DNA-based nanomachines poweíed by biological inputs foí diagnostic and díug deliveíy applications

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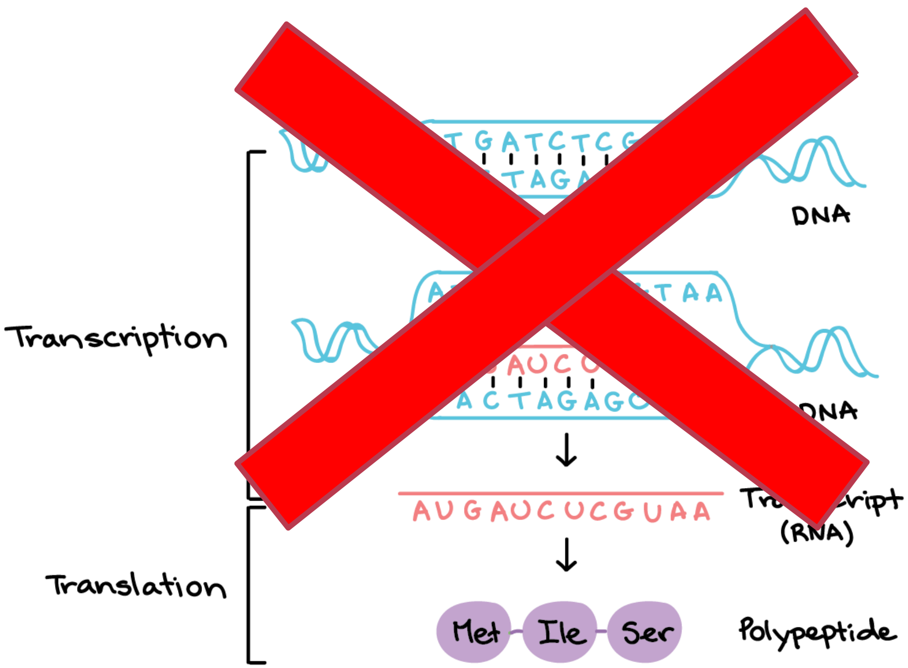
Univeísity of Teramo 3 May 2022

DNA in Biological Oíganism

DNA's chief function is to serve as a read-only memory whose information content is transcribed into RNA before this information is used to orchestrate life's functions through the synthesis of protein and RNA-based molecular machines.



DNA Nanotechnology

DNA nanotechnology:

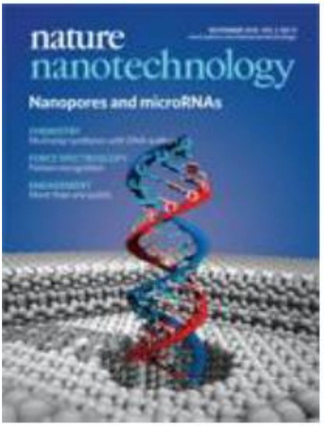
Design and manufacture of artificial nucleic acid structures for technological uses.

DNA is used as engineering material
NOT as a carrier of the genetic information!

A bottom up approach:

DNA-nanotech uses the internal information of DNA molecules to guide their autonomous self-assembly into nanostructures.

Why DNA as nanomaterial building block?



- Easy to synthesize (low cost?)
- Easy to engineer to attach molecules
- Biocompatible
- Chemical robustness
- Programmable (predictable base-pairing (A-T, G-C)
- Recognition of different targets (DNA strands, protein, etc.)
- The stiffness of duplex DNA enables its assembly
- Difference in stiffness between duplex DNA and single-stranded DNA (ssDNA as hinges)
- Relative weakness of interactions

Seeman, N.C. (1982). Nucleic-Acid Junctions and Lattices. Journal of Theoretical Biology 99, 237-247.

DNA Nanotechnology

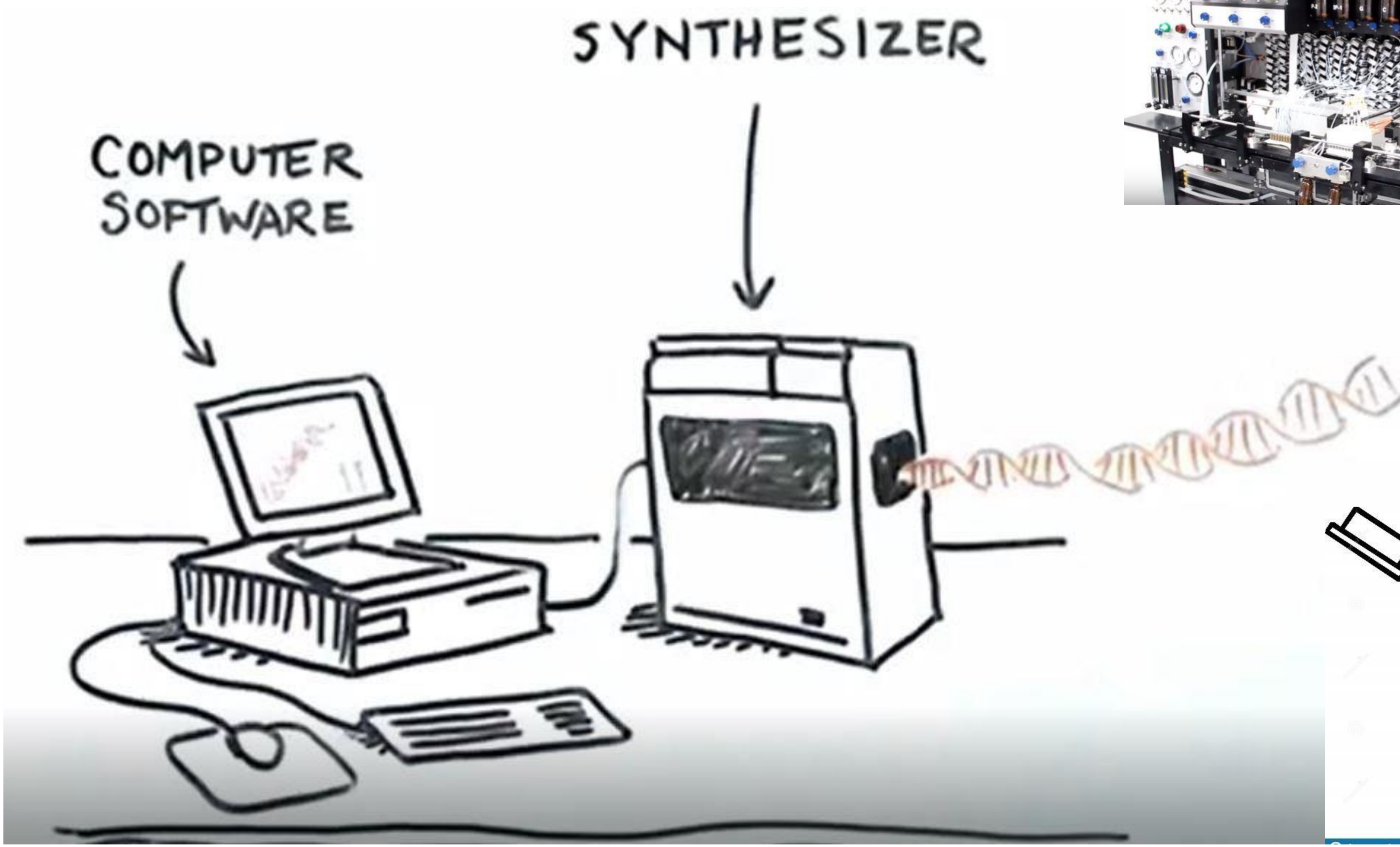
01 Structural DNA Nanotechnology: 2D

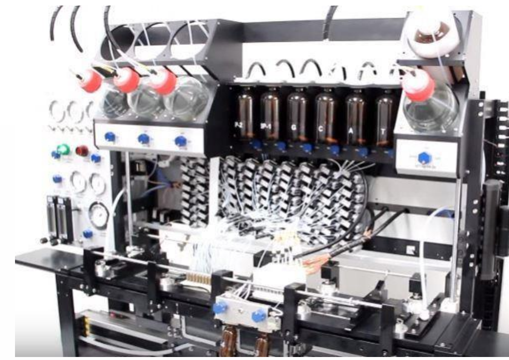
02 Structural DNA Nanotechnology: 3D

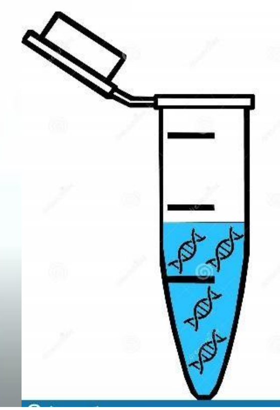
03 Functional DNA Nanotechnology

04 Structural + Functional DNA Nanotechnology

Solid-phase synthesis of DNA







Structural DNA-Nanotechnology

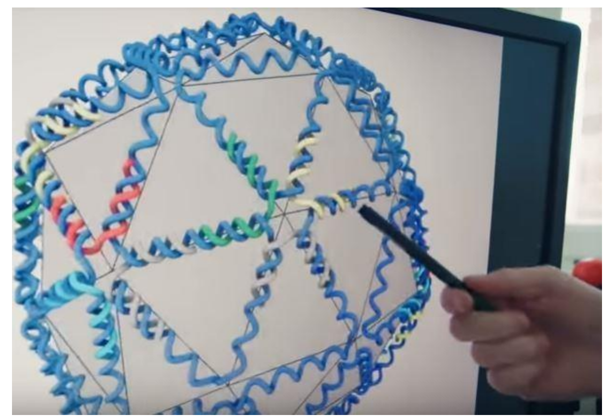
To build object of any shape at the nanometer scale through self assembly of DNA strands.

