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NanoInnovation

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Designing innovation by bio-inorganic self-assembly

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Bio-inorganic self-assembly: why?

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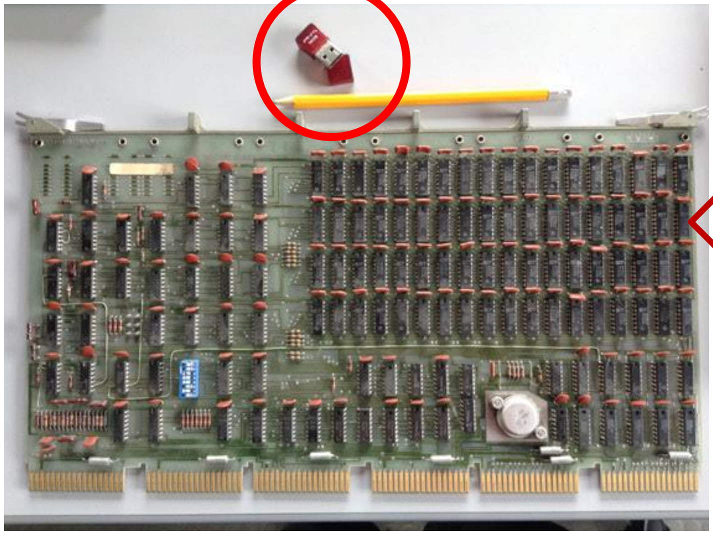
Different approaches to build micro- and nano-devices are needed

1) More than Moore

The number of transistors in a dense integrated circuit doubles approximately every two years (Gordon Moore, 1965)

2015, saturation



1976 *vs* 2013
16 Kb vs 16 Gb
info density 150x10^6 higher

But also:

2) "Top down" fabrication technologies in electronics are highly energy intensive (expensive) → **Fabrication of each laptop requires 2x10^{10} Joule of energy (> than a car)**

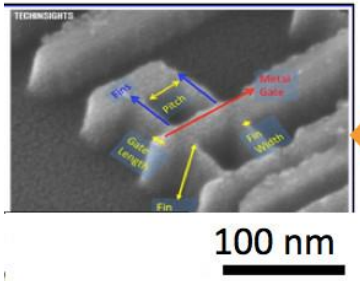
3) High production levels to sustain the costs → **2 billions of new mobile phones in 2013!**

4) Sustainability problems in terms of disposal and waste; environmental pollution → **90 millions of new smart TV and 500 millions of computers**

5) "Bottom up" molecular electronics not yet available for production → **All natural systems are built by "bottom up" processes!**

"Top down" *vs.* "bottom-up" technologies

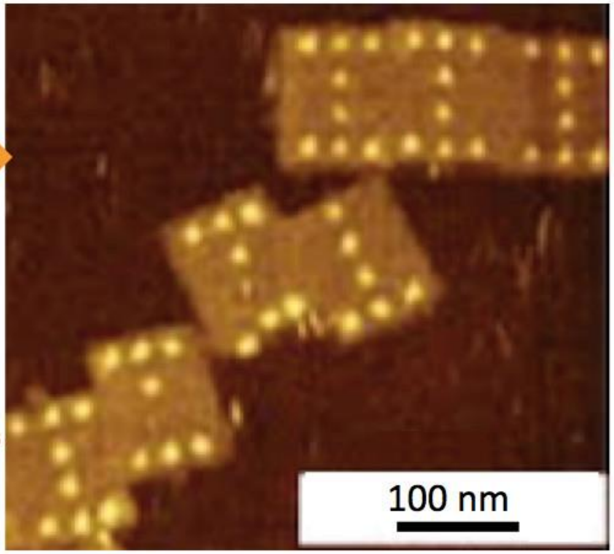
one bit of information



A three-gate transistor, 22 nm technology

same scale

Streptavidine molecules on rectangular DNA origami breadboards



up to 400 topological bits per nanoboard + large n. of different functions

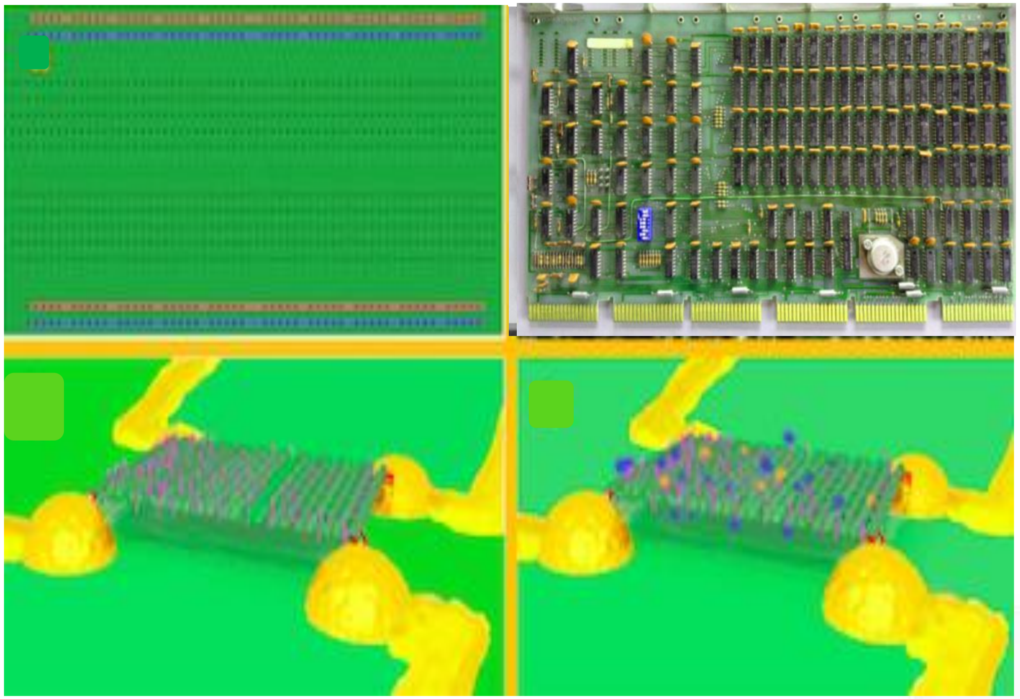
Auto-assembly and adhesion properties of DNA and proteins

- Bottom-up synthesis in a parallel process: MANY DEVICES AT THE SAME TIME!
- "bottom-up" bio-mimetic technologies: low energy to build devices
- Biomaterials disposal may be easier than for silicon and metals

NanoLego: steps to order and connect molecular components

Fiberglass boards, polymer, metals and silica components, soldered together (35 cm x 20 cm)

