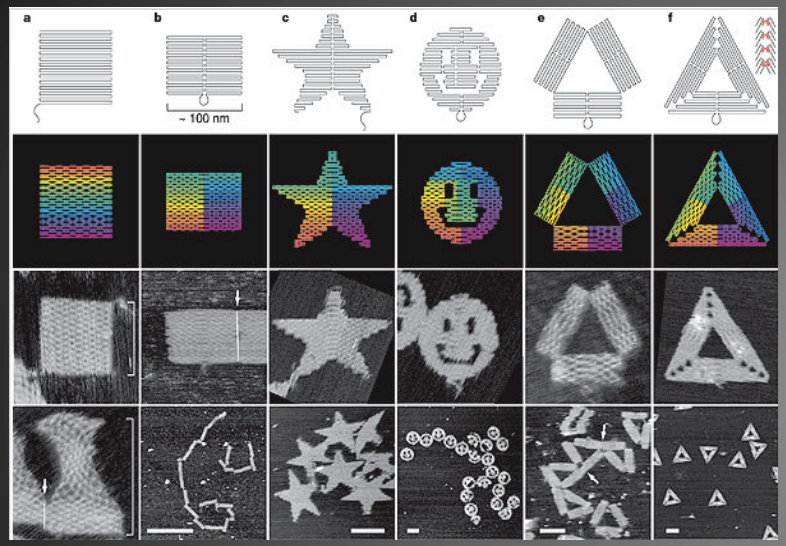
Selfassemblage: architectures with multiple stacked origami

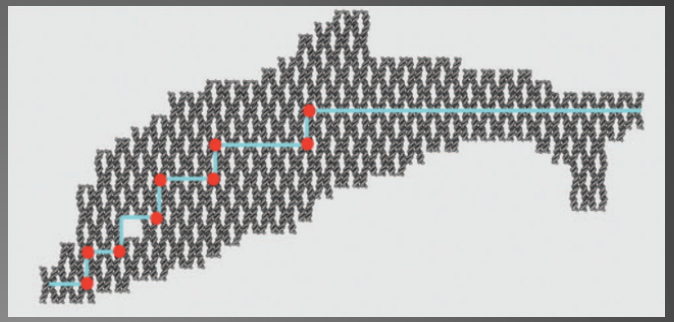


DNA Nanotechnology
Endo Group
WPI-iCeMS Kyoto University
Sugiyama Lab

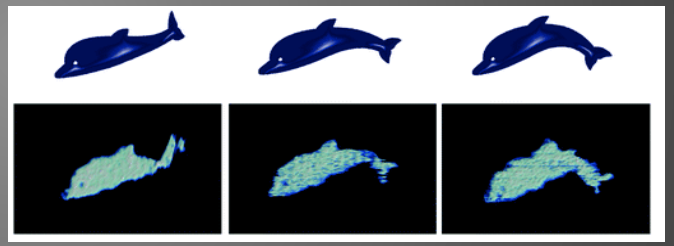
Yoshida-ushinomiyacho, Sakyo-ku,
Kyoto 606-8501

Virtually any shape can be obtained by the DNA origami method! Even 3D and dynamic

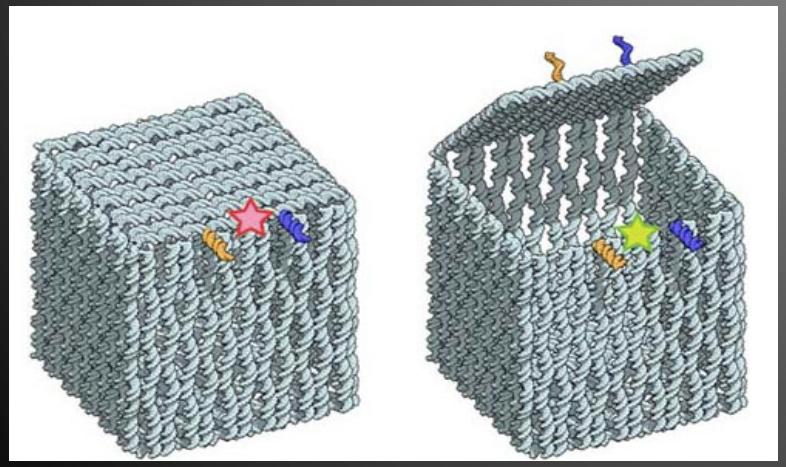




Design



AFM

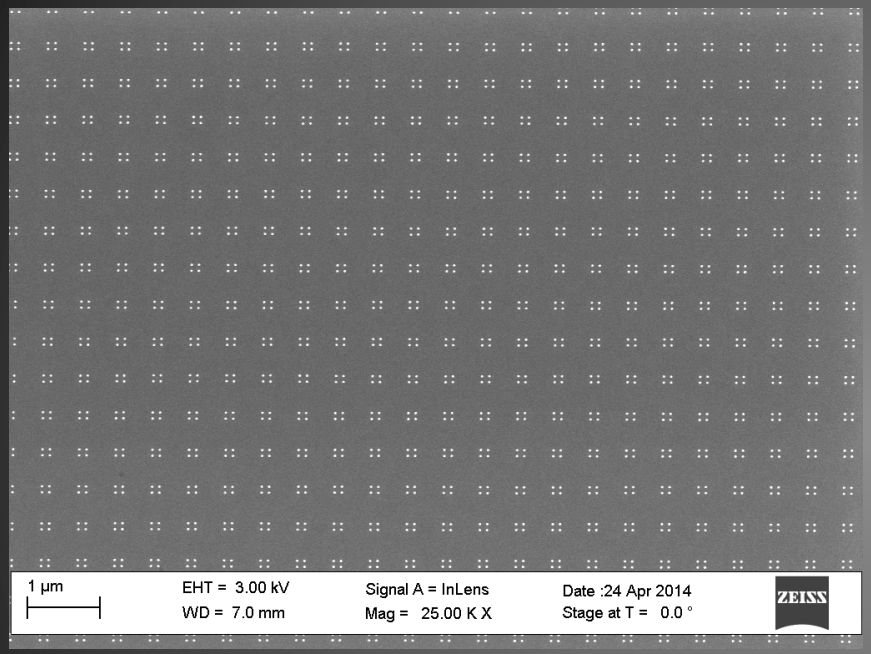


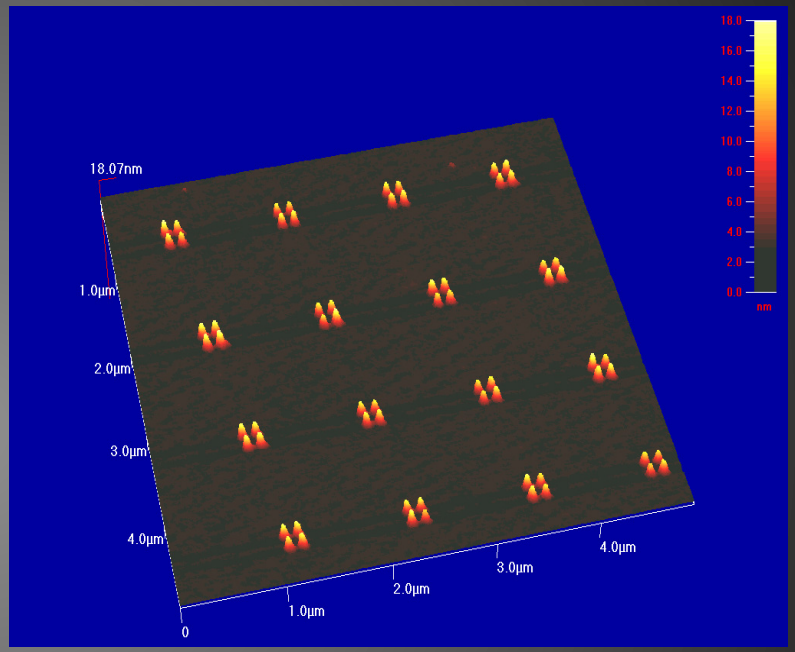
Different stapling oligonucleotides in the tail of the dolphin or in the locking stitches cause the tail to flip up or the box to open.
Applications in nanomedicine!!!

All this is wonderful but…
randomly deposited on surface
(or dispersed in the solution)

- Can we address these shapes onto predesigned locations?
- Can we input or extract signals from biochemical reactions occurring at specific nanometrically addressed locations?
- How precisely can we locate components on these DNA nanostructures?
- Can we use both faces of a figure?

Electron beam lithography can help us to anchor DNA nanostructures…

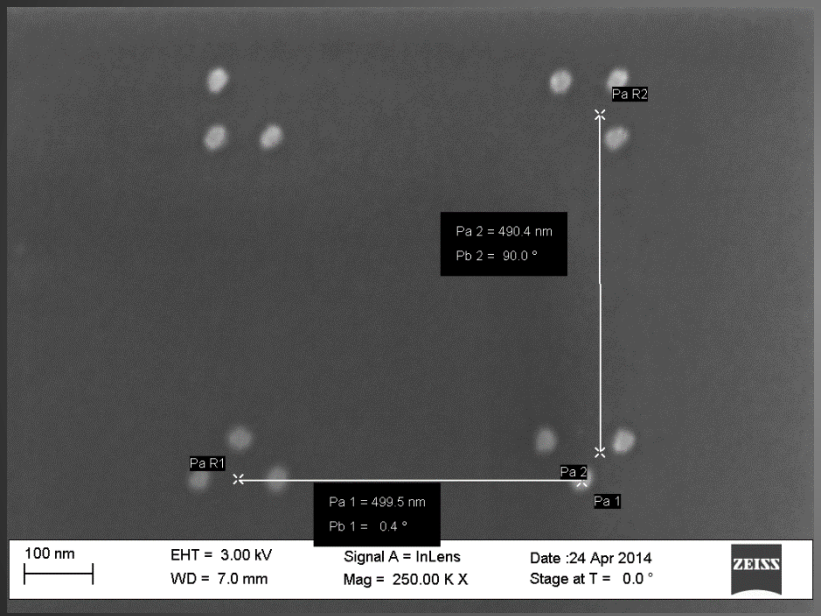


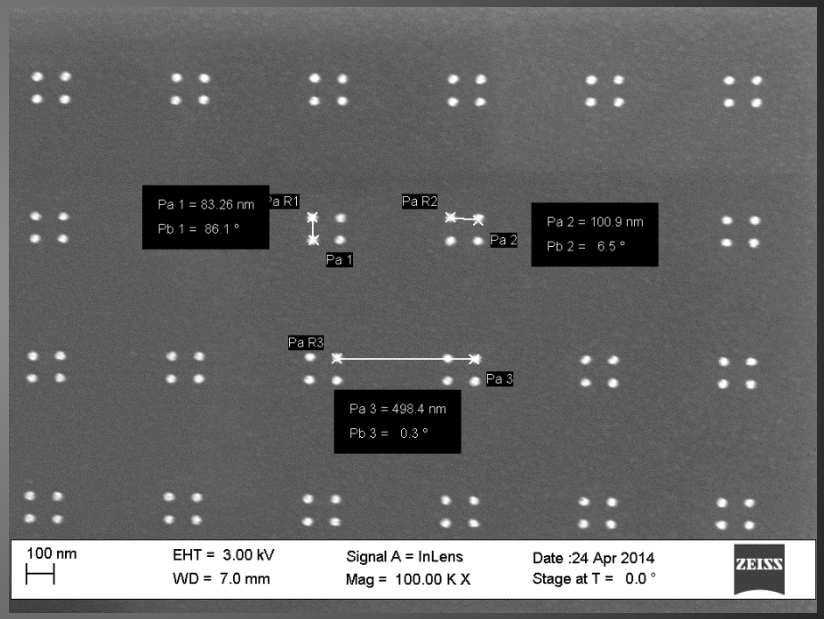


High quality e-beam lithography for gold anchoring nanopads

Jeol 9300 e-beam at the nanofab facility of CNMS Oak Ridge. AFM at the Casaccia labs