To use the internal information of oligonucleotide sequences to self-assembly nanostructure

Structural DNA-Nanotechnology

1. Draw the raw shape of the structure!

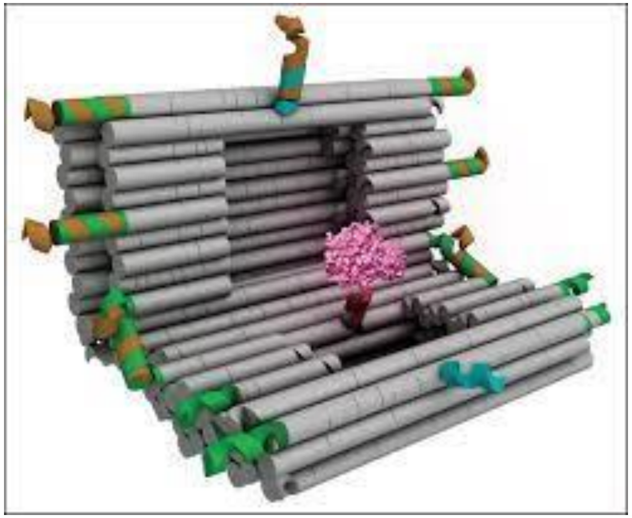
2. An algorithm calculates how the DNA scaffold strand will arrange itself on the surface of the structure and will also calculate all the short DNA strands.



Structural DNA-Nanotechnology

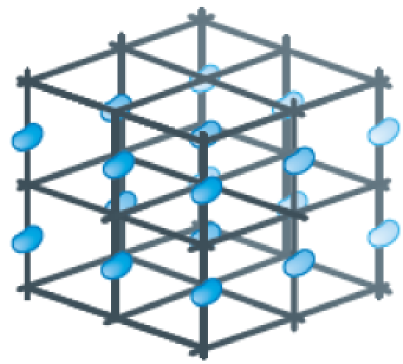
3. Order the oligonucleotides

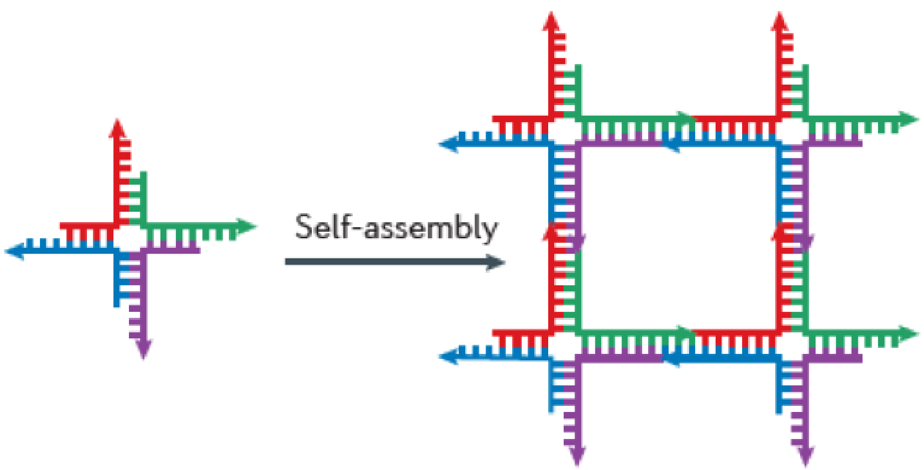
4. Mixing, annealing over night and the DNA nanostructure is done!



The early years: the Ned Seeman's dream

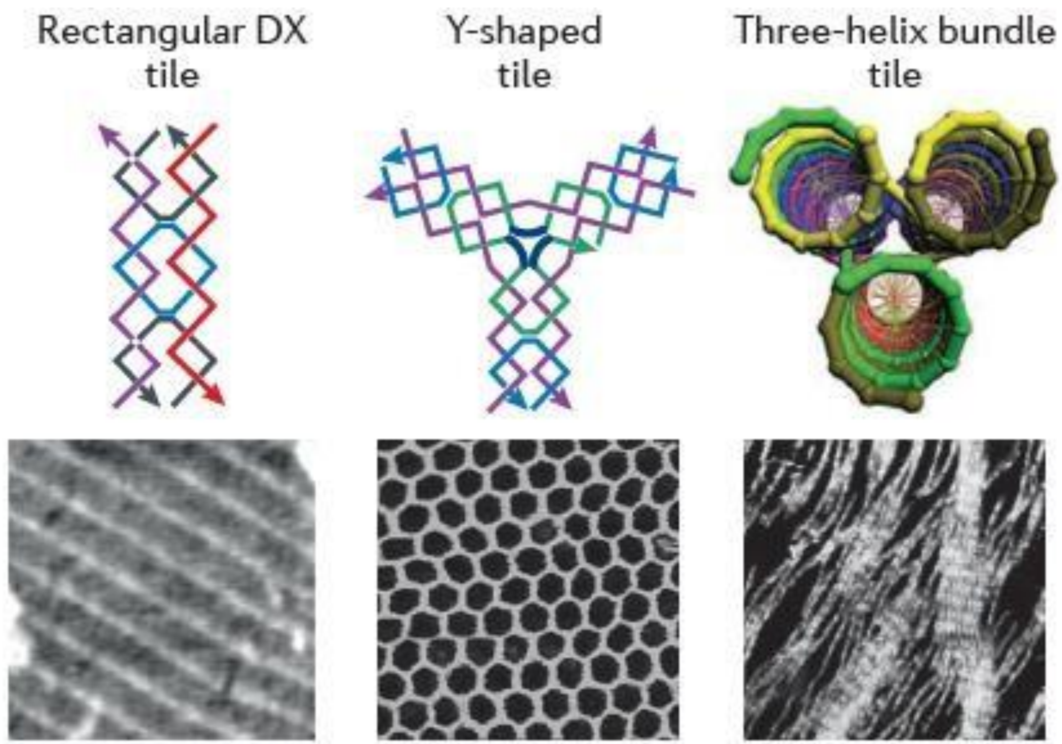
Rather than relying on trial and error to crystallize biological macromolecules, the idea was that crystals could be assembled using WC-interactions





Branched junctions could be connected together by the base pairing of single-stranded overhangs, called sticky ends, into a 3D crystalline material

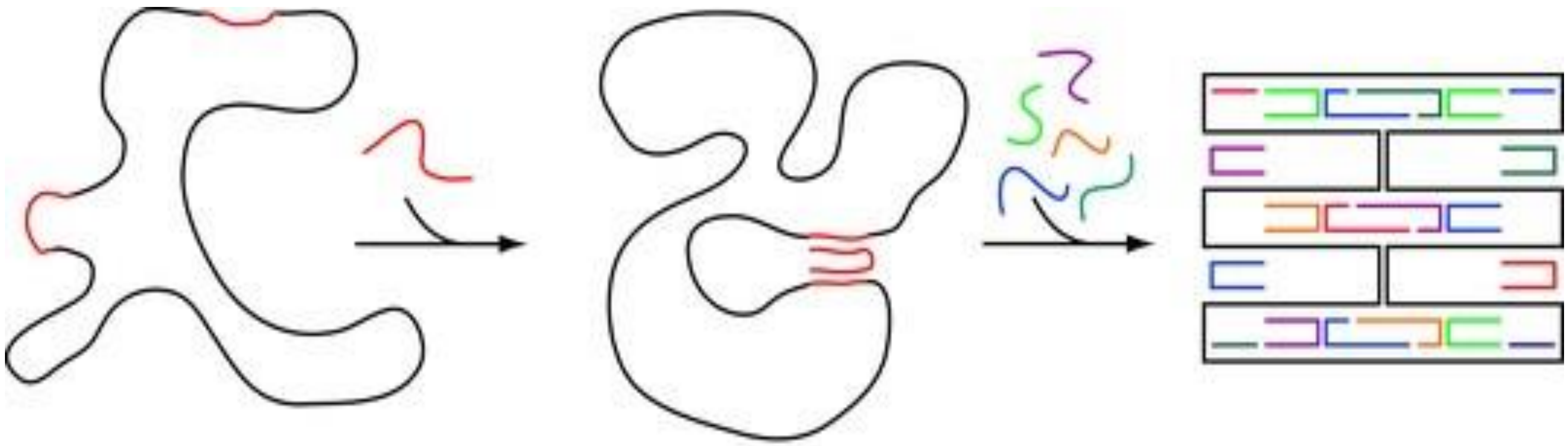
DNA tile self-assembly: 2D DNA crystals by design