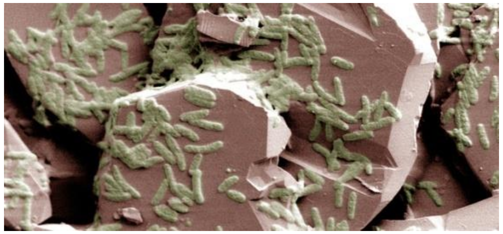
Self-assembly: DNA board (100 nm x 70 nm); proteic components on DNA; DNA on gold electrodes



Bio-inorganic interactions

Based on selective links between peptides (due to charge and conformation) and inorganic materials. Spread in nature:

Geobacter, coating iron oxide minerals



Shewanella oneidensis, that attaches to iron oxide clays

Bacterial biofilm

Bio-mimetics

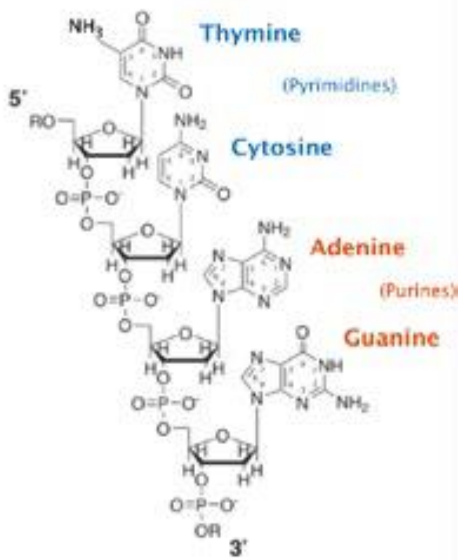
Selection of peptide sequences with high affinity and specific for adhesion to different materials (noble metals, oxides, minerals, semi conductors)

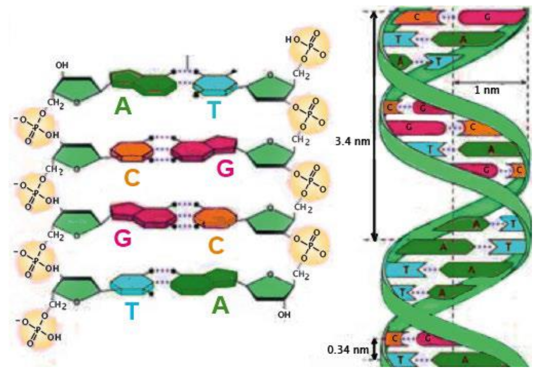
Ideas of application

- **Organic on inorganic: DNA nano-board on gold as support and viceversa**
- **Inorganic on organic : functional components (like quantum dots) on DNA nano-board**

DNA molecules to build nano-boards

From double strand discovery (Watson e Crick, 1953) to Nanotechnologies (Seeman, 1982)





ds diameter: 20 A° (2 nm)

distance between the basis: 3.4 A°

10-10.5 nucleotide pairs per helix turn (~3.5 nm)

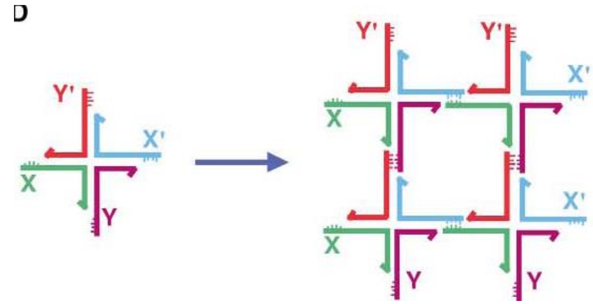
Specific pairing between nucleotide basis (ss) → double strand (ds)

DNA exact replication and expression : **Universal system of genetic information**

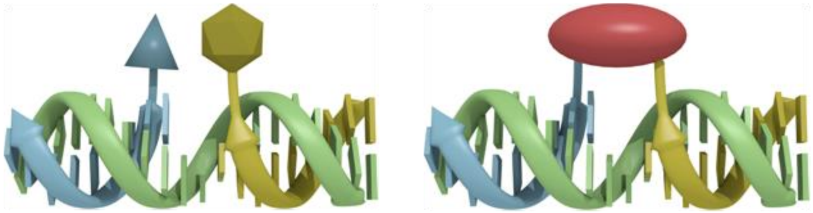
Auto-assembled structures from programmed nucleotidide sequences: **DNA Nanotechnologies**

The “DNA breadboards” concept

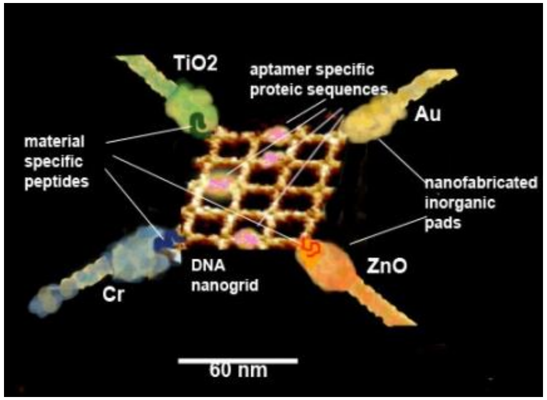
Self-assembly of the DNA “breadboards”: base complementarity and sticky ends allow building of DNA nanostructures

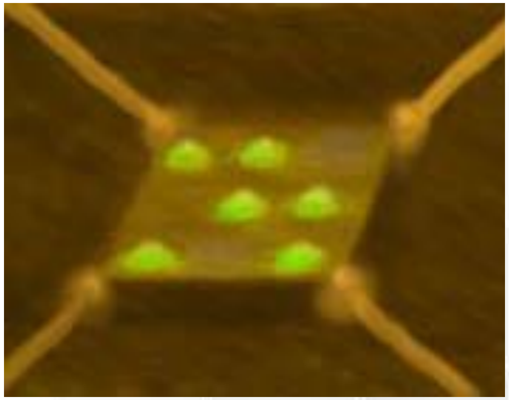


Functionalization: proteins as smart components, and DNA aptamers as connectors



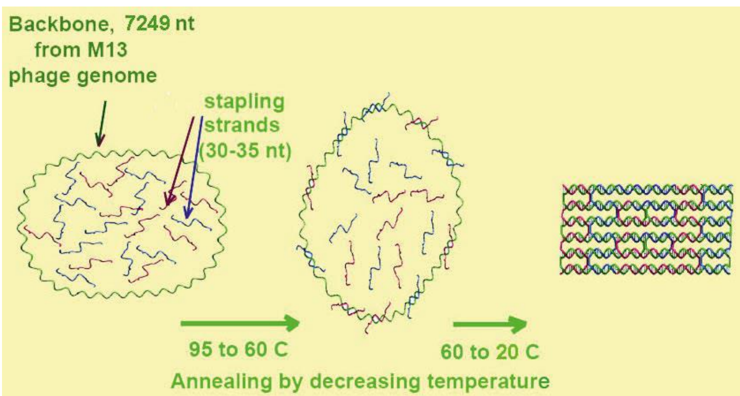
Immobilization on inorganic surfaces: examples of mechanical connections via material selective peptides linked to DNA grids; gold connections by thiols on DNA “origami”