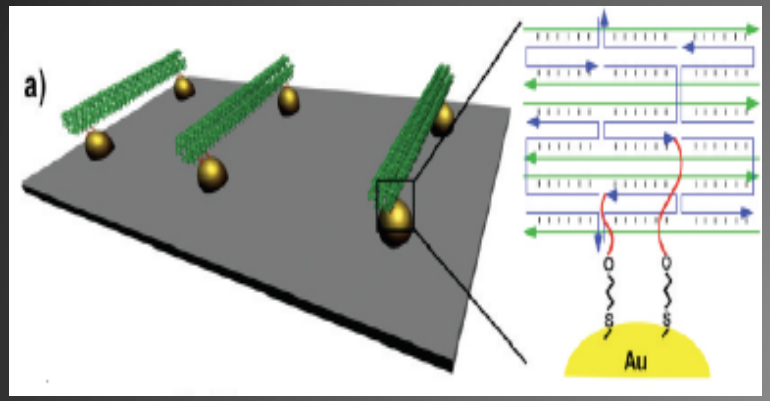
… on gold nanopads for docking of triangular and rectangular DNA origami



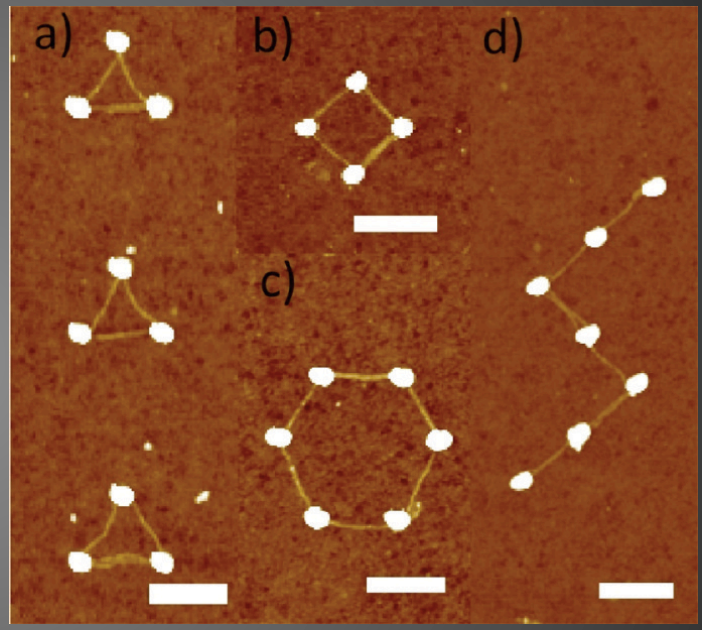


25 nm diameter dots, spacing 80 nm center-center
Intergroup spacing 500 nm

… and we can exploit the sulphur-gold bond



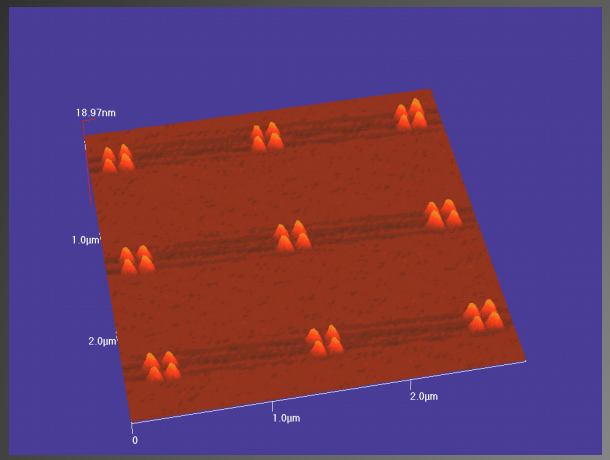
400 nm long nanotubes made of DNA origami anchored to gold



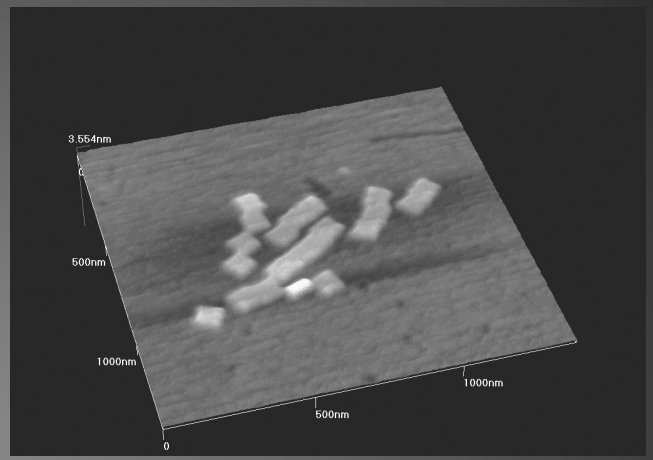
Bar is 300 nm, nanodots about 80-100 nm

- **Ebeam lithography for gold islands (40-120 nm) + DNA nanotubes (Hao Yan 2010)**

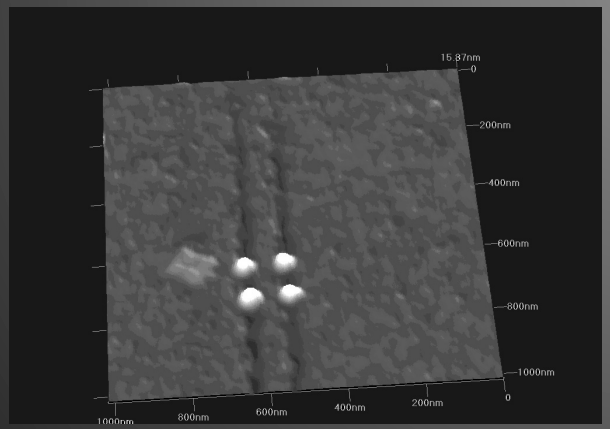
So, what elements do we have so far?



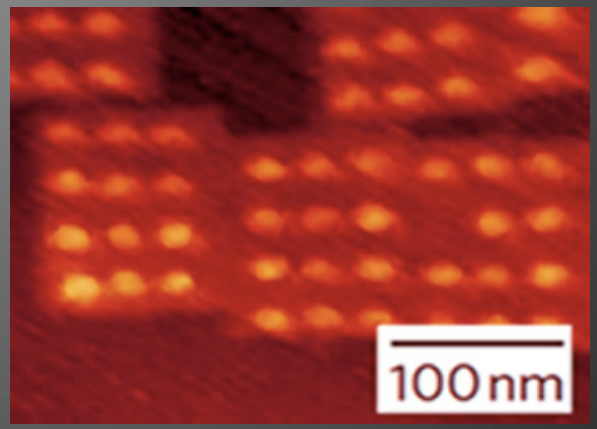
Gold nanoanchors arrays



DNA origami “nanoboards” on mica



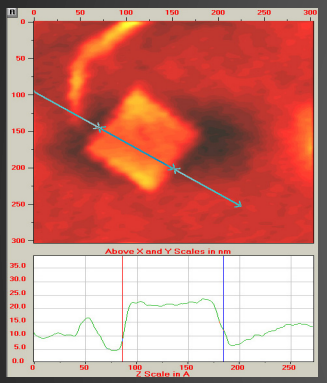
DNA Origamis on their Si substrate

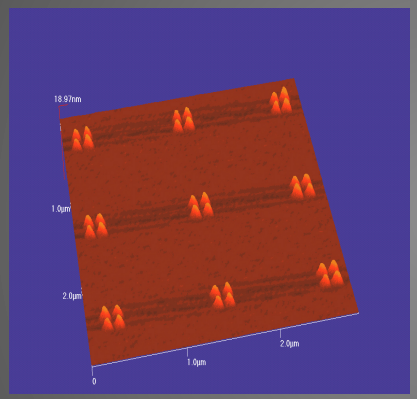


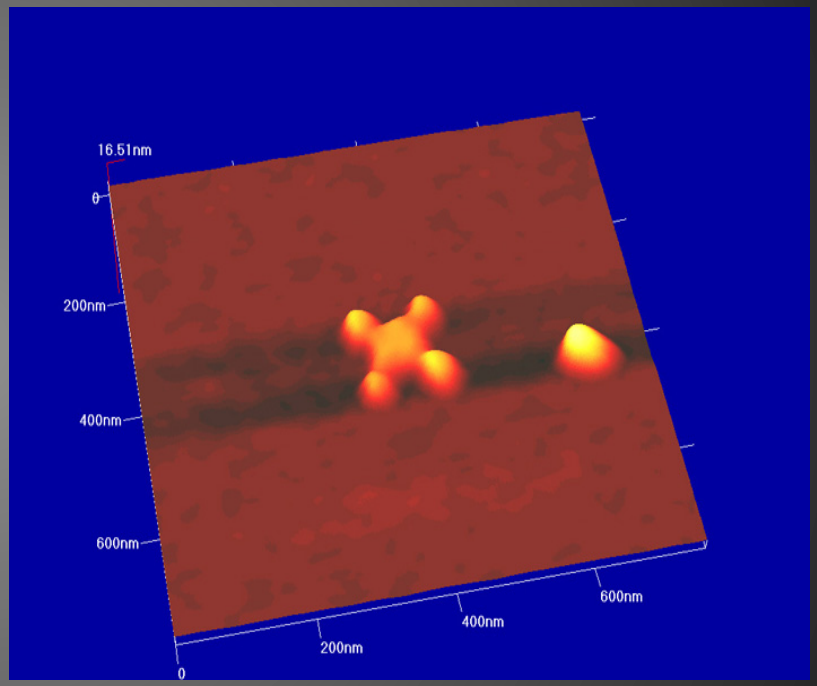
Proteins on DNA origamis on mica (cDNA Aarhus)

Putting these together …

! 75 mM $MgCl_2$!