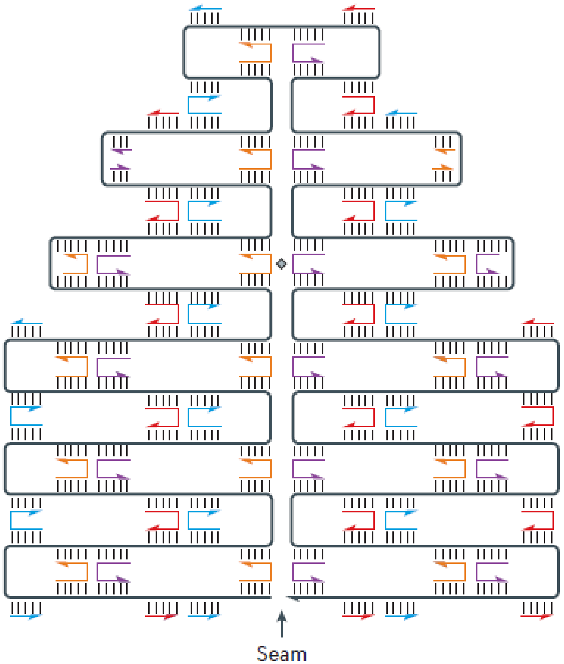


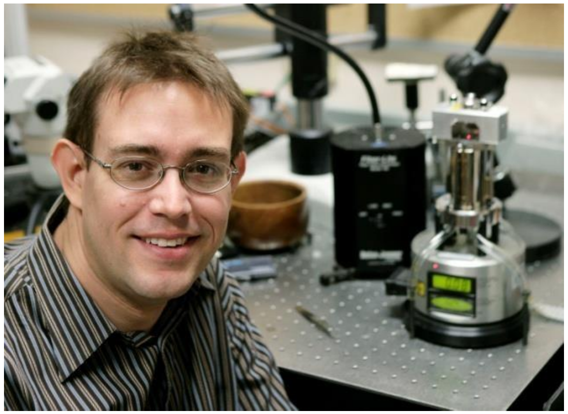
1) Making arms with unique sequences (limited branch migration)
2) Three-arm junctions , and double-crossover (DX) molecules comprising two DNA double helices linked together (geometric rigidity, proper stability and topology)

Lin, C. et al. DNA tile based self-assembly: building complex nanoarchitectures. *ChemPhysChem* **7**, 1641–1647 (2006).

DNA Origami



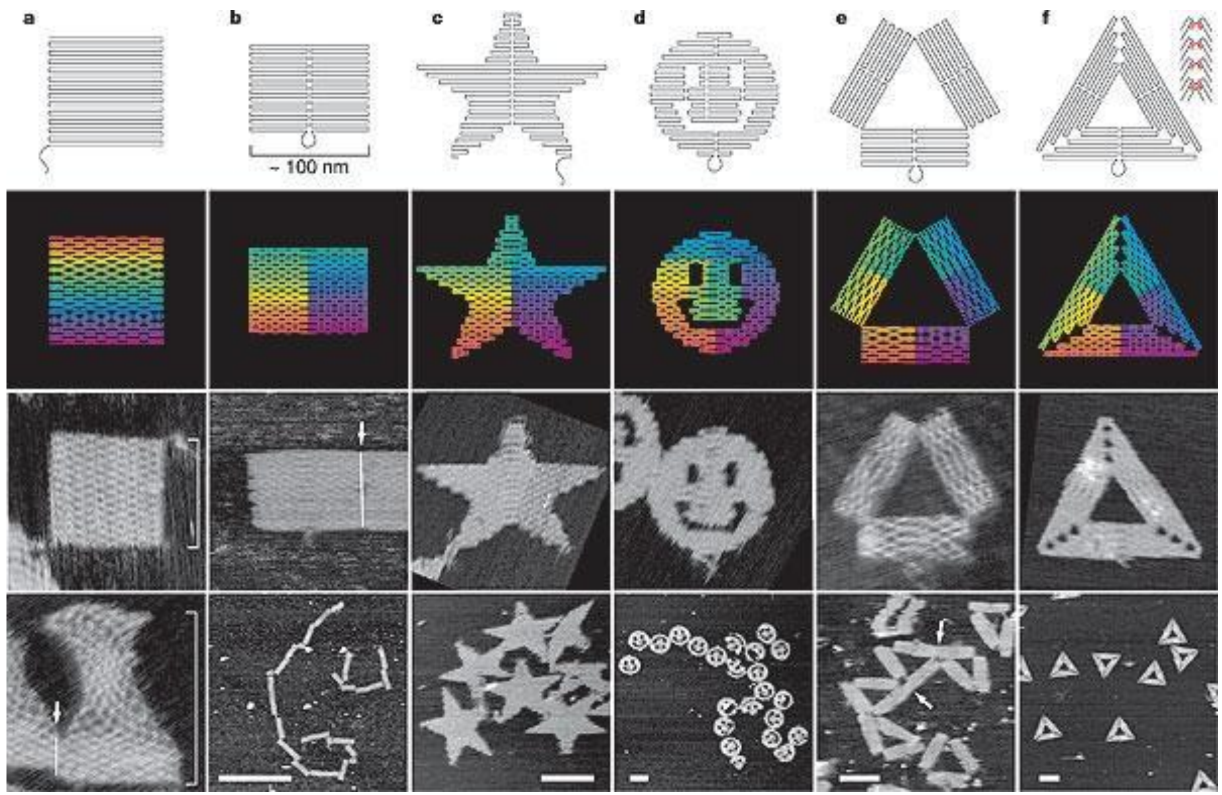




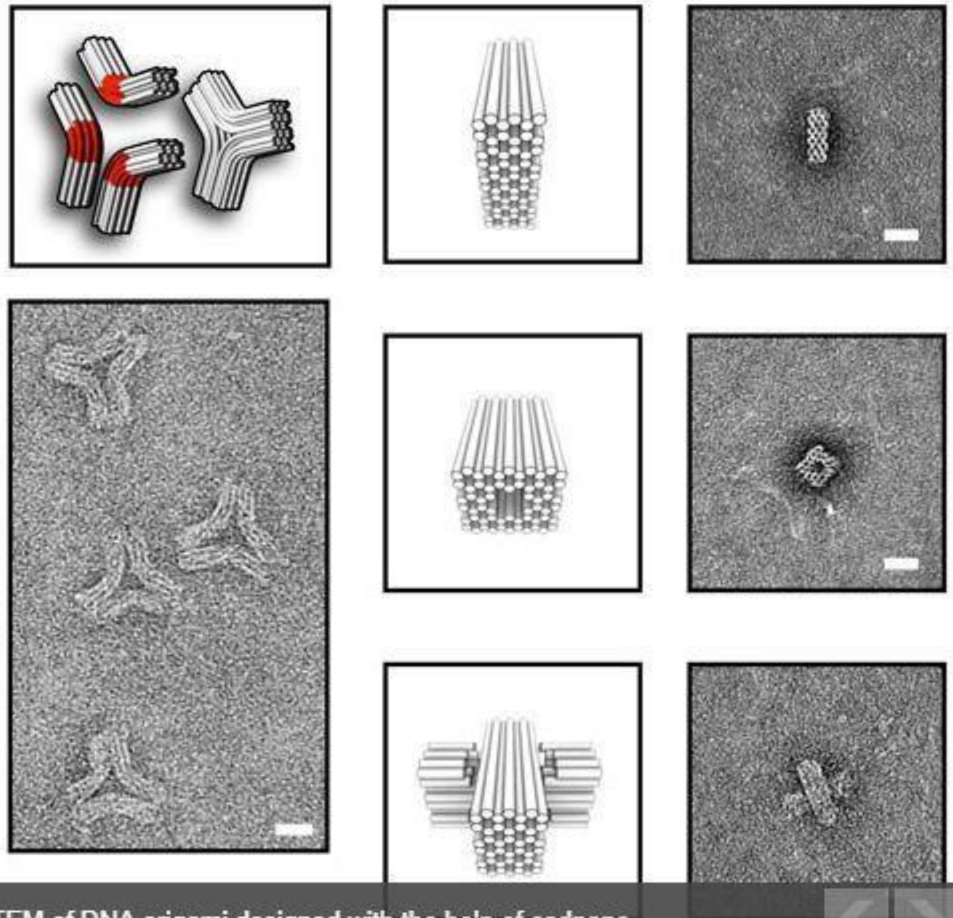
Rothemund, P. W. Folding DNA to cíeate nanoscale shapes and patteíns. ***Natuíe*** 440, 297–302 (2006).