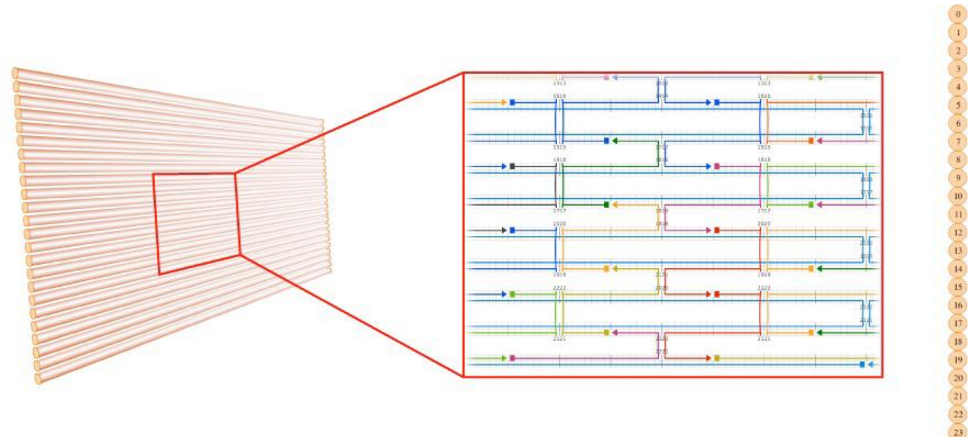




DNA origami self-assembly

Bacteriophage M13 ssDNA folding by means of base pairing of selected regions with complementary oligonucleotides (staples strands)





M13 ssDNA 10 nM → scaffold

216 oligonucleotides 200 nM each→ staples

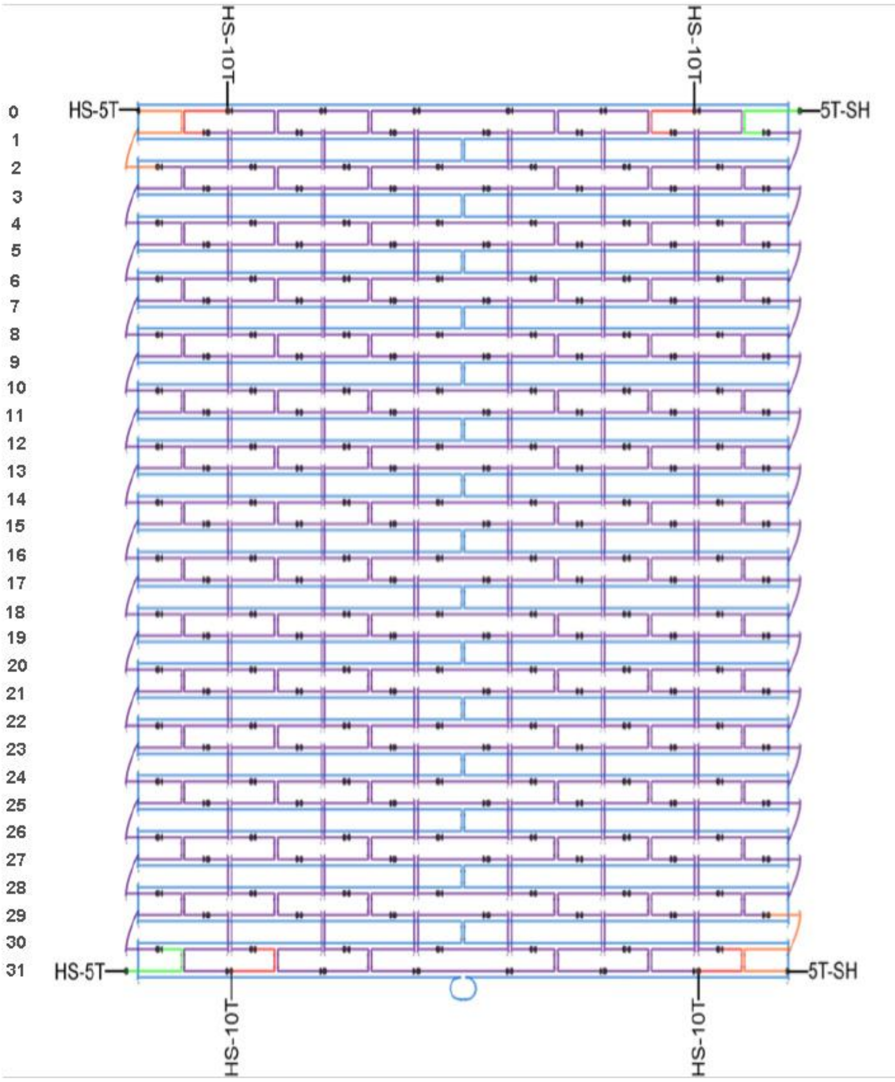
8 oligonucleotides TH (-SH group) → anchorage

Buffer TAE + Mg^{2+} , volume 25 μl

DT = 1° C /min

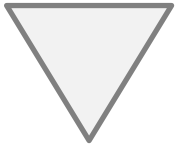
85° → 55° C (1h) → 45° C (1h) → 15° C (1h) → 4° C

Self-assembled origami



measures: 95 × 75 nm^2

- **M13mp18 ssDNA (scaffold)**
- **216 oligo (staples)**
- **8 oligonucleotides with thiol groups** (2 at each corner) for anchorage to gold nanodots



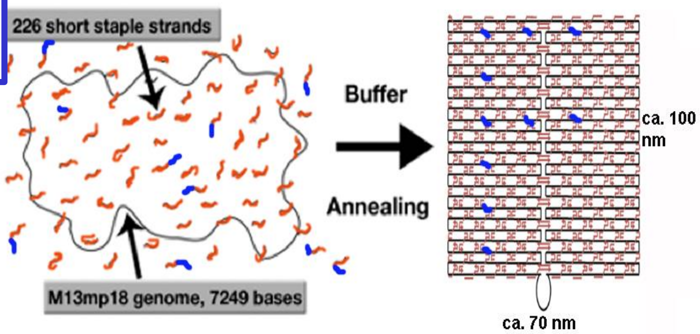
5T-SH (3') **5T-SH (5')** **10T-SH (5')**

Specific sequences in selected sites to link origami with:
- **functional molecules**
- **inorganic surfaces**