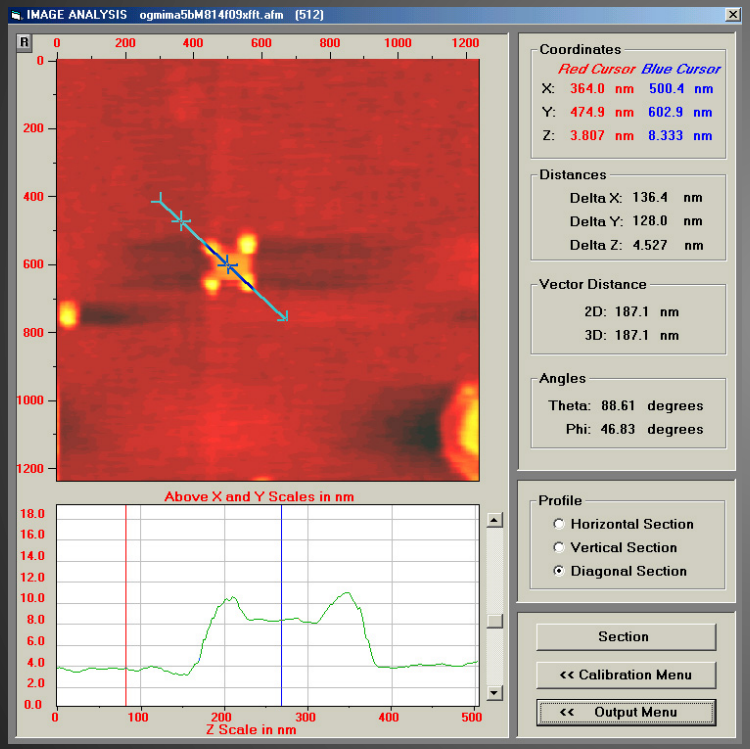


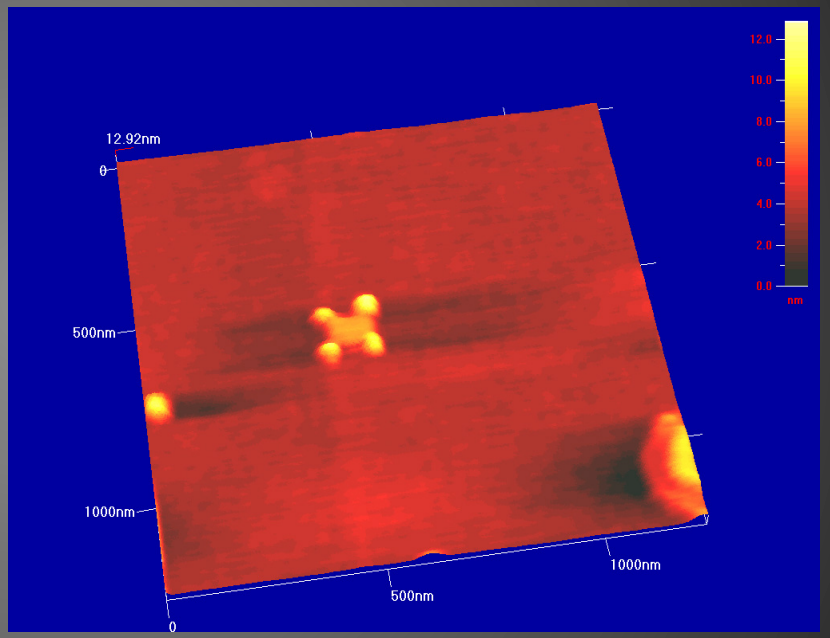
74 nm x 70 nm
2 nm thick

80x80nm;
1000 nm group to group
7.5 nm tall
25 nm diameter

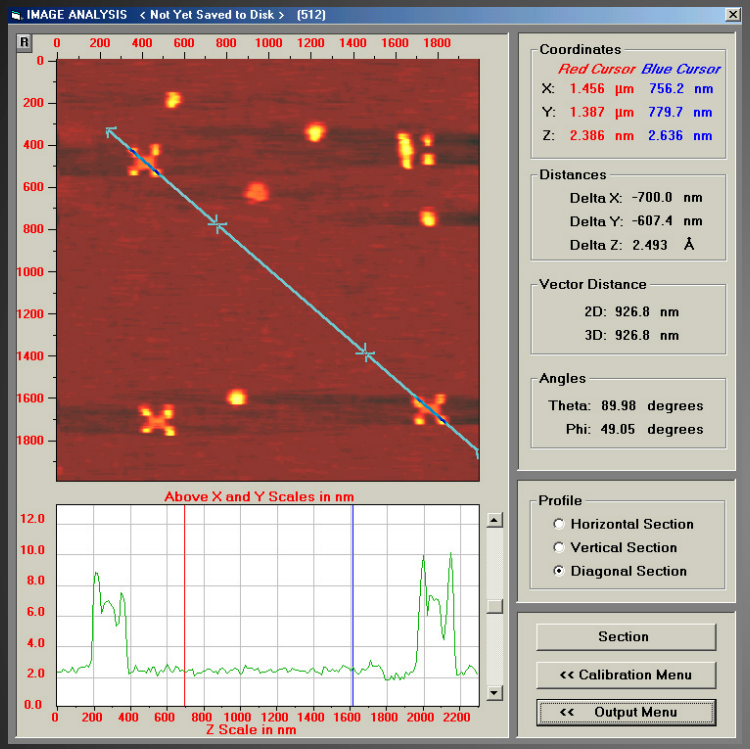
Origamis are suspended at 85% of the nanopillars height

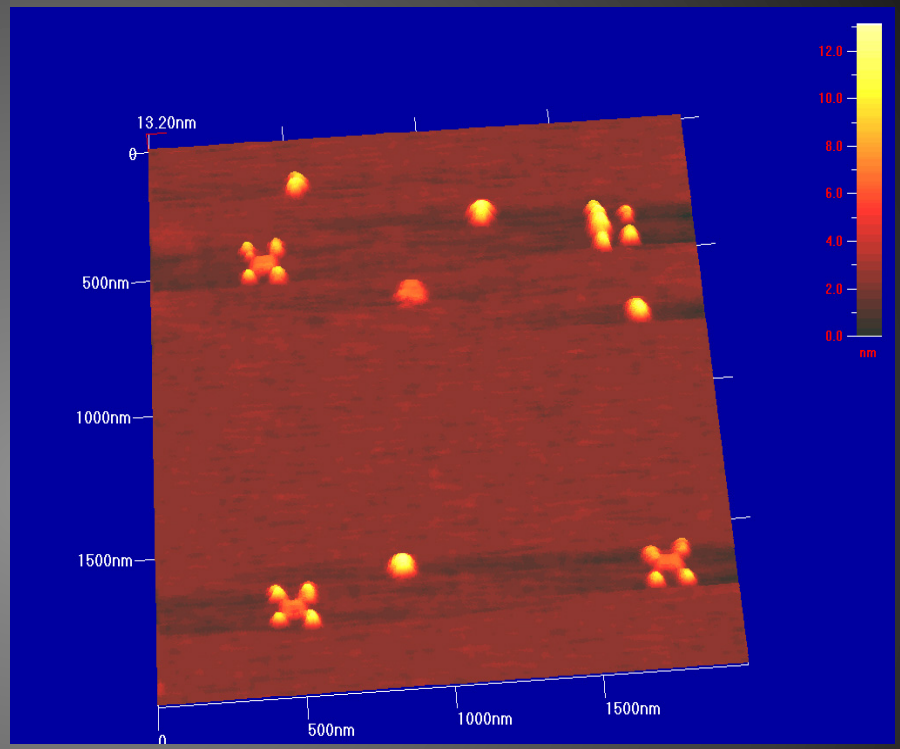
AFM characterization of immobilized DNA origami “breadboards”…





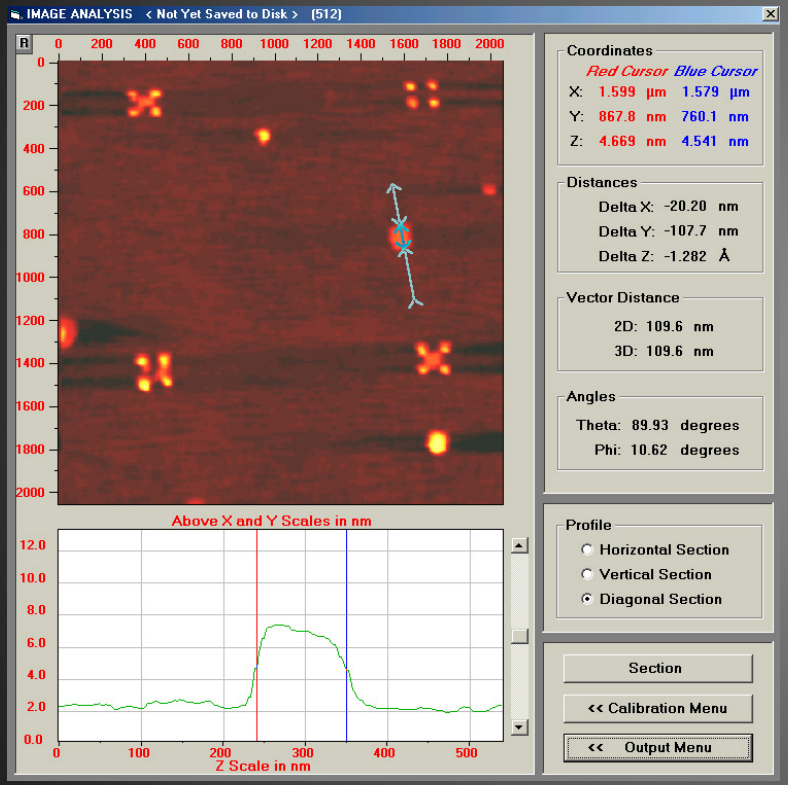
…and more



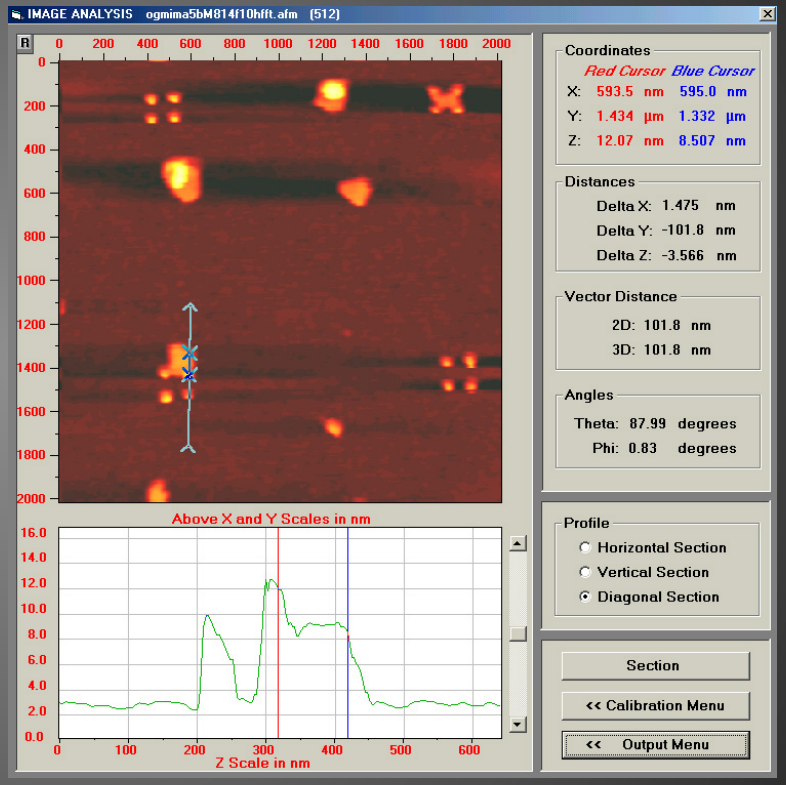


Sections show that immobilized origamis are mostly convex, hanging on average at 85% of the pillars' height: This may allow for use of the bottom face!

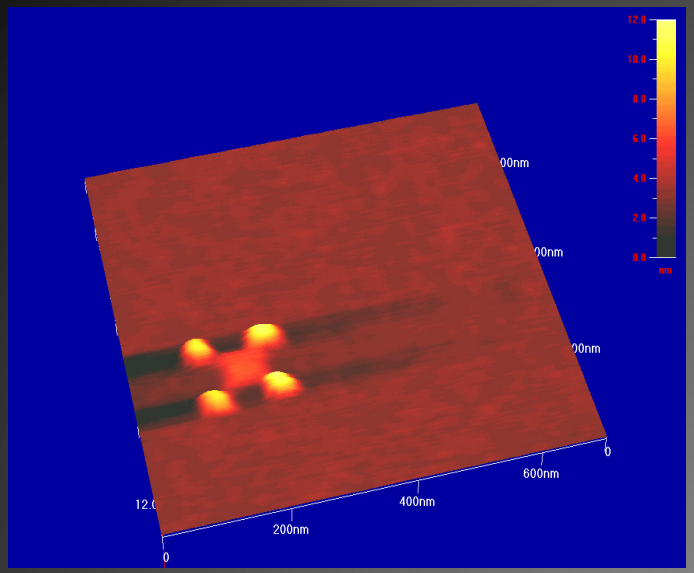
…and more



Cursor distance 88 nm



Cursor distance 81 nm NB:
stands horizontal 6 nm above surface!
Stacking or electrostatics?