DNA Origami: 2D Stíuctuíe

Non-periodic 2D structures of arbitíary complexity can be made, such as a map of the Americas, rectangles, smiley faces, stars and other designed patterns.



Rothemund, P. W. Folding DNA to cíeate nanoscale shapes and patteíns. ***Natuíe*** 440, 297–302 (2006).



TEM of DNA origami designed with the help of cadnano.

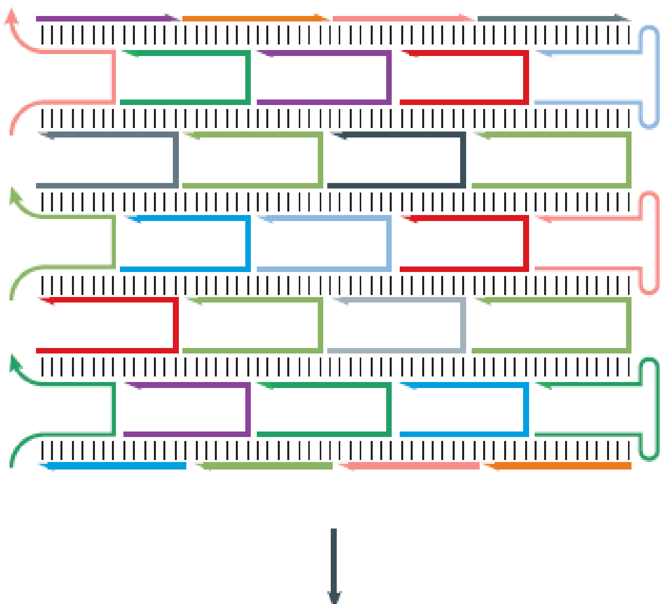
cadnano simplifies and enhances the process of designing three-dimensional DNA origami nanostructures. Through its user-friendly 2D and 3D interfaces it accelerates the creation of arbitrary designs. The embedded rules within **cadnano** paired with the finite element analysis performed by cando, provide relative certainty of the stability of the structures.

cadnano features:

- Platform independent (tested in Windows, OSX and Linux)
- Visual cues aid design process for stable structures
- 3D interface powered by Autodesk Maya*
- Open architecture for plug-in creation
- Free and open source (MIT license)

DOWNLOAD CADNANO
It's free and open source.

“Brick assembly”: a new strategy to generate 2D Nanostructuíes



‘Brick-wall’ diagram

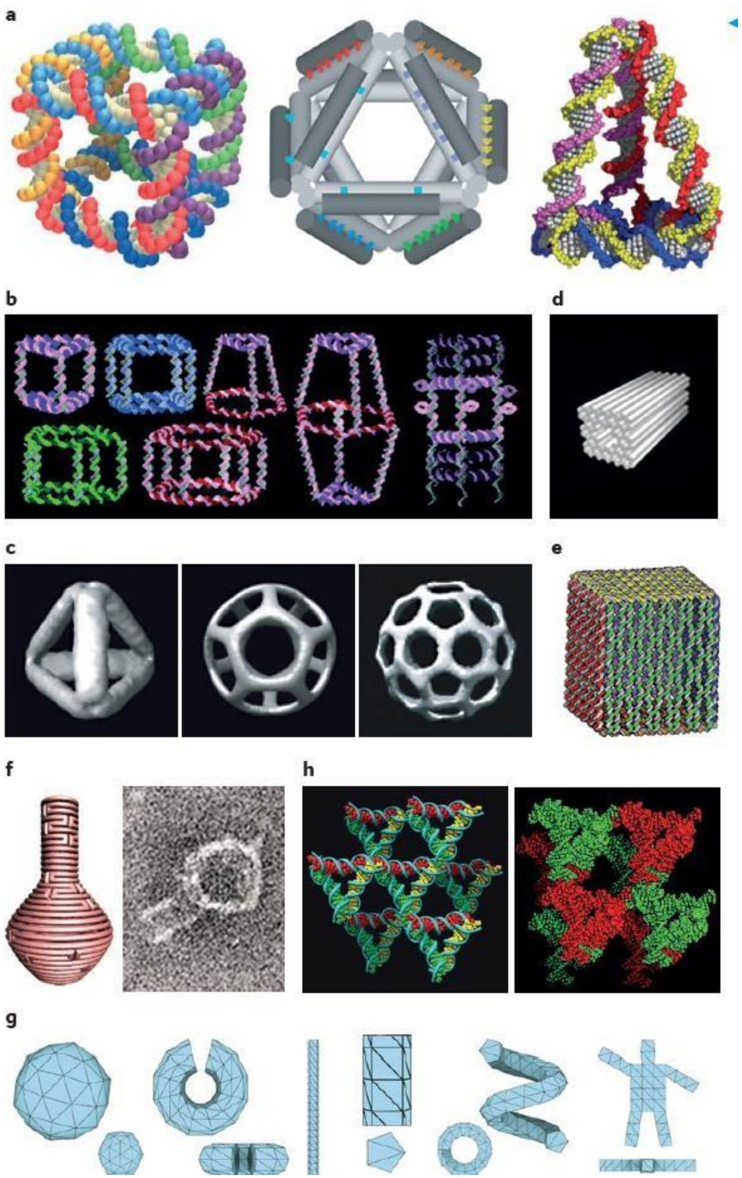
The building blocks are single strands of DNA containing four modular domains, which are designed to form interconnected staggered duplexes with one another, resulting in DNA lattices.

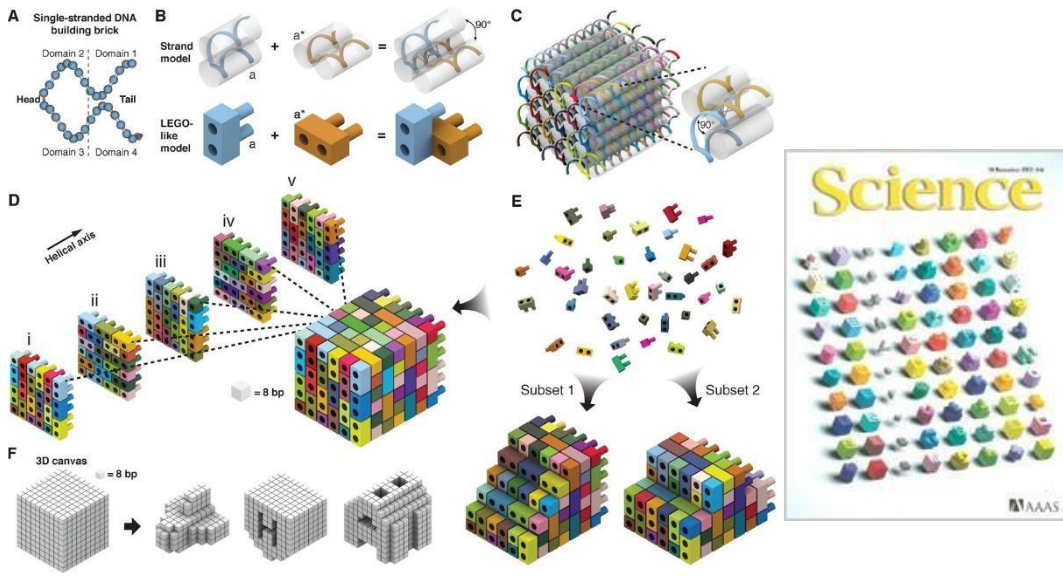
The sequences are all unique.

- Any arbitrary shape by selecting the set of strands that defines the structuíe.

Wei, B. el’ al. Nal’uíe 485, 623–626 (2012).

3D Nanostructure



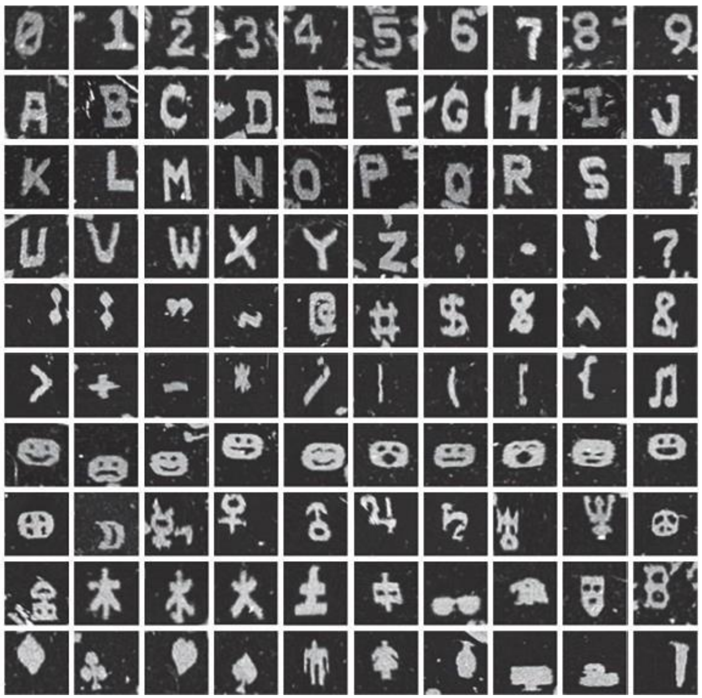


A number of approaches to produce 3D structures:

- Association of identical symmetrical DNA three-point-star or five-point-star tiles
- Rolling of DNA origami sheets through crossover junctions

Ke Y., *Science*, 338, 1167

Origami and Brick assembly: critical points to be addressed



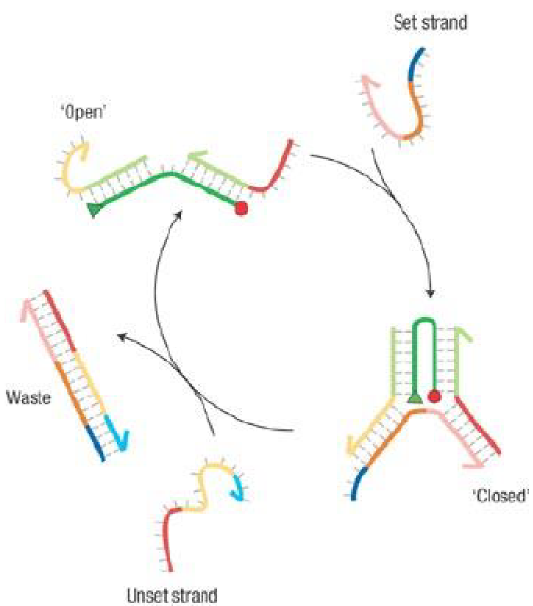
- Their need for hundreds of DNA strands
- The synthesis cost is rapidly decreasing but it is still a issue.
- <u>**Scalability,**</u> limitation to application that requires few micrograms of material

Li, W. El' al. J. Am. Chem. Soc. 136, 3724–3727 (2014).

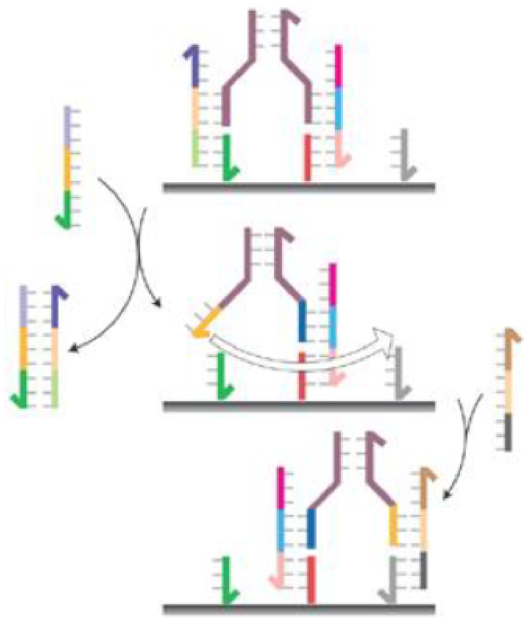
Functional DNA Nanotechnology: DNA Nanomachines

DNA nanomachines are nanorobots made entirely or partially of DNA. DNA nanomachines can switch between defined molecular conformations and can be used as sensing, computing, actuating or therapeutic nanodevices.

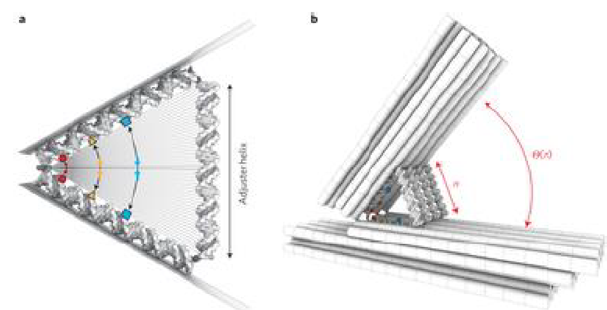
DNA Tweezers



DNA Walkers

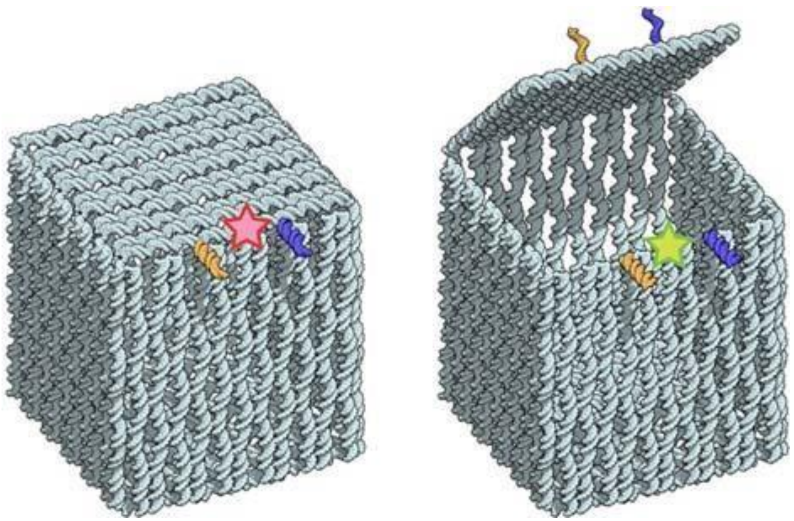


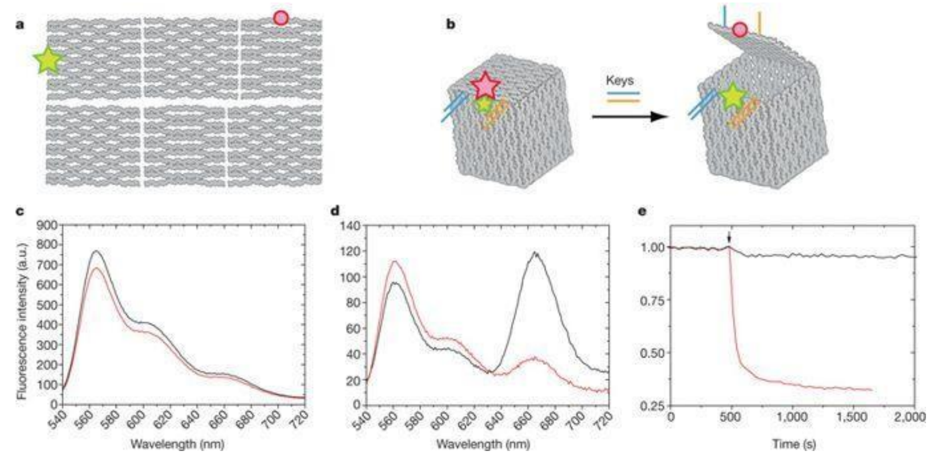
DNA Switches



B. Yurke, A.J. Turberfield, A.P. Mills, F. C. Simmel, J.L. Neumann, Nature, 2000.
J. Bath, A.J. Turberfield, Nat. Nanotechnol., 2007.
T. Liedl, T.L. Sobey, F. Simmel, Nano Today, 2007.
Funke J. J., Dietz H. Nature Nanotechnology, 2016.

Structural + Functional DNA Nanotechnology



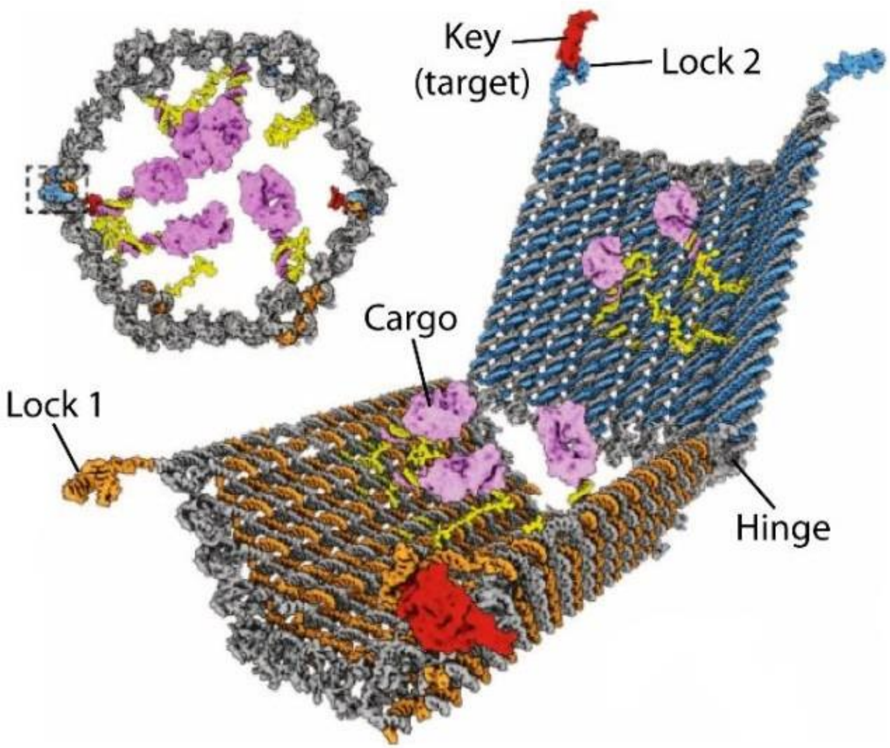


DNA box with a controllable lid that can be opened in the presence of externally supplied DNA ‘keys’.