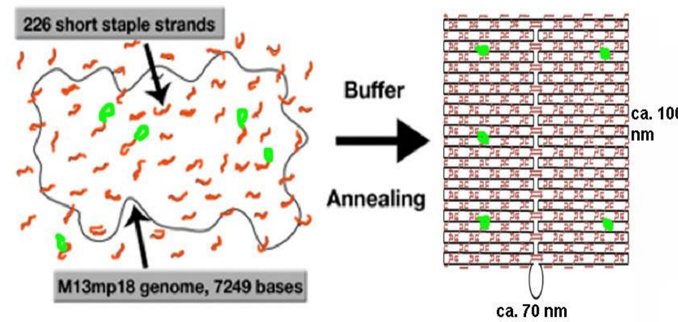
Functionalization of DNA origami

Molecular self-assembly → origami architectures with:

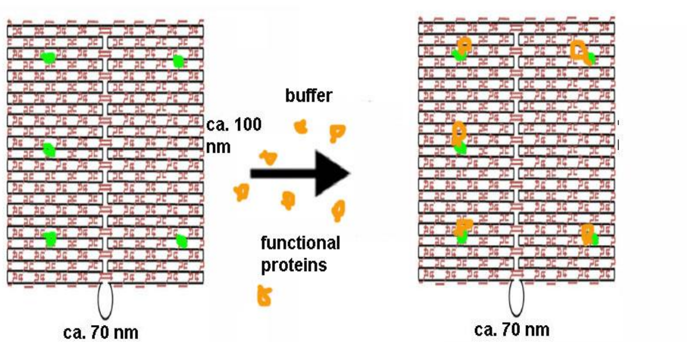
A “sticky” DNA extensions



B protein-specific DNA aptamers

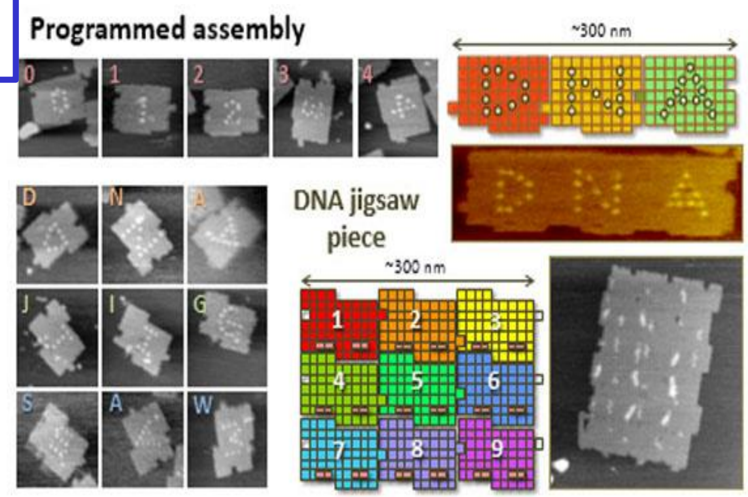


C aptamer bound functional proteins

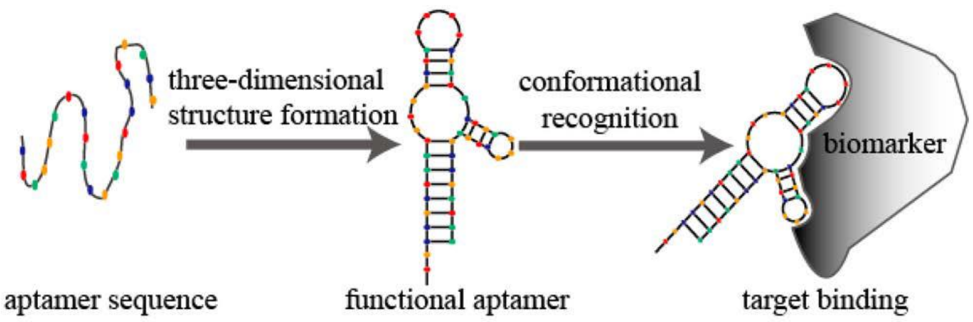


one specific, selectively addressable, sticky location every 6 nm approx., 220 locations per origami (7.000 nm^2), ~ 30.000 per μ^2

D multiple stacked origami

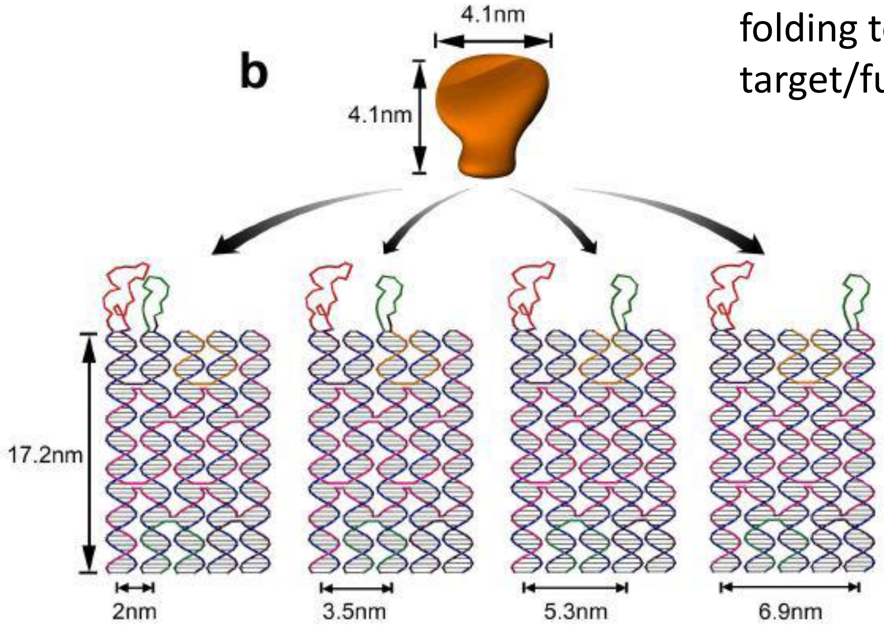


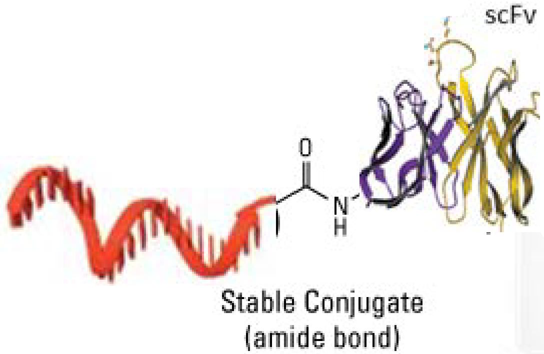
Aptamers and antibodies as connectors



Aptamers
single strand DNA molecules (length 30-70 nt) with specific tridimensional structure