12.0
10.0
8.0
6.0
4.0
2.0
0.0
nm
00nm
00nm
00nm
600nm
400nm
200nm
0

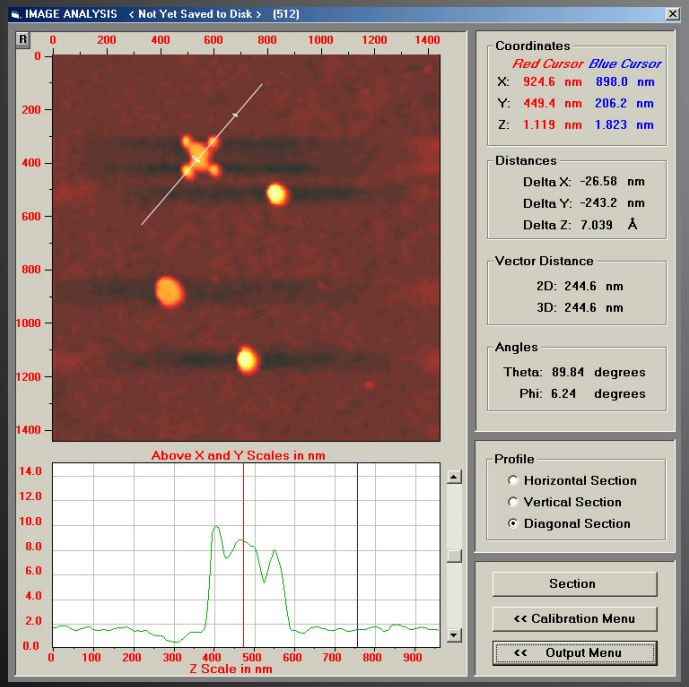


IMAGE ANALYSIS < Not Yet Saved to Disk > (512)
Coordinates
Red Cursor Blue Cursor
X: 924.6 nm 898.0 nm
Y: 449.4 nm 206.2 nm
Z: 1.119 nm 1.823 nm
Distances
Delta X: -26.58 nm
Delta Y: -243.2 nm
Delta Z: 7.039 Å
Vector Distance
2D: 244.6 nm
3D: 244.6 nm
Angles
Theta: 89.84 degrees
Phi: 6.24 degrees
Above X and Y Scales in nm
Z Scale in nm
Profile
Horizontal Section
Vertical Section
Diagonal Section
Section
<< Calibration Menu
<< Output Menu

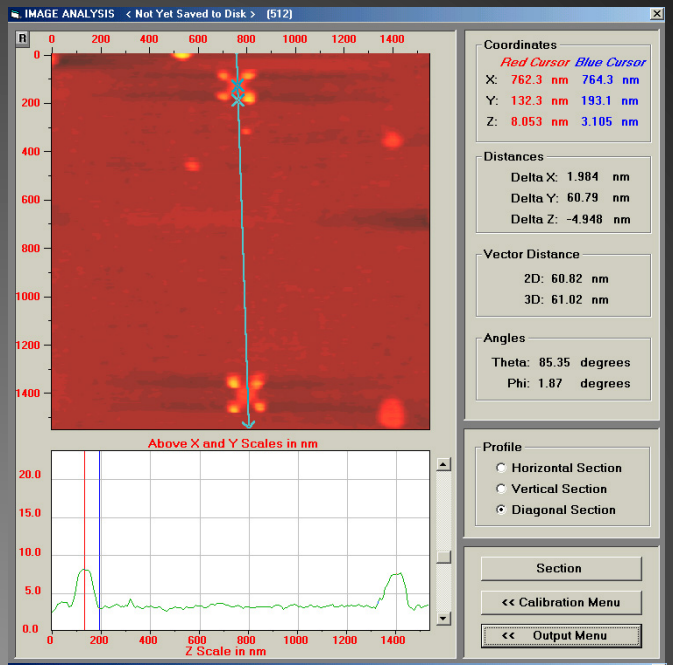


IMAGE ANALYSIS < Not Yet Saved to Disk > (512)
Coordinates
Red Cursor Blue Cursor
X: 762.3 nm 764.3 nm
Y: 132.3 nm 193.1 nm
Z: 8.053 nm 3.105 nm
Distances
Delta X: 1.984 nm
Delta Y: 60.79 nm
Delta Z: -4.948 nm
Vector Distance
2D: 60.82 nm
3D: 61.02 nm
Angles
Theta: 85.35 degrees
Phi: 1.87 degrees
Above X and Y Scales in nm
Z Scale in nm
Profile
Horizontal Section
Vertical Section
Diagonal Section
Section
<< Calibration Menu
<< Output Menu

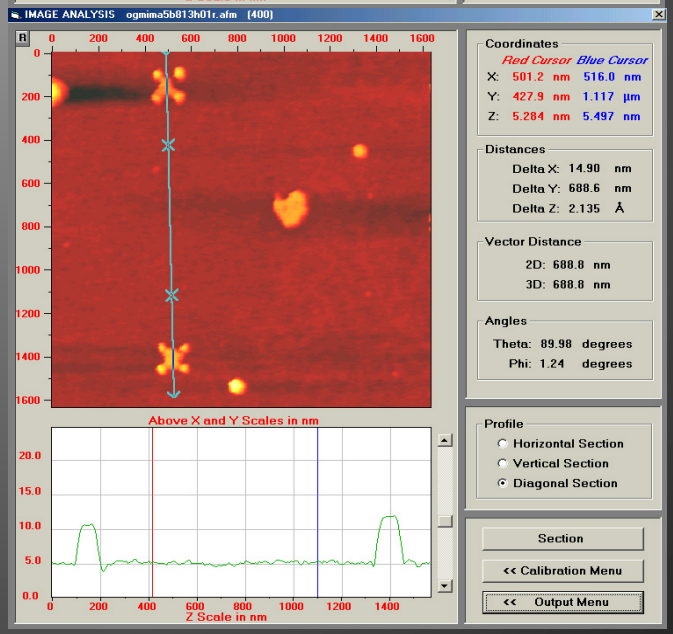
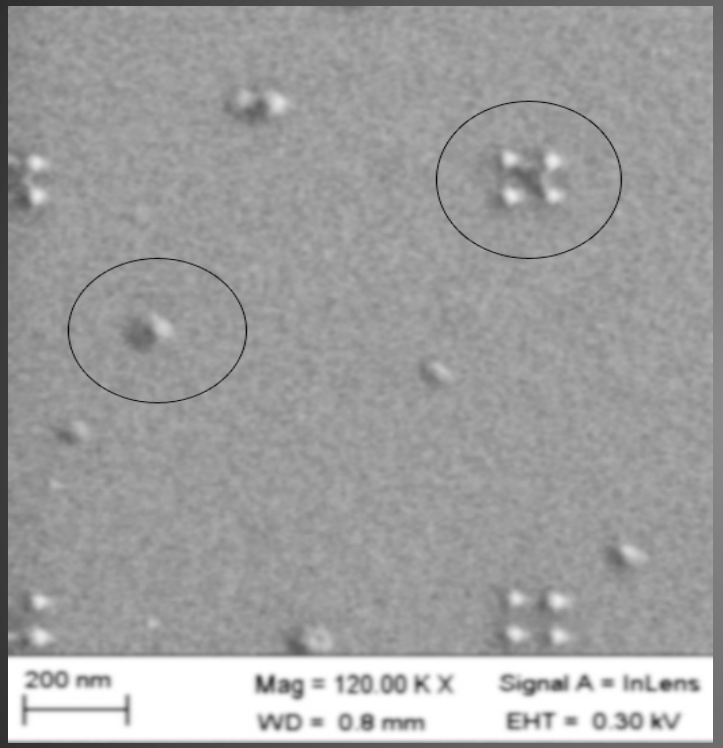
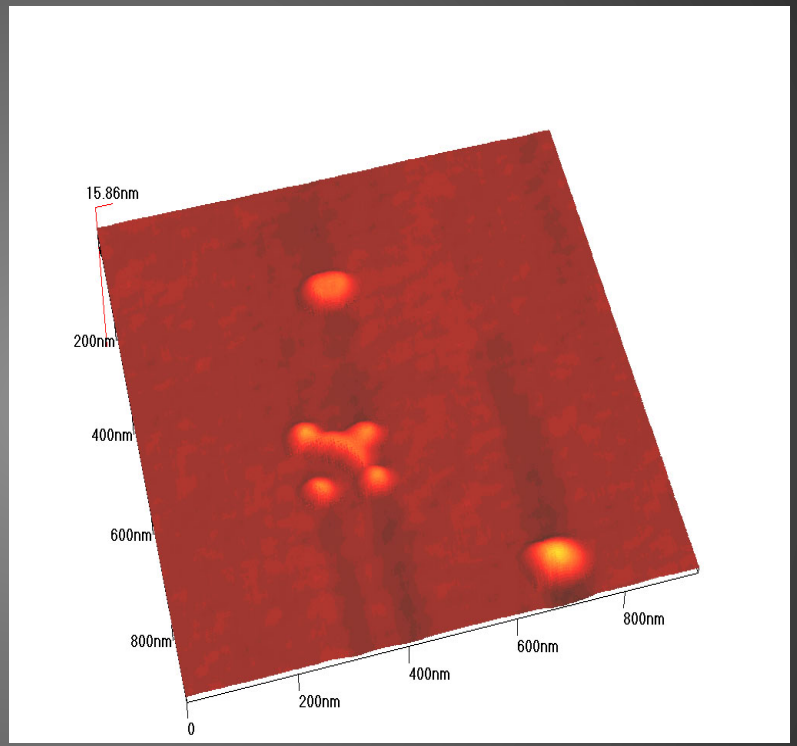


IMAGE ANALYSIS ogmima5b813h01r.afm (400)
Coordinates
Red Cursor Blue Cursor
X: 501.2 nm 516.0 nm
Y: 427.9 nm 1.117 µm
Z: 5.284 nm 5.497 nm
Distances
Delta X: 14.90 nm
Delta Y: 688.6 nm
Delta Z: 2.135 Å
Vector Distance
2D: 688.8 nm
3D: 688.8 nm
Angles
Theta: 89.98 degrees
Phi: 1.24 degrees
Above X and Y Scales in nm
Z Scale in nm
Profile
Horizontal Section
Vertical Section
Diagonal Section
Section
<< Calibration Menu
<< Output Menu

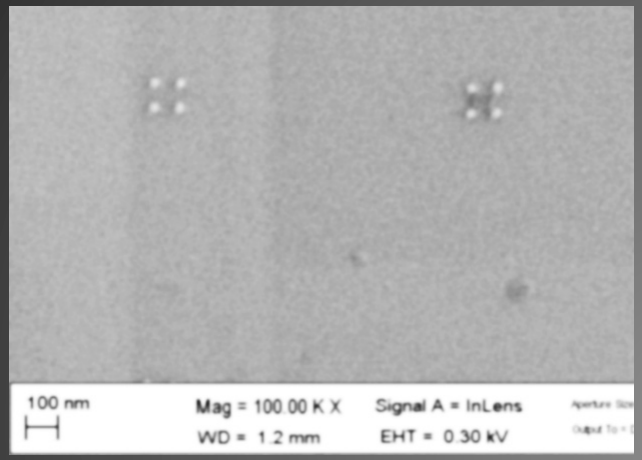
Imaging by SEM at 300 V and comparing to AFM