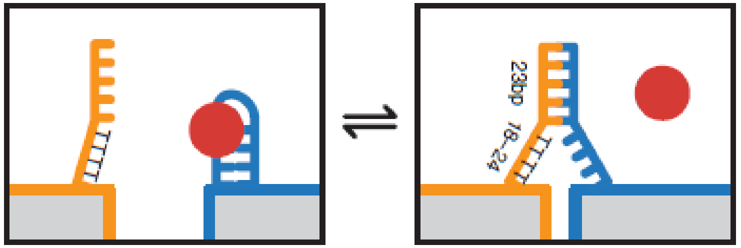
the lids of the DNA box have the potential to be uniquely programmed to respond to complex combinations of oligonucleotide sequences

Andersen, E.S. 2009 *Nature* 459 (7243): 73–76.

DNA Nanorobot for targeted transport of Molecular Payloads

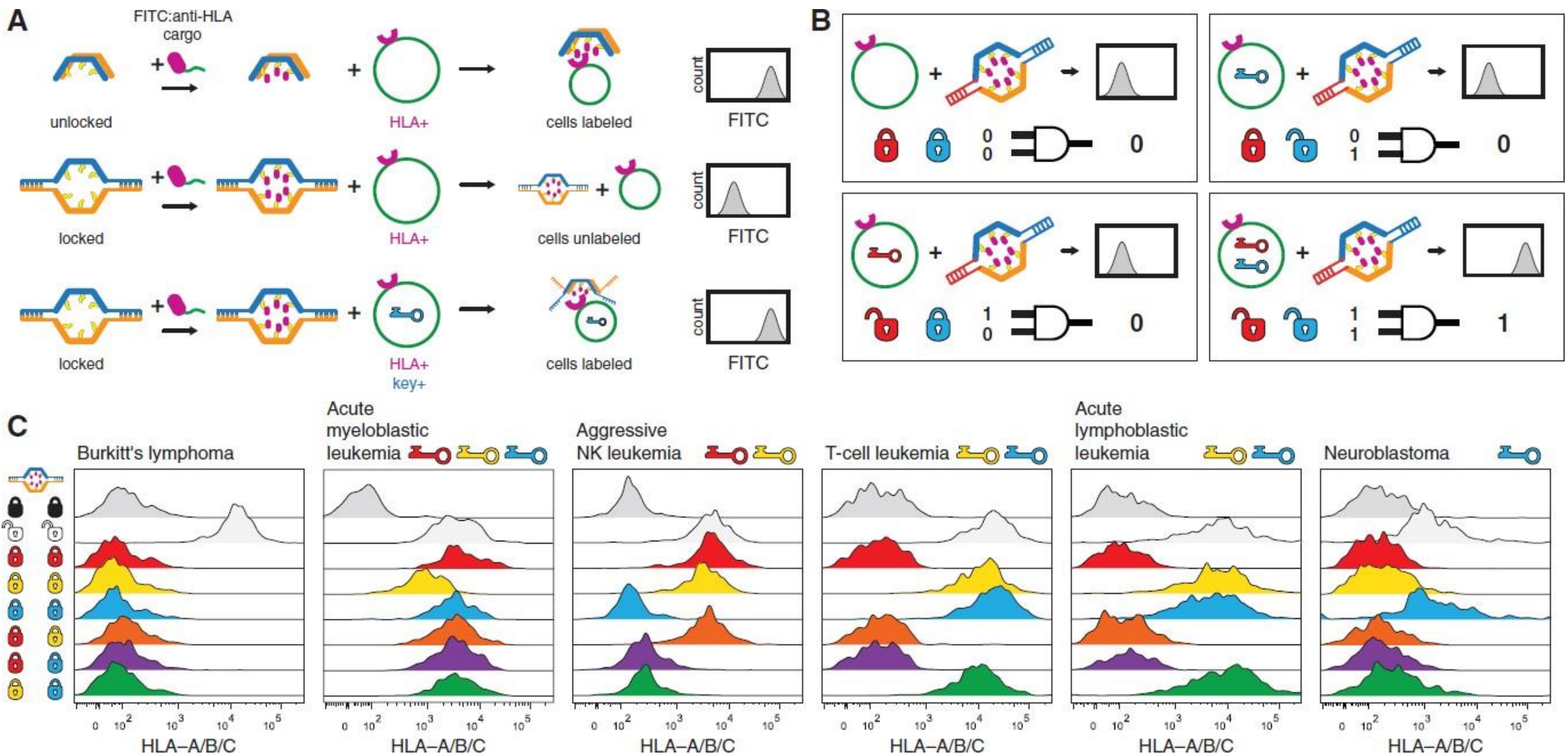


- **transport of molecular payload**
- **Sensing cell surface inputs and trigger activation**
- **Reconfigurtaion of its structuure for payload delivery**

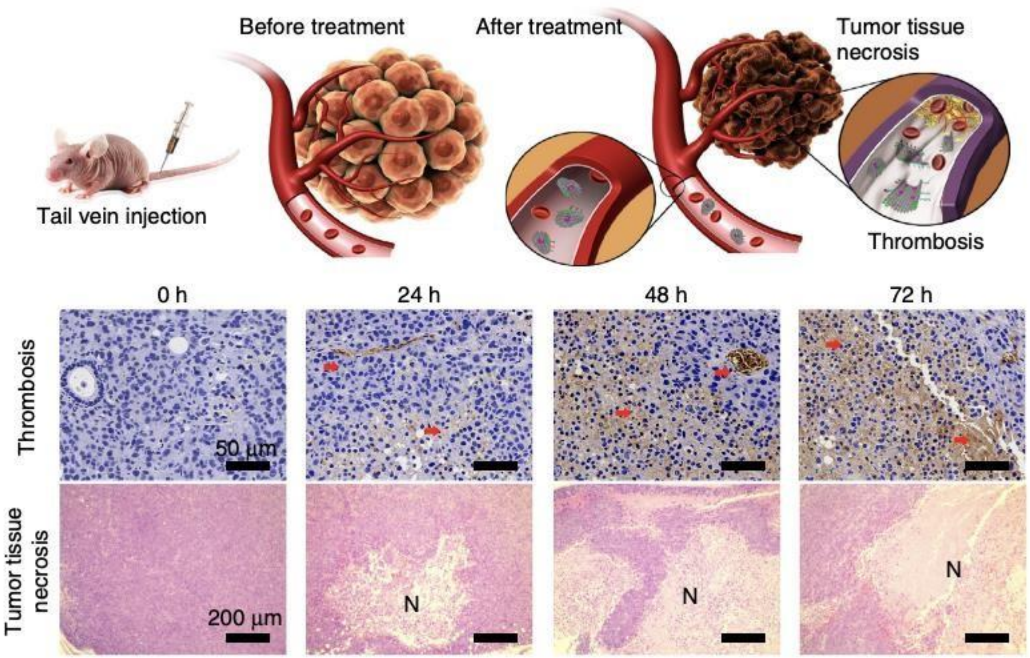


Douglas, Shawn M.; Bachelet, Ido; Church, George M. 2012 *Science* 335 (6070): 831–834.

Robots loaded with fluorescently labeled antibody fragments against human leukocyte antigen (HLA)–A/B/C were mixed with different cell types expressing human HLA-A/B/C and various "key" combinations. In the absence of the correct combination of keys, the robot remained inactive.



DNA nanorobot functions as a cancer therapeutic in response to a molecular trigger in vivo

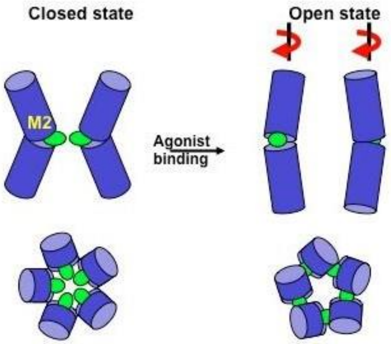


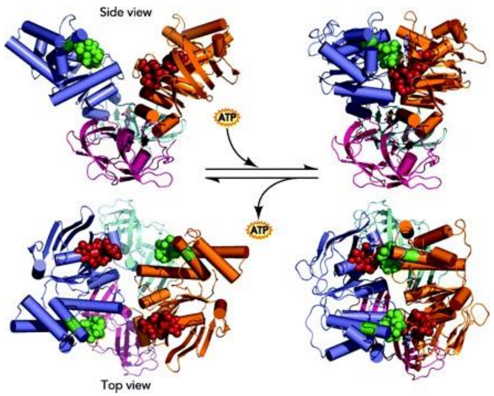
Autonomous DNA robot programmed to transport payloads and present them specifically in tumors. Nucleolin-aptamers thrombin delivery to tumor site induce necrosis

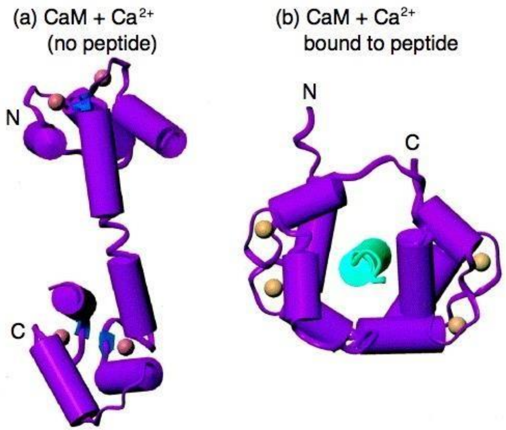
Li S., 2018 ***Nat. Biothecnol.*** 36 (3): 258-264.