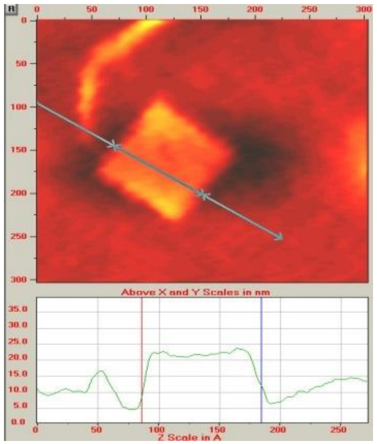
Extended staples by aptamers with the sequence resulting in the desired folding to link the target/functional molecule



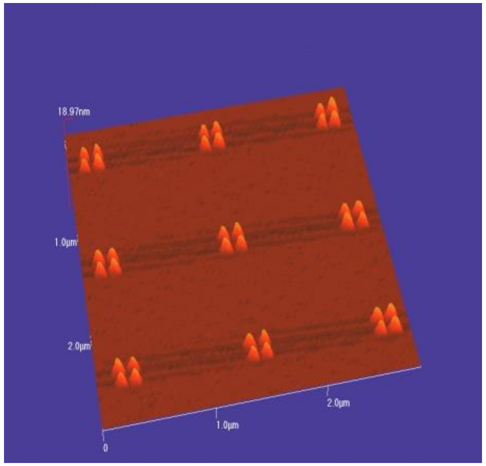


DNA linked to **antibody**

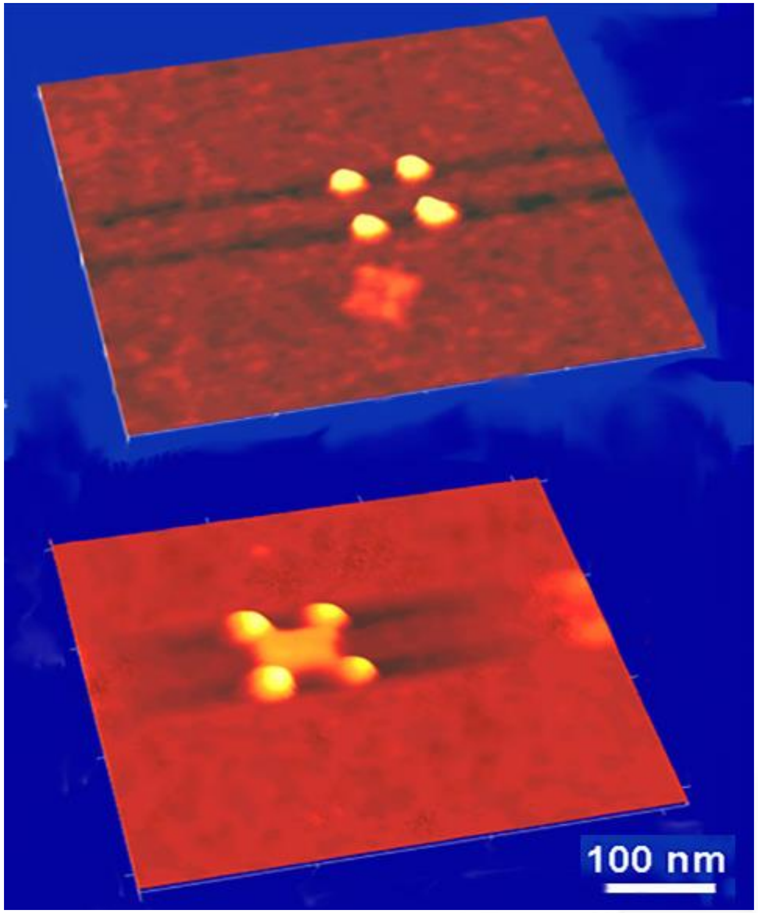
Immobilization of DNA nano-boards



Origami
74 nm x 70 nm
2 nm thick



Gold nano-anchors arrays
Each nanopillar (NP):
7.5 nm tall
25 nm diameter
80x80nm spacing
1000 nm group to group



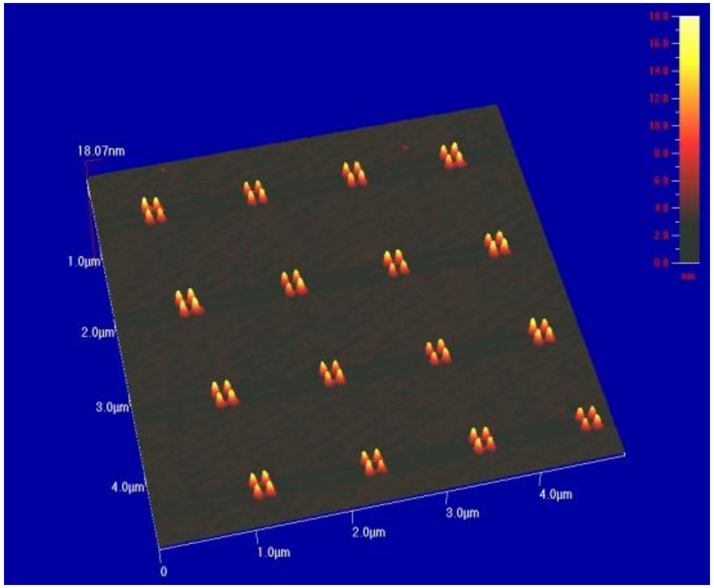
DNA Origami on the substrate
Suspended at 85% of NP height

Publications

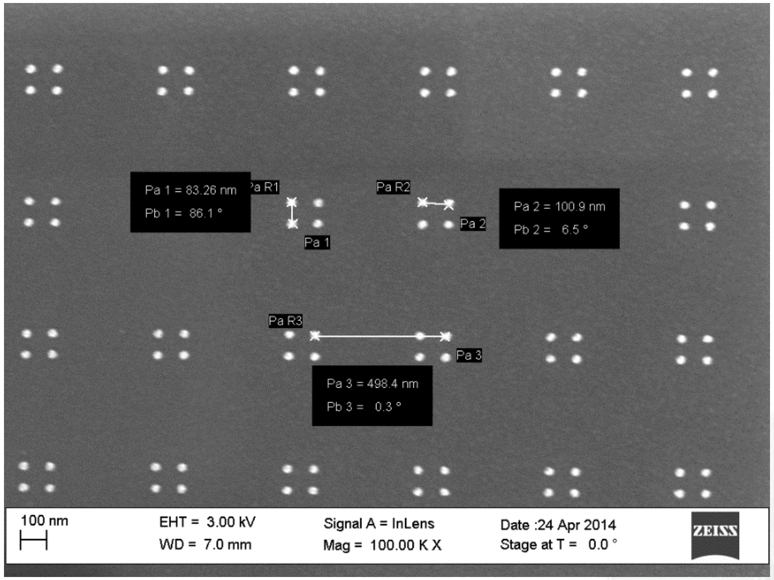
- **Wang et al. EAI,Energia, Ambiente e Innovazione 3/2015**
- **Morales et al. Small, 2015**