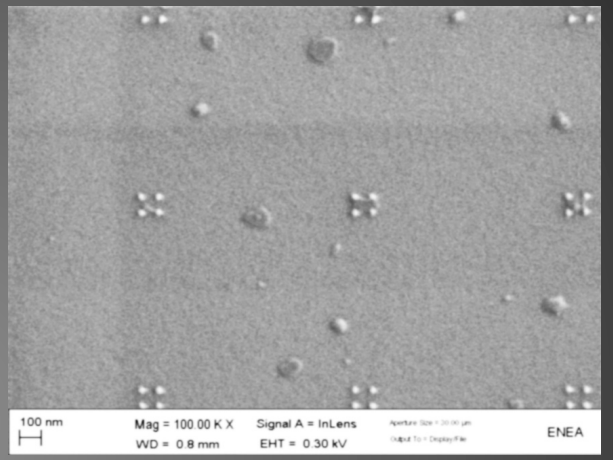


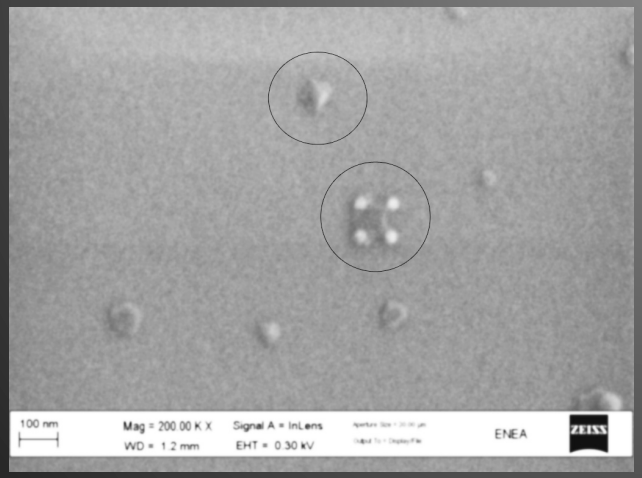


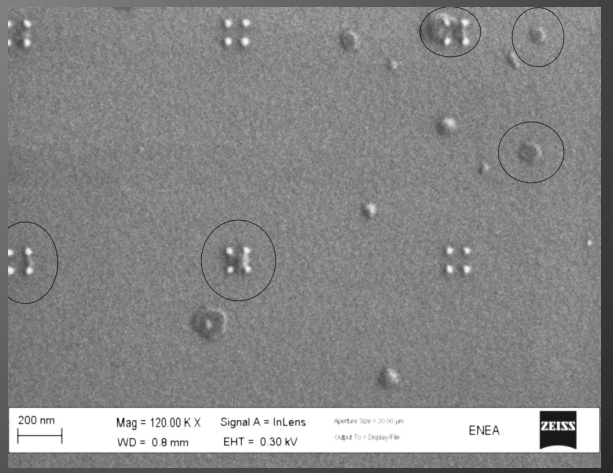
Broken or folded along its diagonal ?

More SEM imaging to have tip-size independent information on x,y





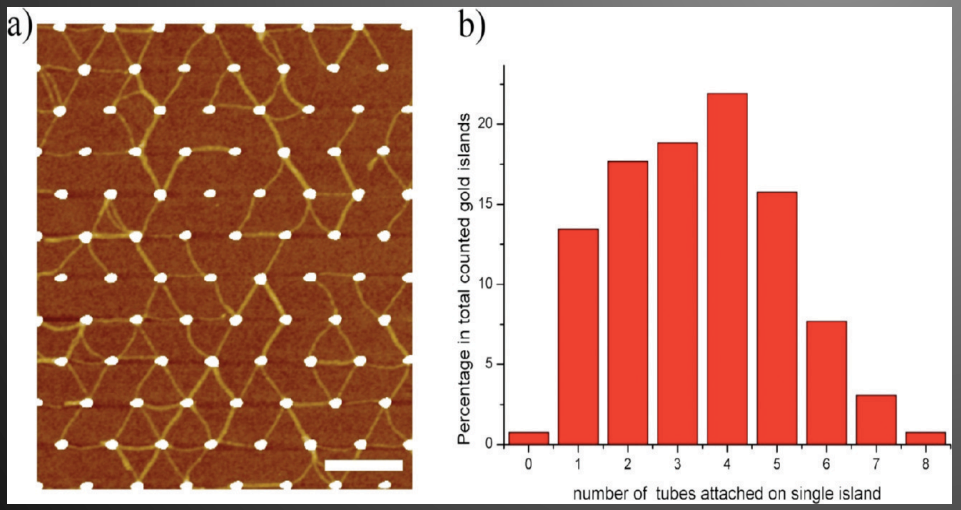


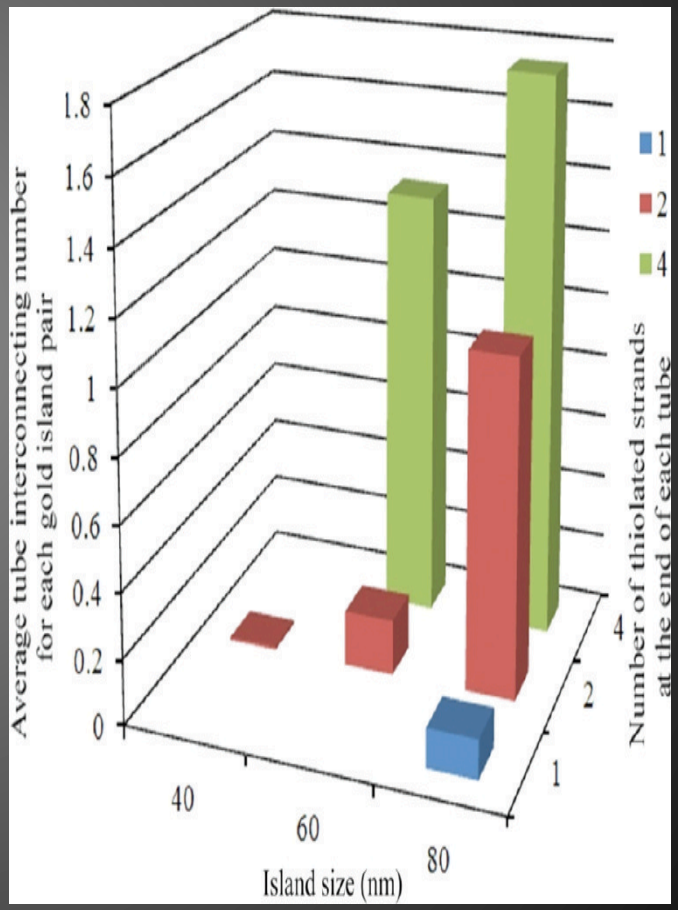


Important parameters

(Hao Yan et al. Nanoletters 2012)

- Size of the gold nanodots
- N. of available thiols
- Concentration of DNA origami
- Counterions concentration
- Time of “incubation”



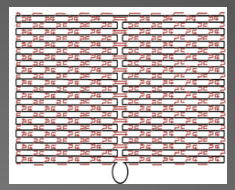


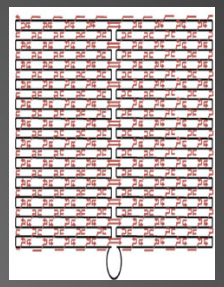
Some interesting results

- Origami immobilized on nanopillars are not damaged by AFM probe tapping
- Immobilized origami observed by AFM and by SEM look similar
- When they are bound to nanopillars they mostly appear convex: repulsion by the substrate?
- Thiolated origami often fold in two, both along the diagonal and along the axis
- Origami immobilized onto gold nanopillars seem very stable, they look the same after 11 months

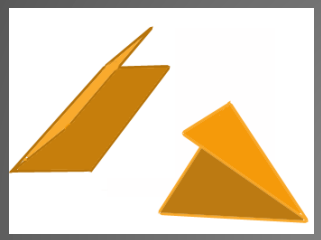
We also learn about “misbehaviours” of immobilized DNA origami

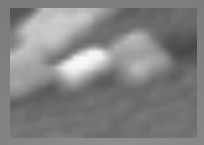
- Squeezing/stretching:



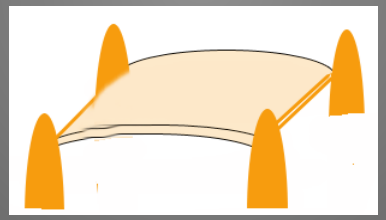


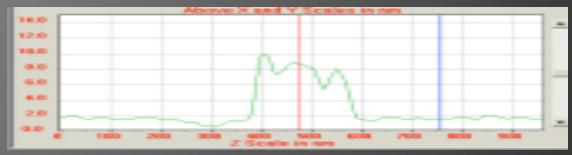
- Folding:



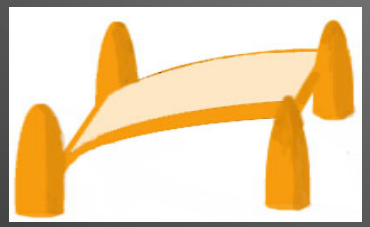


- Bending:

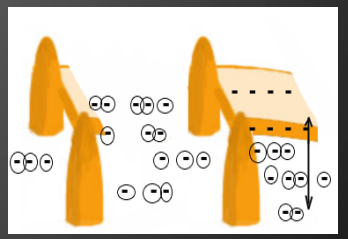




- Sloping:

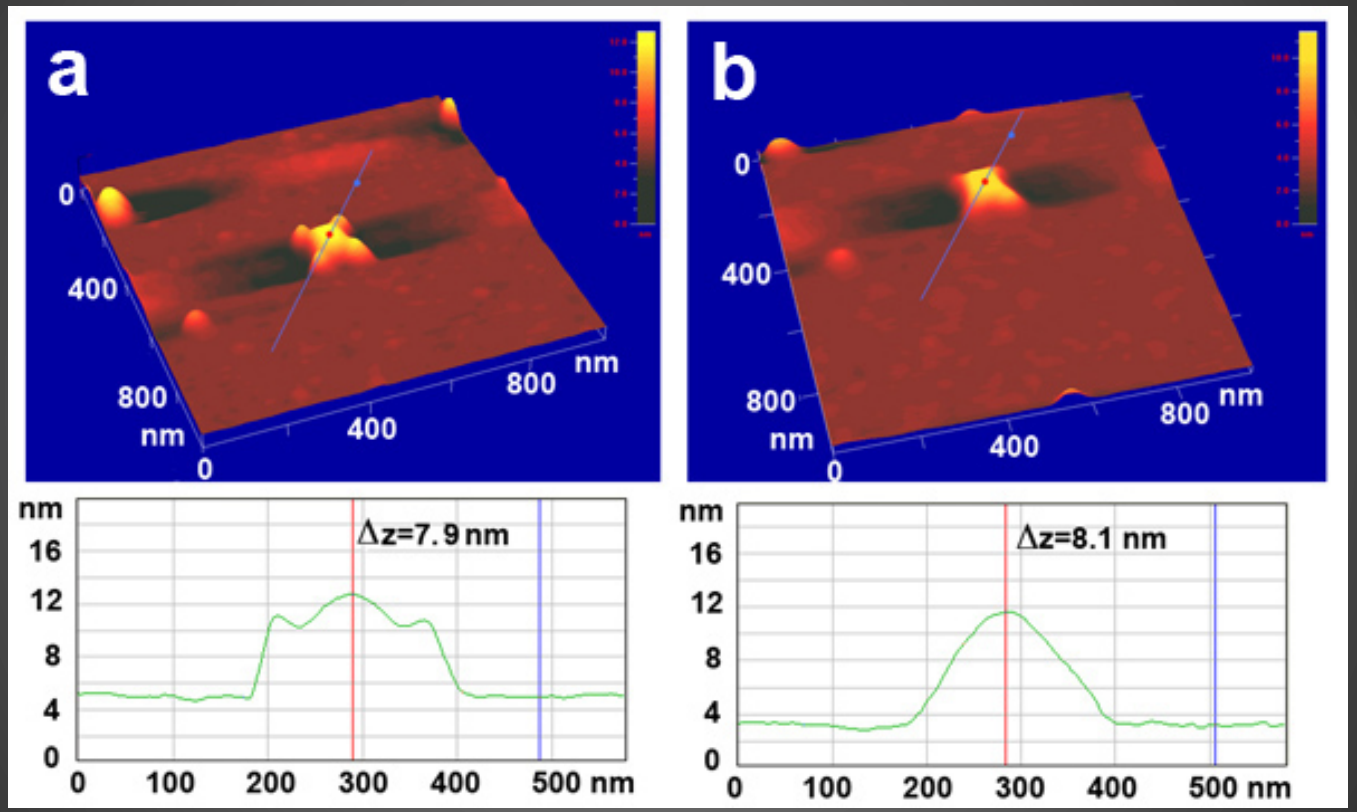


Hanging:



?