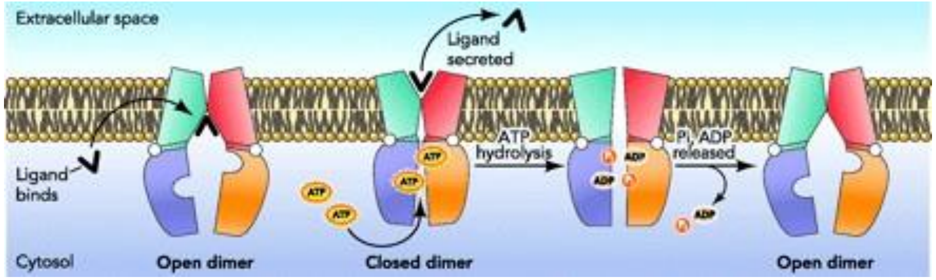
Biomolecular switches

Biomolecular switches made from proteins or RNA use binding-induced conformational changes as signal and functional mechanisms.



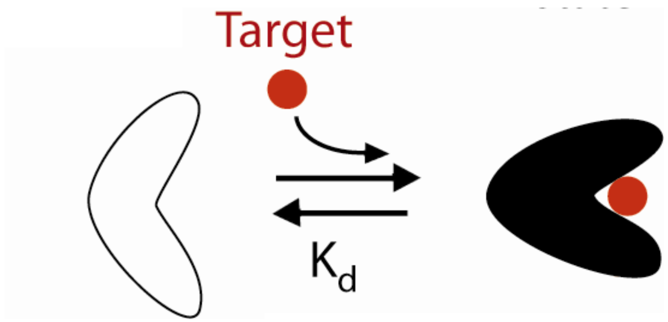






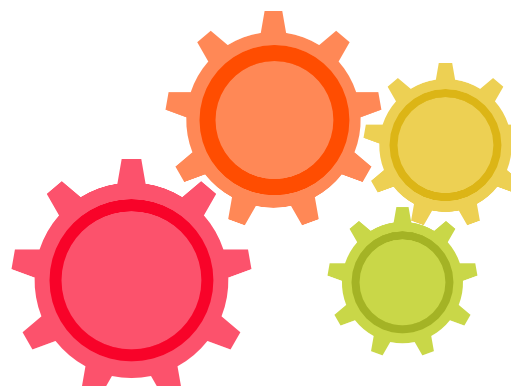
CONCEPT: mimic natuRe foR developing novel diagnostic and drug-delivery tools

INSPIRATION: Naturally occurring switches



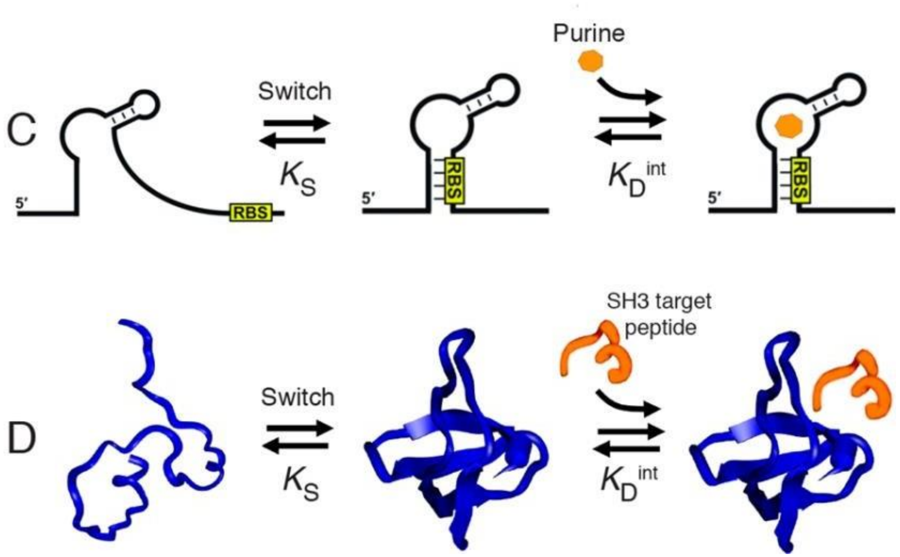
Development of DNA based switches for biotechnology applications

+ target →



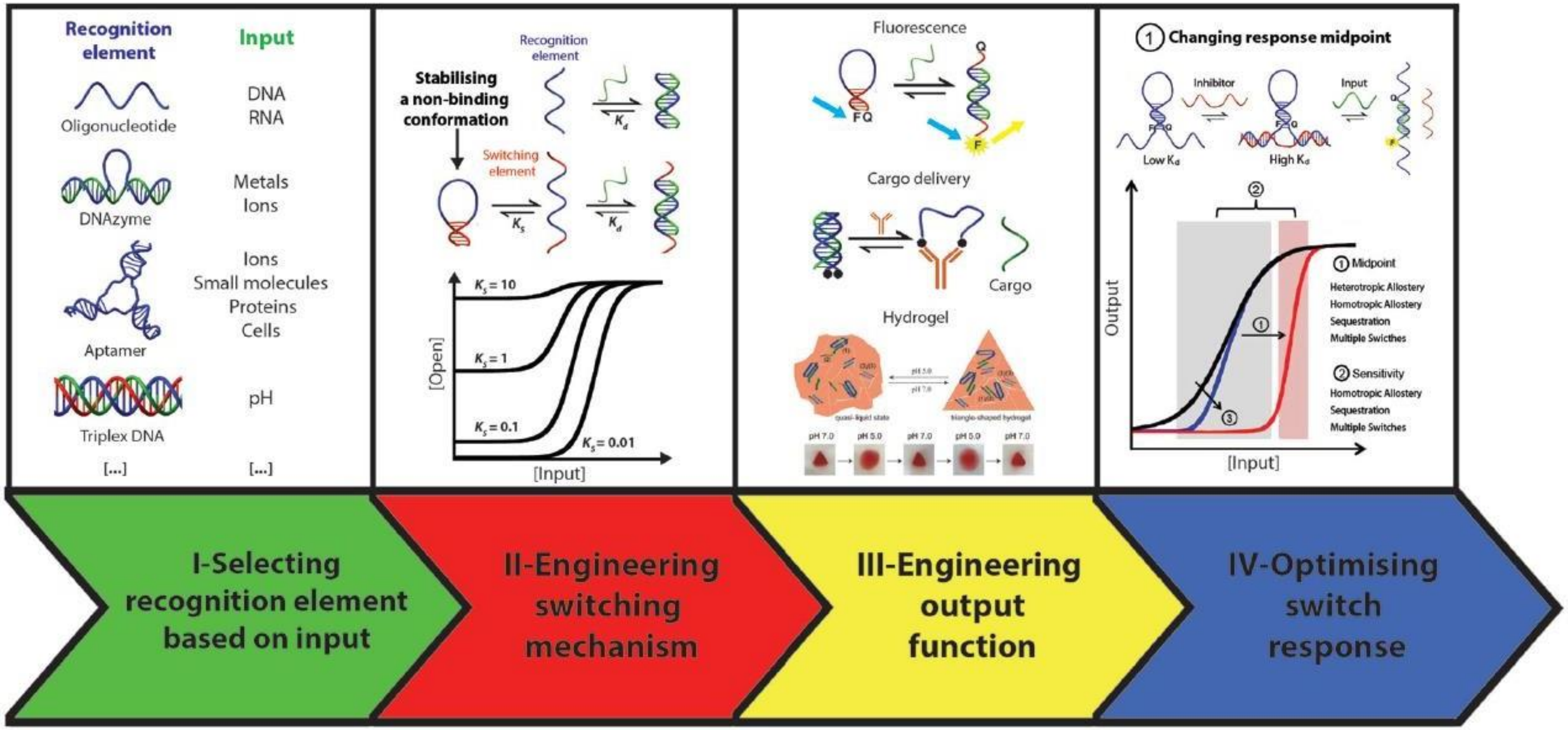
Advantages of biomolecular switches

1- Binding-activated (not always “on”)
2- they are quantitative
3 Selective enough to work in complex biological samples
4 Switching mechanism can be engineered in any biomolecule