Top down lithography for immobilization of DNA nano-boards on Au or Pt

- After synthesis, origami are distributed in the solution or randomly deposited
- To use these shapes as nano-boards, they have to be precisely addressed onto predesigned locations
- This will make possible to exchange signals from biochemical reactions occurring at specific nanometrically addressed locations



High quality electron -beam lithography for gold anchoring nanopads

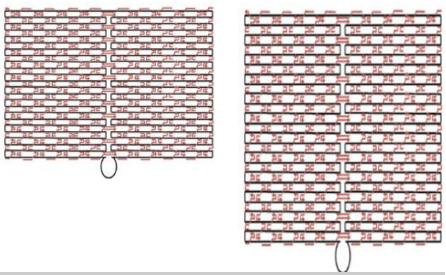


25 nm diameter dots, spacing 80 nm center-center
Intergroup spacing 500 nm

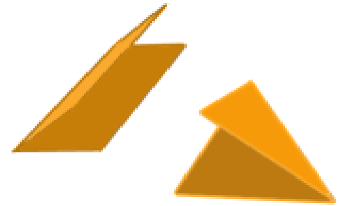
Important parameters

- **Size of the gold nanodots**: a reasonable compromise between precision and probability of docking
- **N. of available thiols**: affects the stability of docking
- **Concentration of DNA origami** to optimize the yield of docking
- **Counterions (Mg^{2+}) concentration** to optimize the yield of docking (both DNA and silicon oxide are negatively charged)
- **Time of incubation**

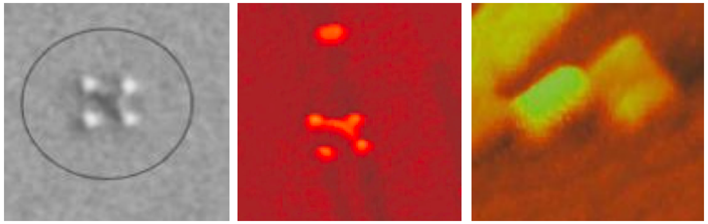
“Misbehaviours” of immobilized DNA origami



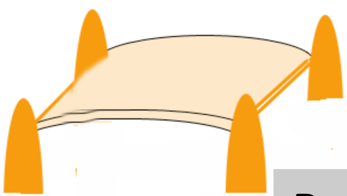
Squeezing/stretching

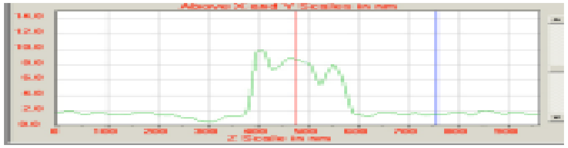


Folding



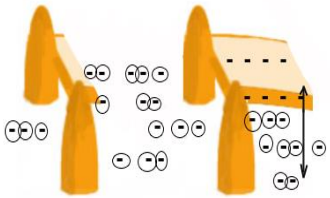
SEM and AFM imaging: broken or folded?



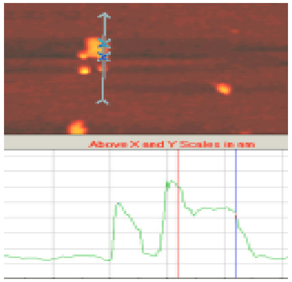


Bending

Sloping



Hanging



Problems

- Samples are often too dirty with buffer residues
- Origami can stack or/and coalesce into lumps
- Origami can adsorb onto substrate (relationship with counterions concentration, Mg^{2+})
- Solutes precipitate under the origami → preventing use of lower face
- The estimated percentage of correctly immobilized DNA origami is ~ 10%: low probability of setting on small nanoanchors

Possible solutions and future perspectives

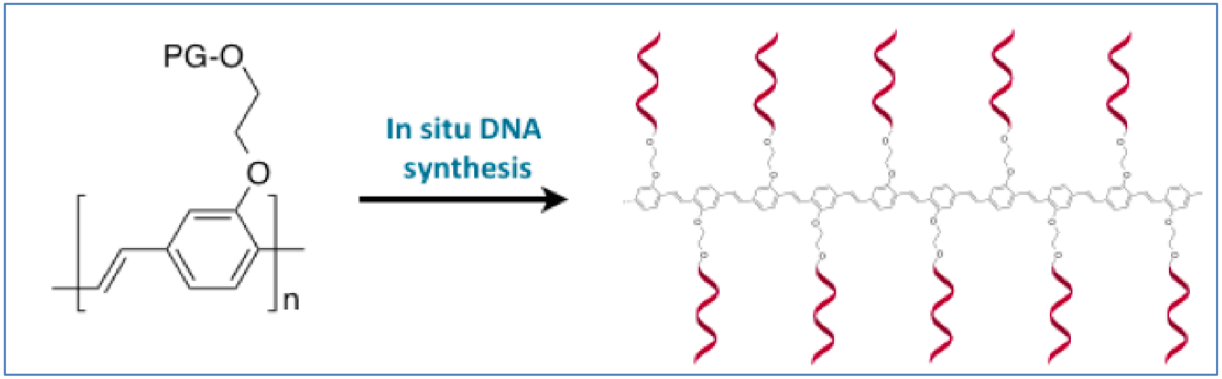
Connecting nano-dots electrically

- to drive DNA bread-boards more efficiently in position and controlling orientation

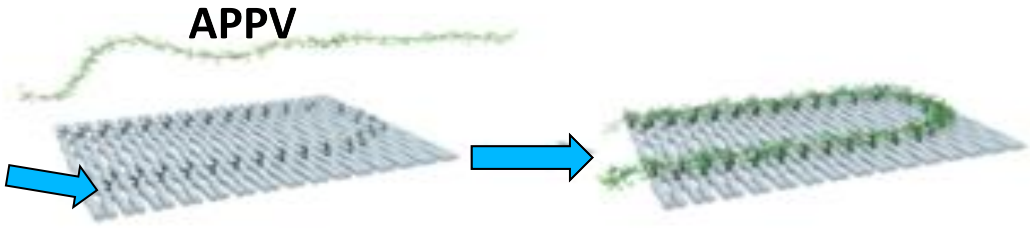
- to gain input-output of electrical signals