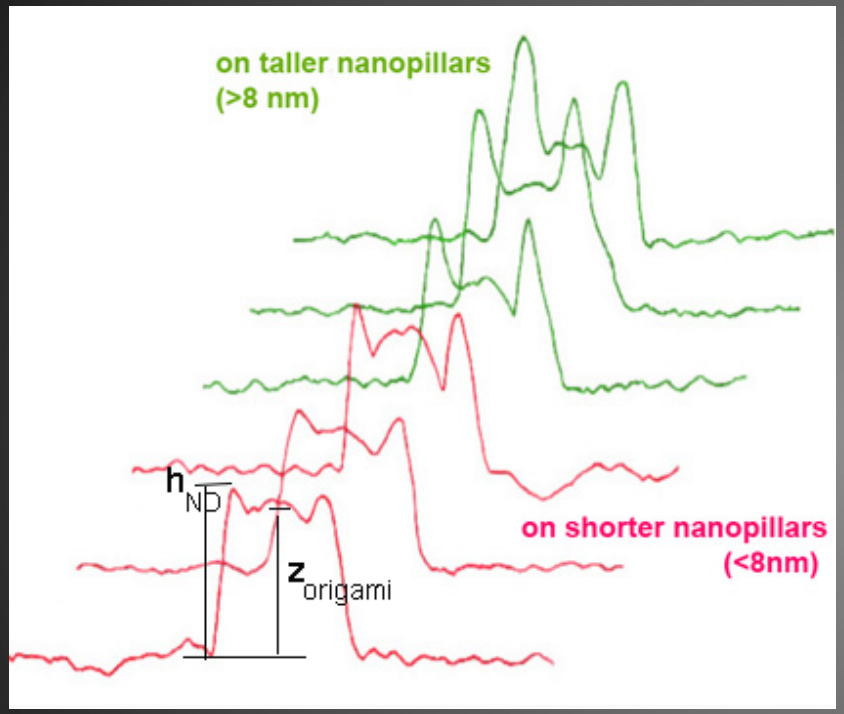
Hammering hard by AFM on suspended origami

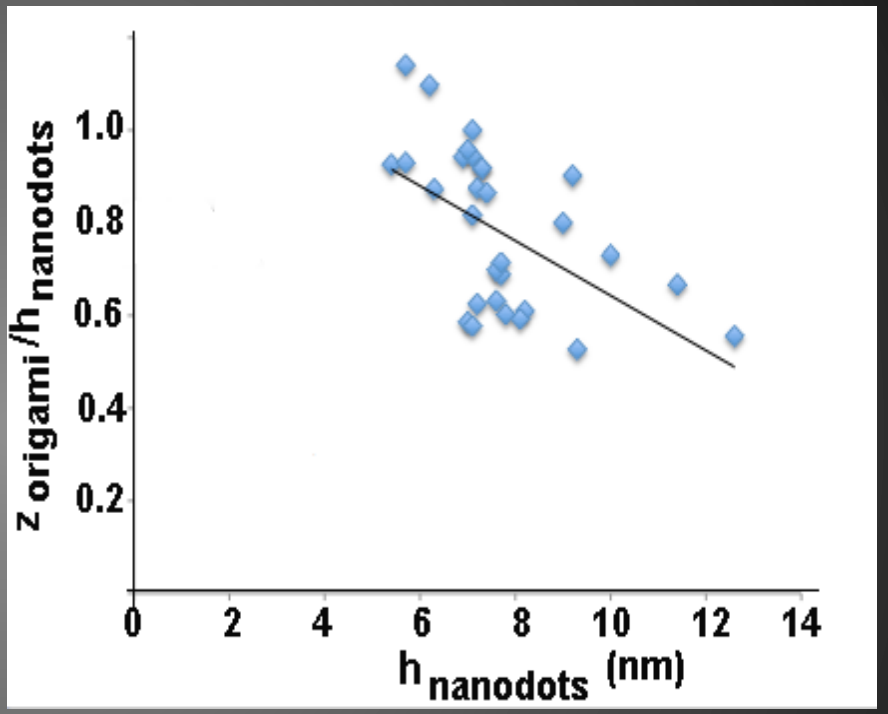


Gentle tapping Hard “hammering” (3 scans)

Surprising! DNA more robust than gold? There must be some support below!

DNA breadboards distance from substrate





Distance from substrate depends on counterion concentration and on nanodots height (among taller nanodots origami "sink" more than among short ones)

Problems

- Our samples are often too dirty with buffer residues
- Origami can stack or/and coalesce into lumps
- Origami adsorb also onto substrate (controlled to a certain extent by counterions concentration)
- Solutes precipitate under the origami, preventing use of lower face
- The estimated percentage of correctly immobilized DNA origami is of the order of 10% on our specimen (nanoanchors are very small)

Possible solutions and future perspectives

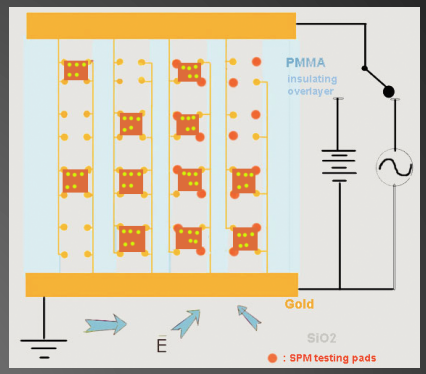
- Connecting nanoanchors electrically:

a) Drives DNA breadboards more efficiently in position, + controlling orientation

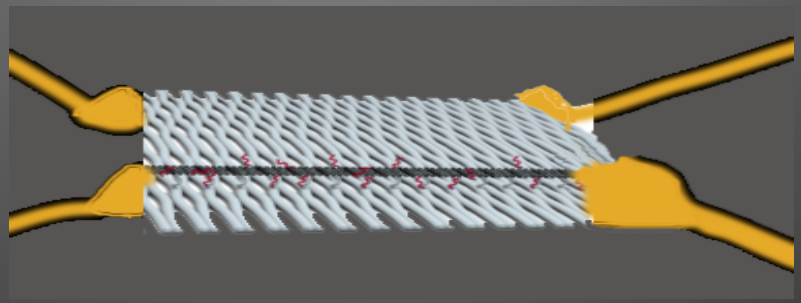
b) Input - output of electrical signals

Next steps

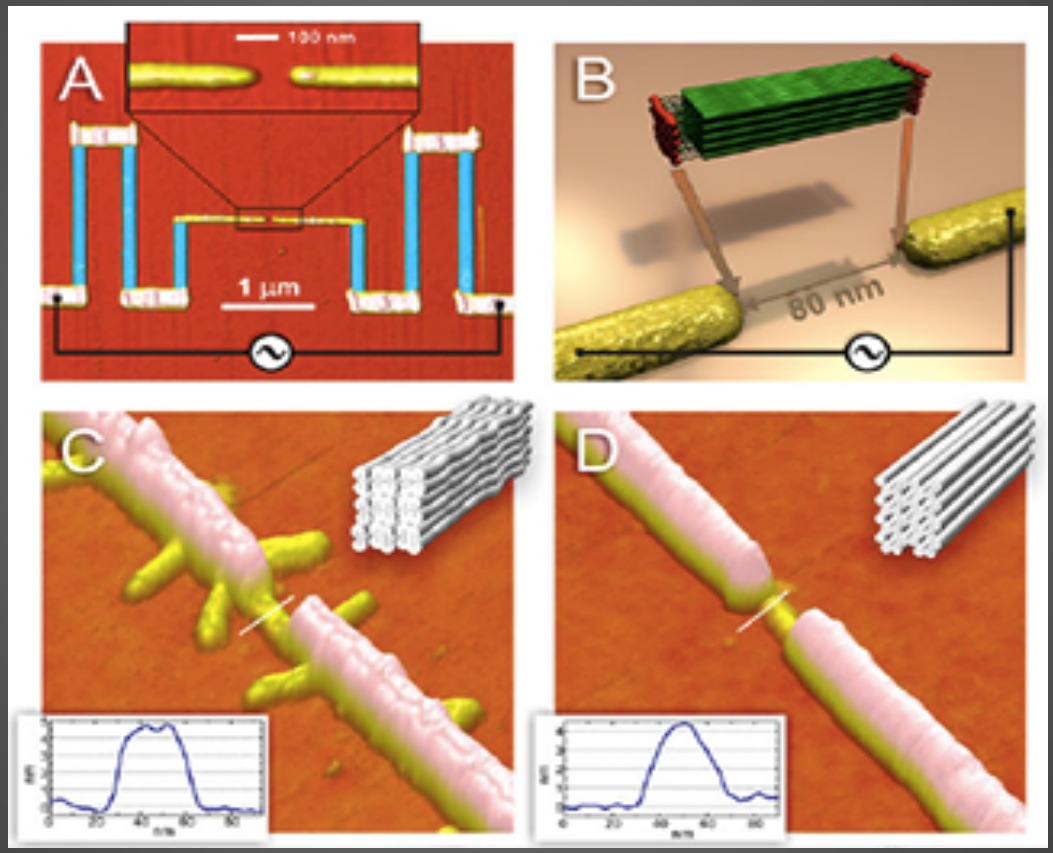
a) Connect nanodots with nanowires and use static fields or dielectrophoresis to deposit origami breadboards



b) Use conductive polymers to define arbitrary electrical paths on DNA origami breadboards



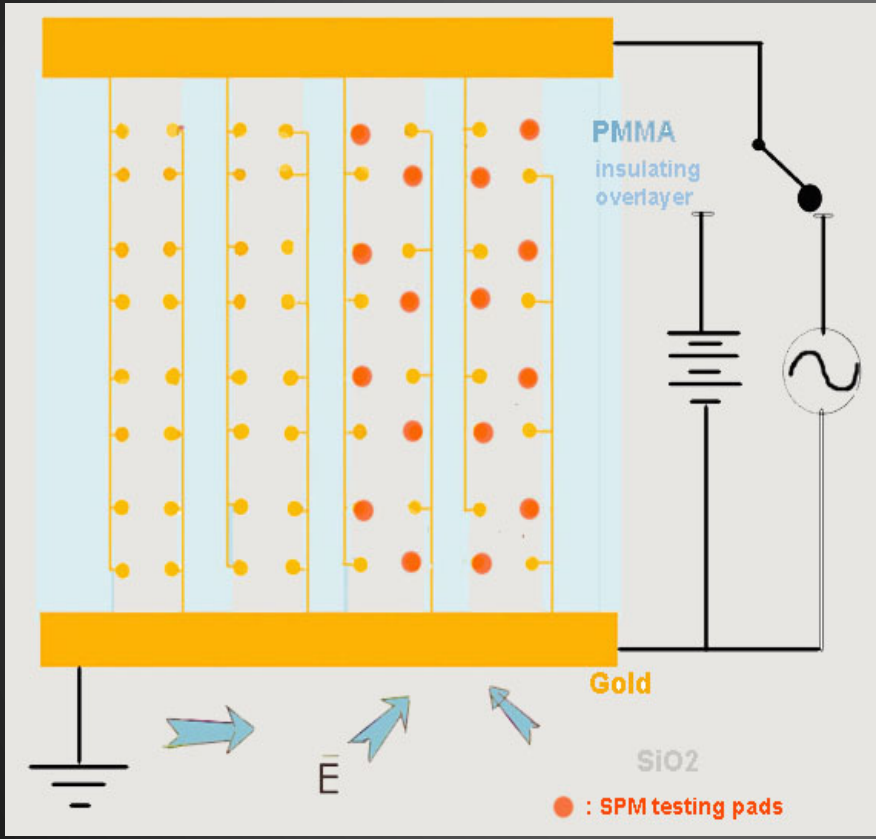
a): dielectrophoresis as a promising solution



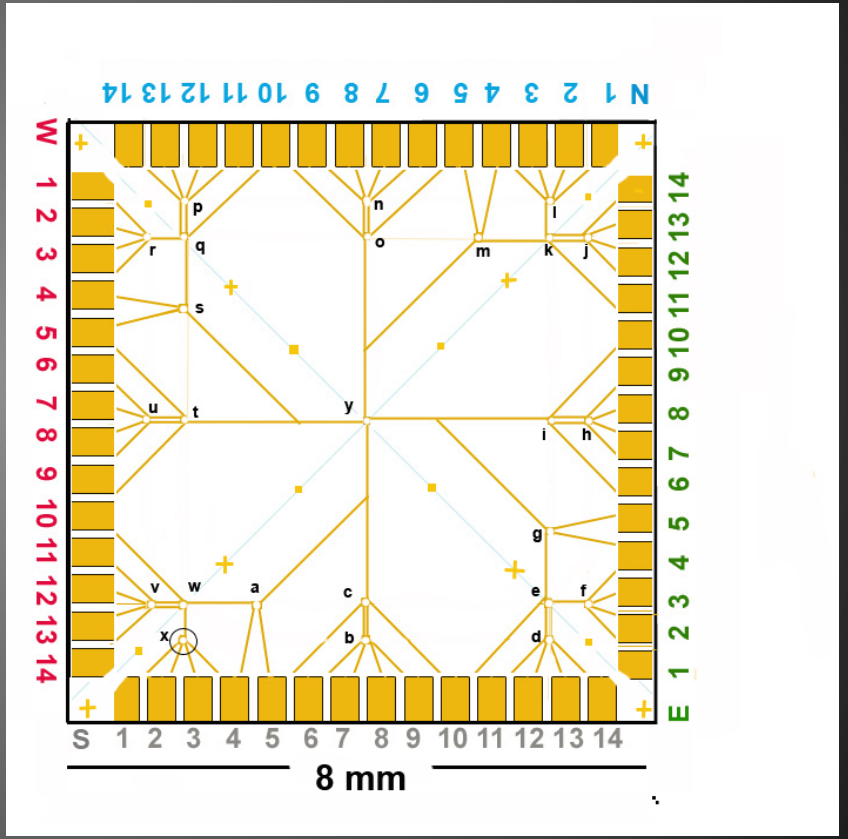
B. Shen, V. Linko, H. Dietz and J. Jussi Toppari

Electrophoresis 2015, 36, 255–262 255

Organizing the device



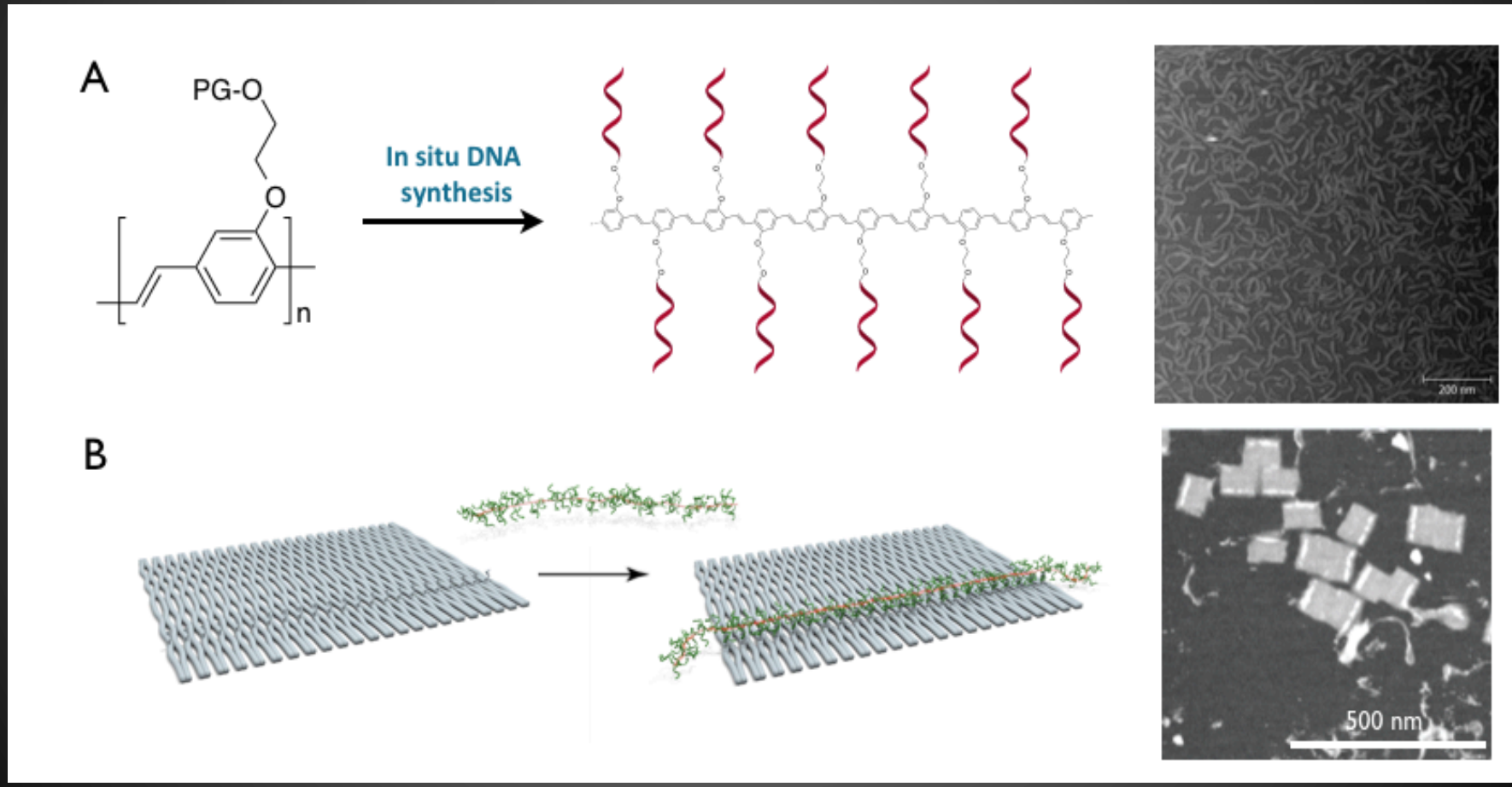
Nanoanchors connected in groups + conductive AFM probing (arrays of 10^4 devices)