Arranging nanowires on the breadboard

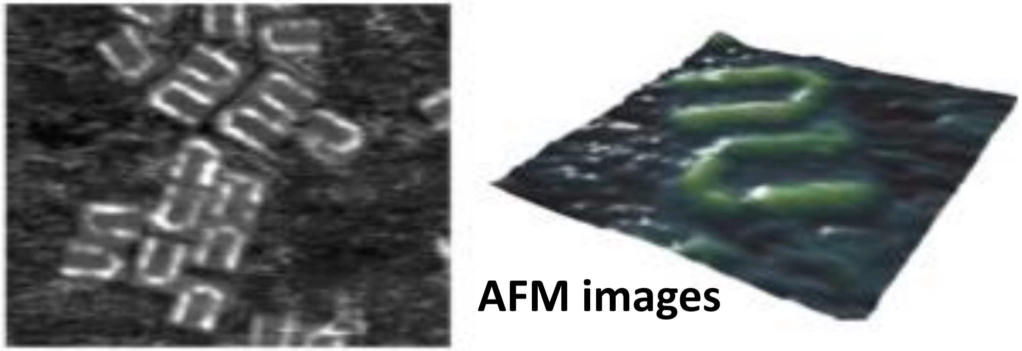
Alkoxy-para-phenylene vinylene, **APPV**



ssDNA complementary to staples extensions



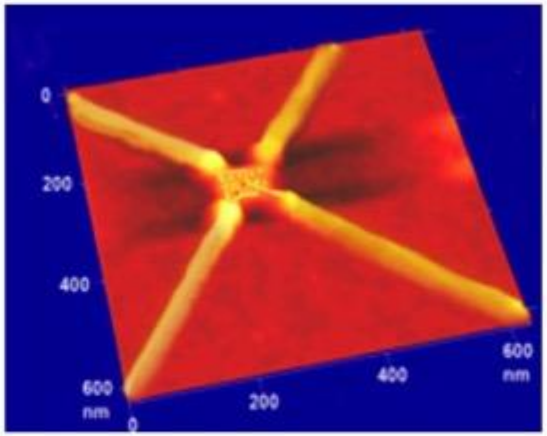
APPV conductive polymer hybridized on specific staples extensions



AFM images

Gothelf et al. 2016

Next goals: electrical connection of breadboards, experiments in molecular electronics



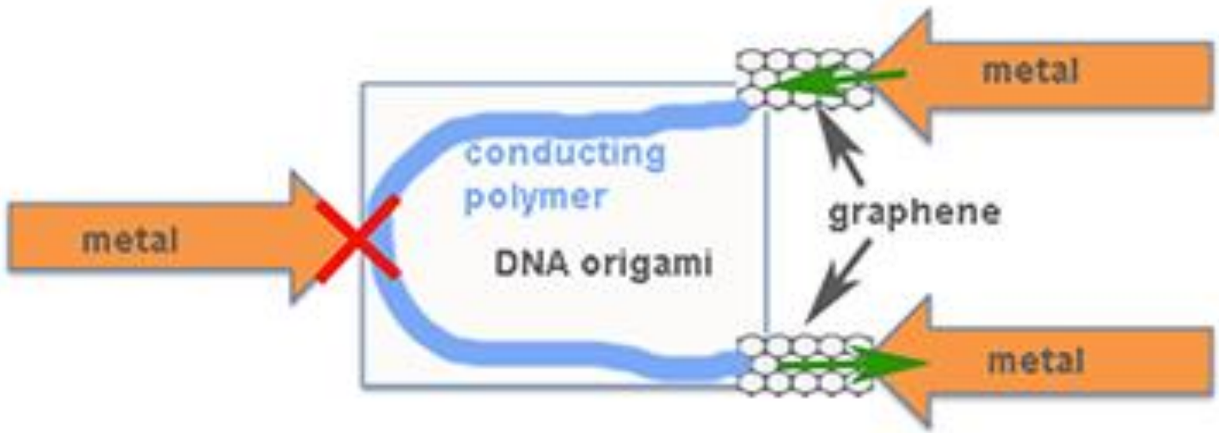
a) Pt and graphene nanoelectrodes instead of gold ones

b) He ion lithography of graphene

c) Dielectrophoresis to increase docking yield and speed

d) Computer simulations and surface specific peptides to dock: 1) breadboards on different substrates

2) Quantum dots on breadboards

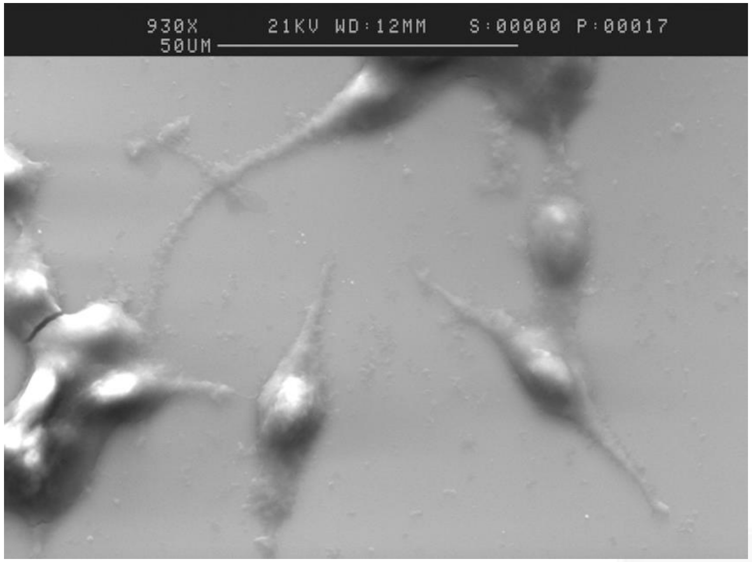


Bio-inorganic self-assembly applications

Specificic bio-bio and bio-inorganic interactions offer a wide range of possible self-assembly based applications

To be explored:

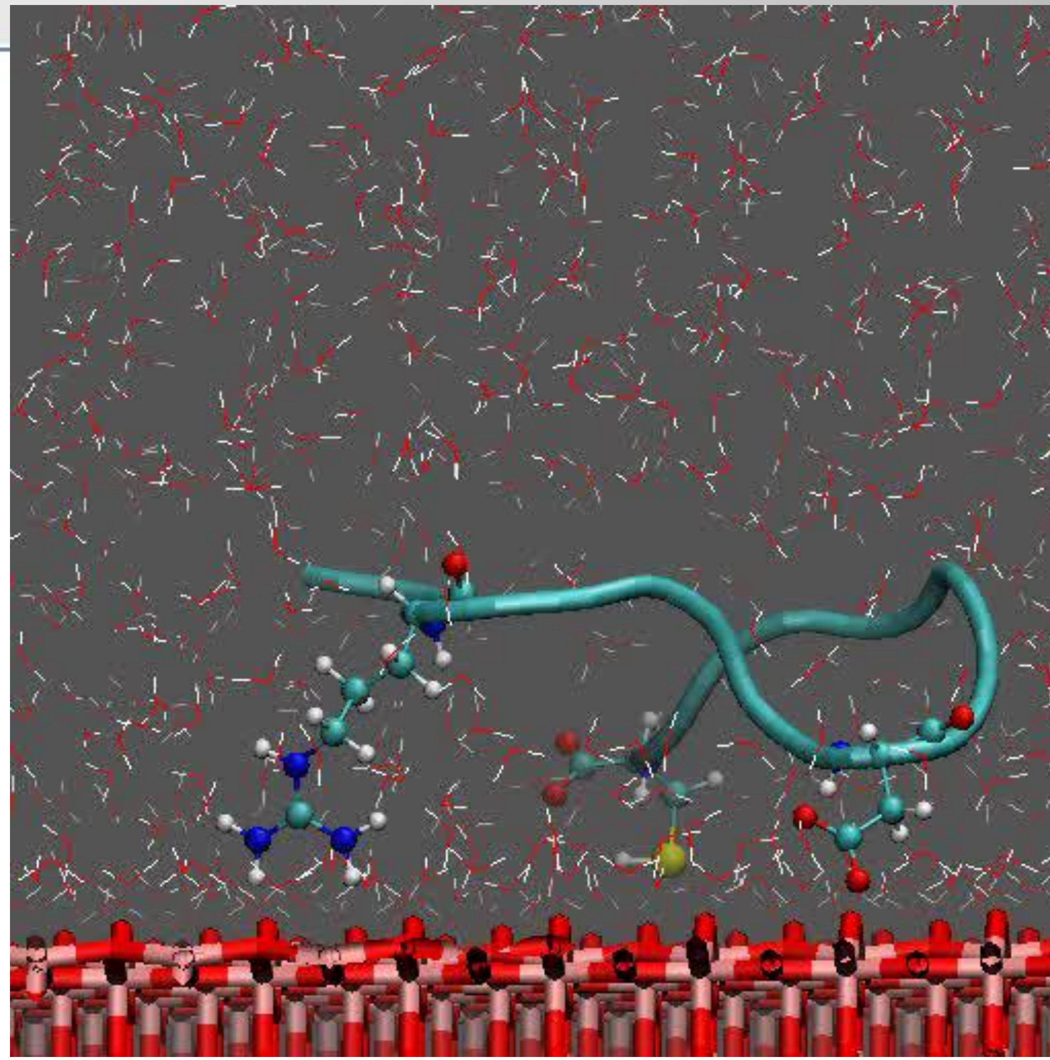
- **Molecular Electronic and ICT**
- **Sensors and biosensors**
- **Nanophotonics and plasmonics**
- **Self-assembly of functional materials**
- **Drug delivery systems**
- **Genomics and proteomics**
- **Neurological and orthopedic implants**
- **Surface-specific adhesives**



Neuroblastoma cells on Silicon

Interaction between specific peptide and titanium di-oxide surface

Classical molecular dynamics simulation of charged aminoacids within the peptide chain on TiO_2 surface, including water

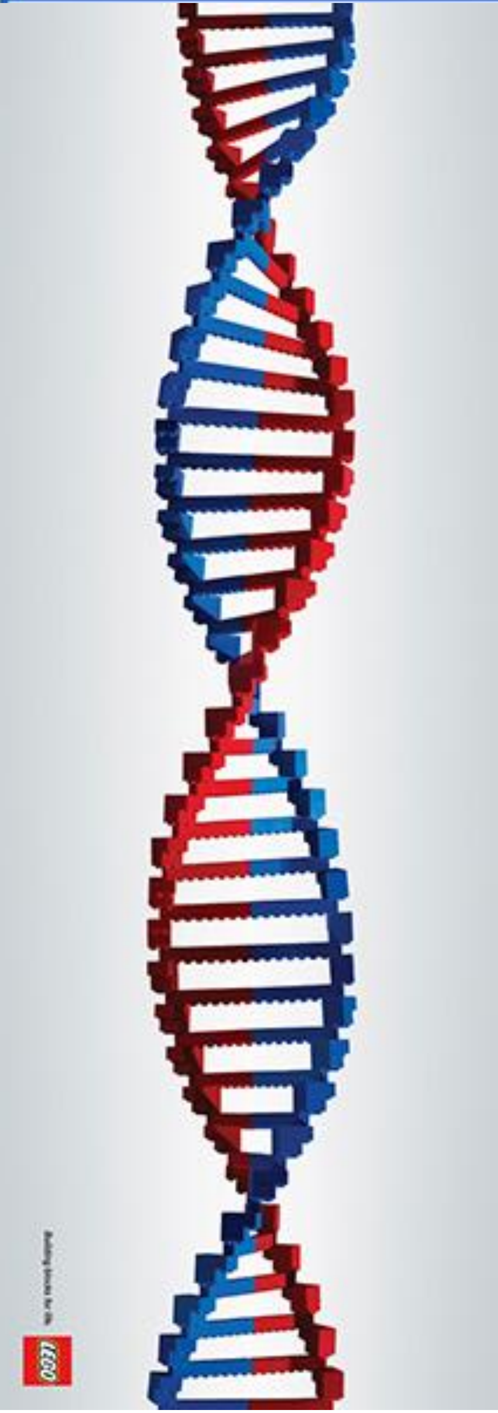


A steered molecular dynamics simulation by GROMACS

The peptide was detached from the surface along the z-direction by pulling on the COM (center of mass) using $k=5000$ kJ/mol/nm^2 and a pull rate of 0.0005 nm/ps. The pulling force builds up until three breaking points are reached, at which the interactions between the cysteine 13, aspartic acid 7 and arginine 3 with the metal surface are disrupted, allowing the peptide to dissociate from the metal surface

The team

- Piero Morales, Selene Baschieri, Chiara Lico, Lucia Mosiello, Bruno Rapone, Massimo Celino, Caterina Arcangeli, Francesco Buonocore (ENEA, Rome)
- Liqian Wang, Wei-hua Han (NAST Center University of Tor Vergata, Rome)
- Scott Retterer, Ilia Ivanov (CNMS, Oak Ridge, USA)
- Kurt Gothelf, Mattia De Stefano, Abhichart Krissanaprasit, Jesper Vinther (cDNA, Aarhus, DK)
- Tibor Hianik (Comenius University, Bratislava, SK)



Thank you!