Four independent connections on each of only 24 devices

b): conjugate conductive polymers

(2,5-alkoxy) paraphenylene vinylene, APPV

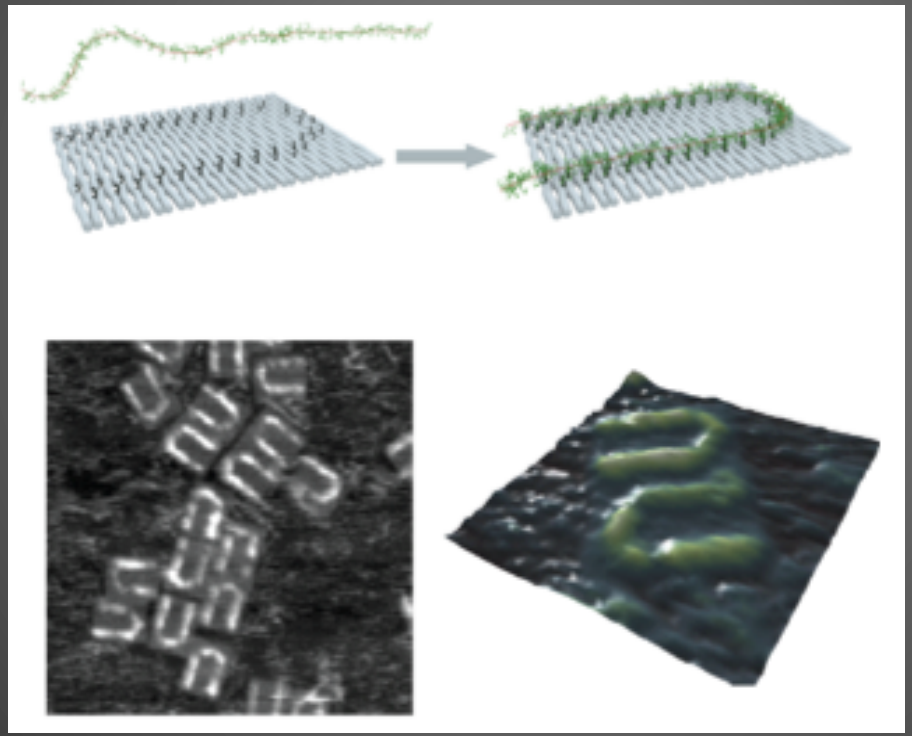


Kurt Gothelf's group at the cDNA Center, University of Aarhus, Denmark

Arranging nanowires on the breadboard

Staples extensions

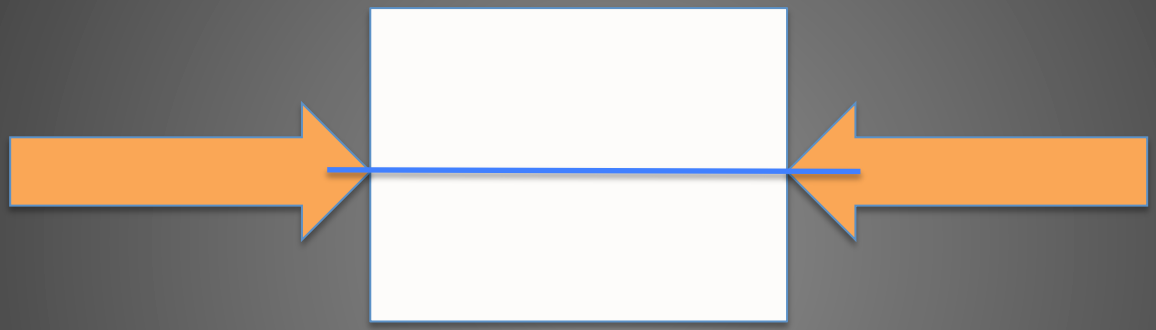
APPV



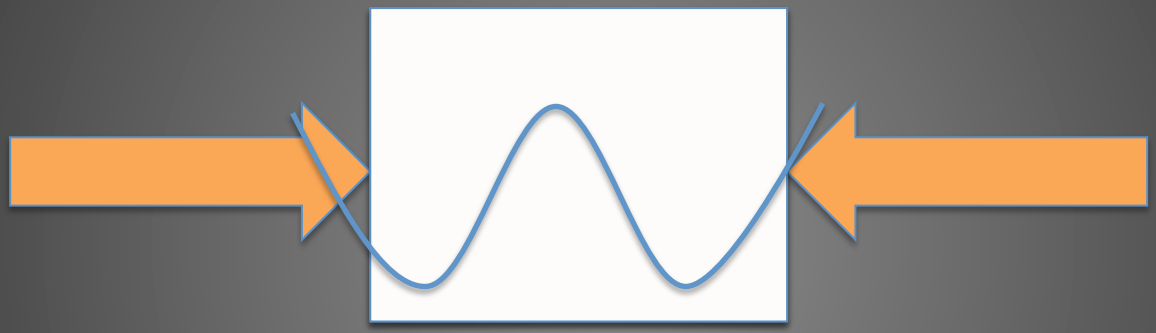
Conductive polymer hybridized on specific staples extensions

AFM

Basic conductivity measurements

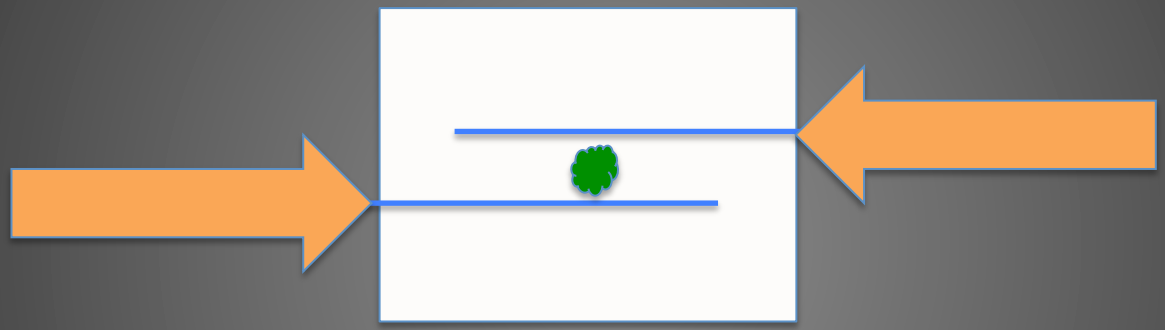


Conductivity vs polymer path

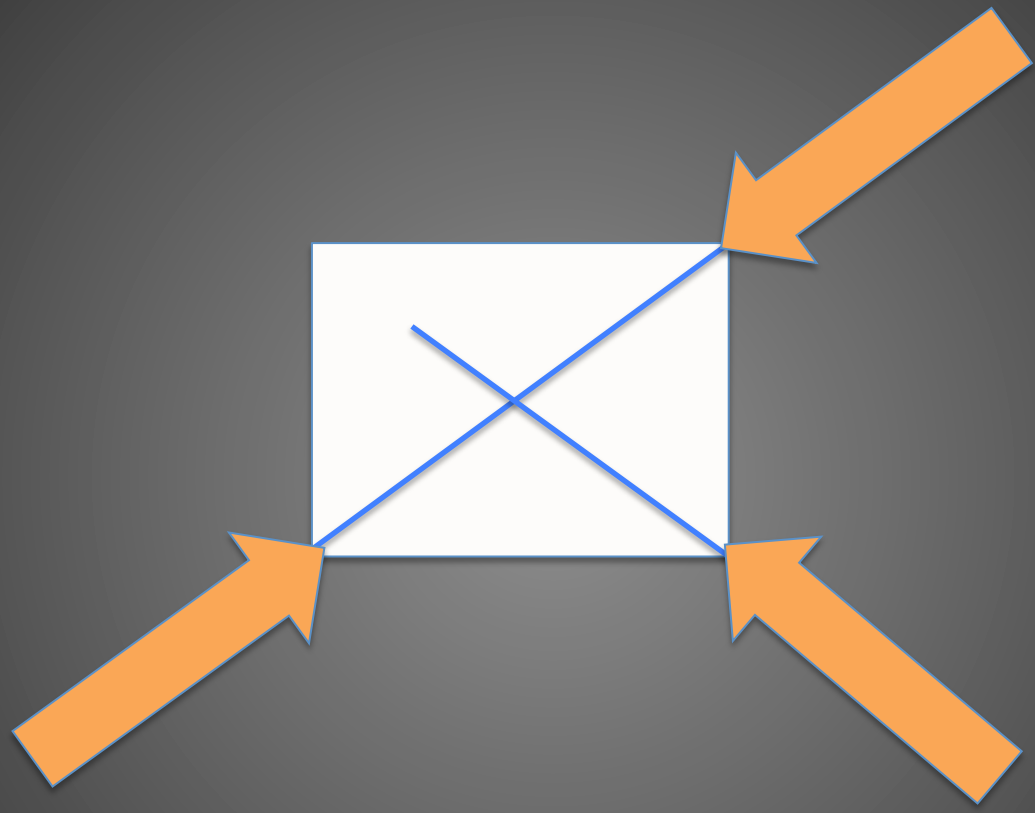


Polymer-polymer electron transfer

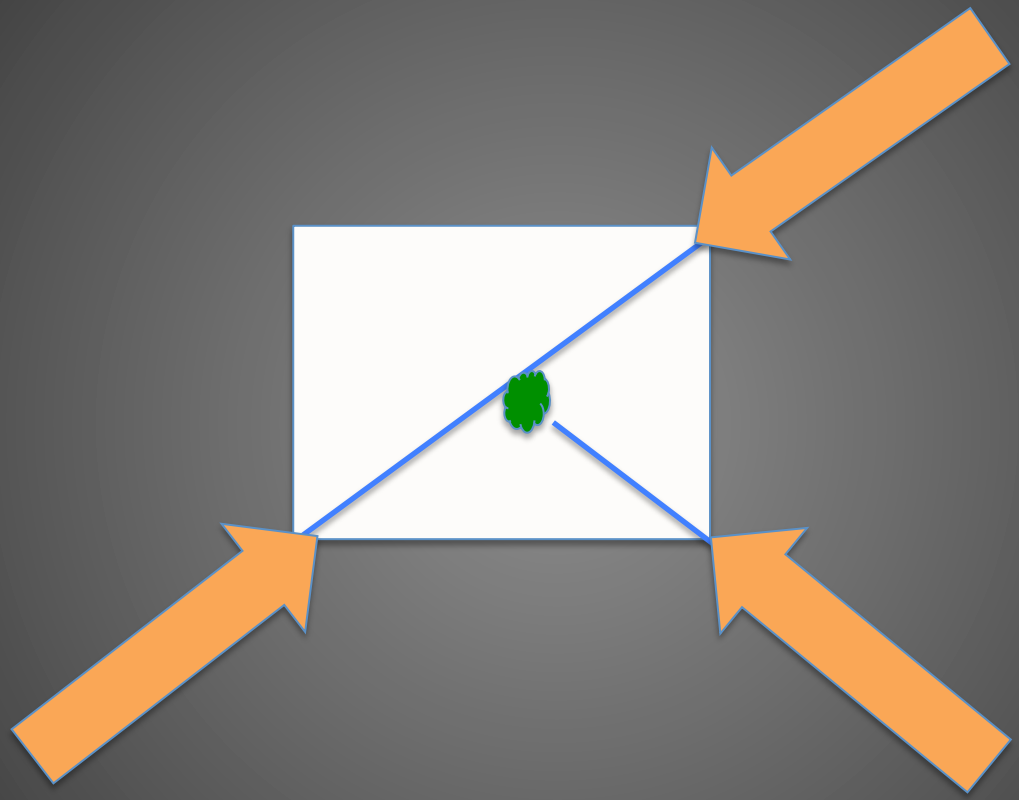
Redox enzyme mediated electron transfer from wire to wire



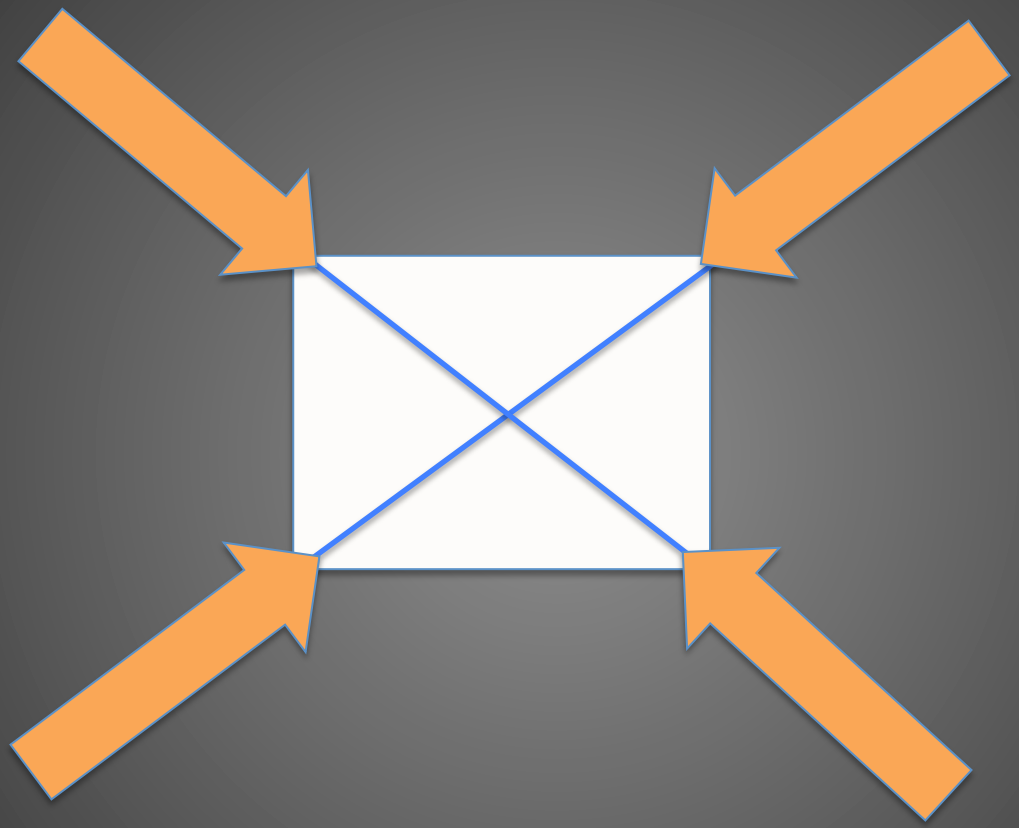
Gated conductivity via three point contacts



Redox enzyme gated conductivity via three point electrode



Gated conductivity via four point contacts



Bringing gold nanoclusters close to one electrode via biomolecular vdW force patterns Probe by FRET SERS TERS (optimize plasmonics)