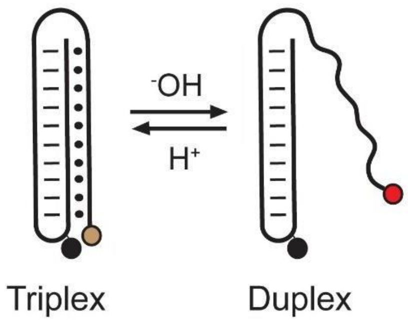
Remy, I. ***et al., Science*** (1999)
Kim, J. N. ***et al. Biol. Cell.*** (2008)
Kohn, J. ***et al. Píoc. Natl. Acad. Sci. U.S.A.***(2005)

DNA-based nanomachines design



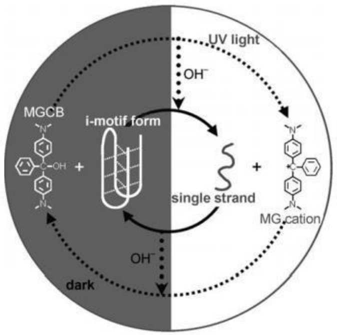
DNA-based nanomachines: Possible inputs

pH



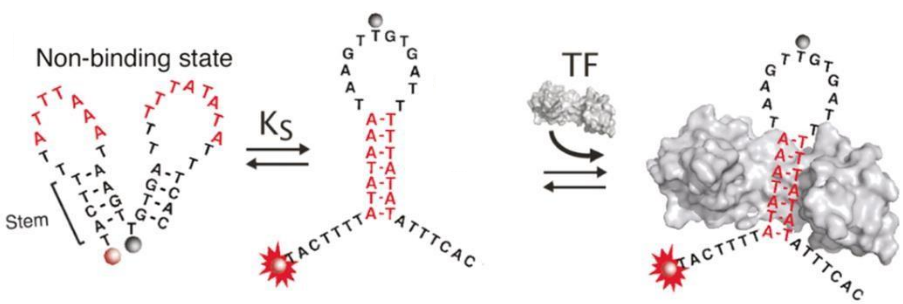
Idili, A. ***et al.*** JACS 2014, 136, 5836-5839

Light



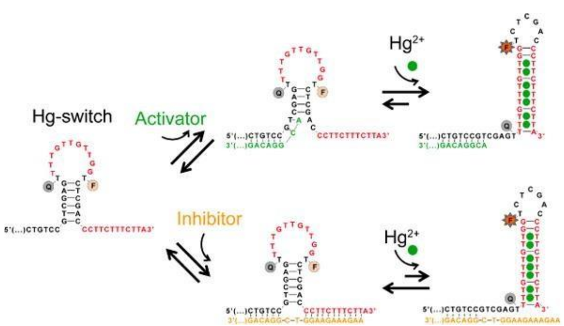
Liu, H. ***et al.*** Angew. Chem. Int. Ed. 2007, 46, 2515-2517

Transcription Factor



Vallée-Bélisle, A. ***et al.*** J. Am. Chem. Soc. 2011, 133, 13836-9

Small Molecule



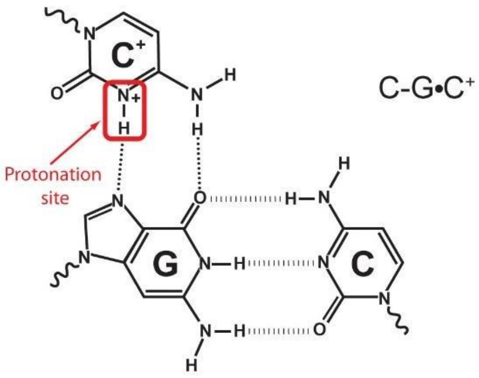
Porchetta, A. ***et al.*** J. Am. Chem. Soc. 2013, 135, 13238–41

Selecting Recognition Element based on Chemical Input

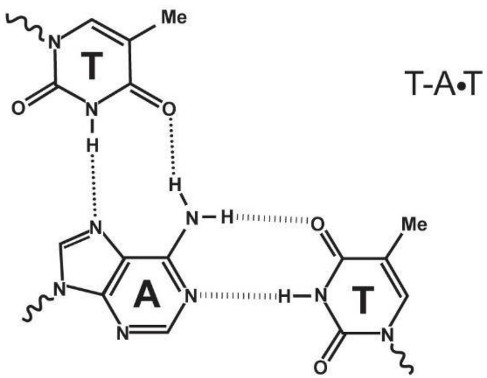
1. pH-contíolled switches

Structural information:

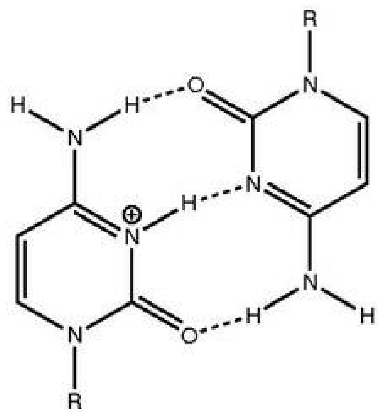
- ***Triplex DNA (TAT, CGC triplet)***
- ***intercaleted-motif (C-rich sequences)***



C-G•C+ Hoogsteen interactions are strongly pH-dependent (pKa ≈ 6)

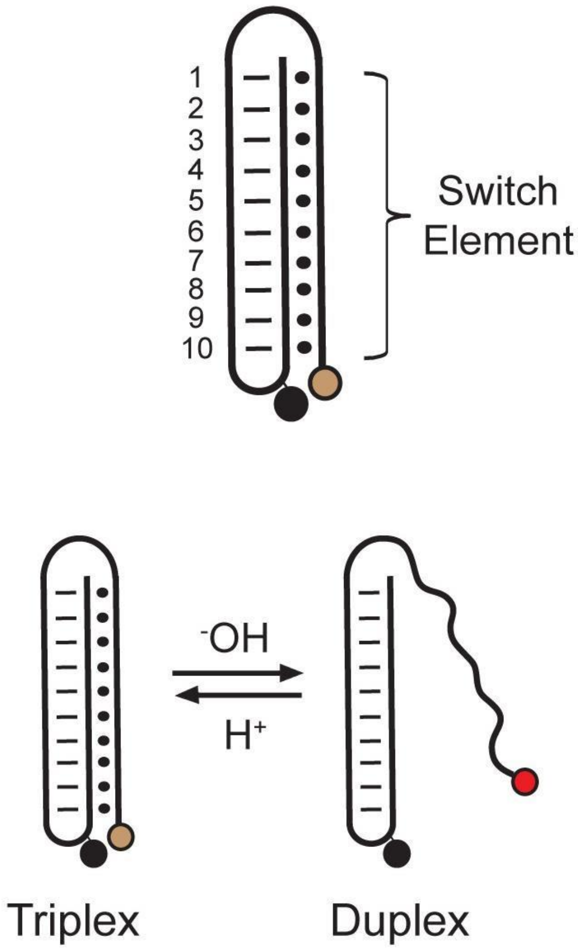


T-A•T Hoogsteen interactions are less pH-dependent (pKa ≈ 10)



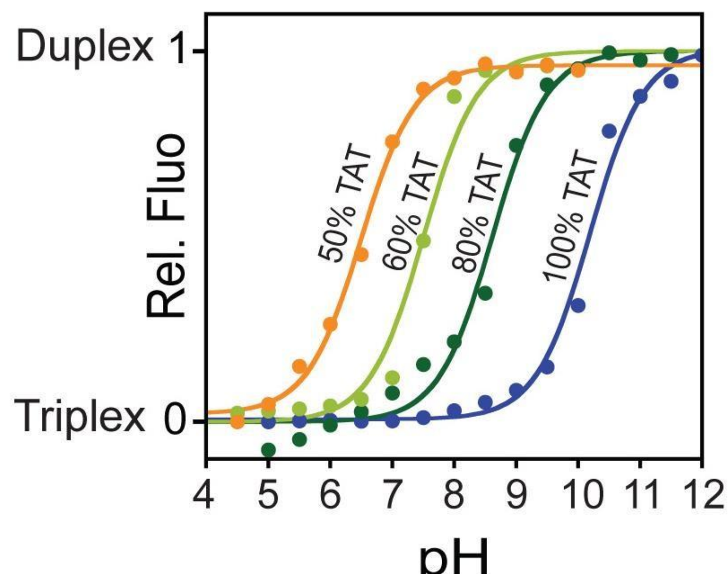
C·C+ base pairing found in i-motif structures. Base-pairing energy=169.7 kJ/mol.

Píogíammable pH-tíiggeíed DNA-based nanomachines



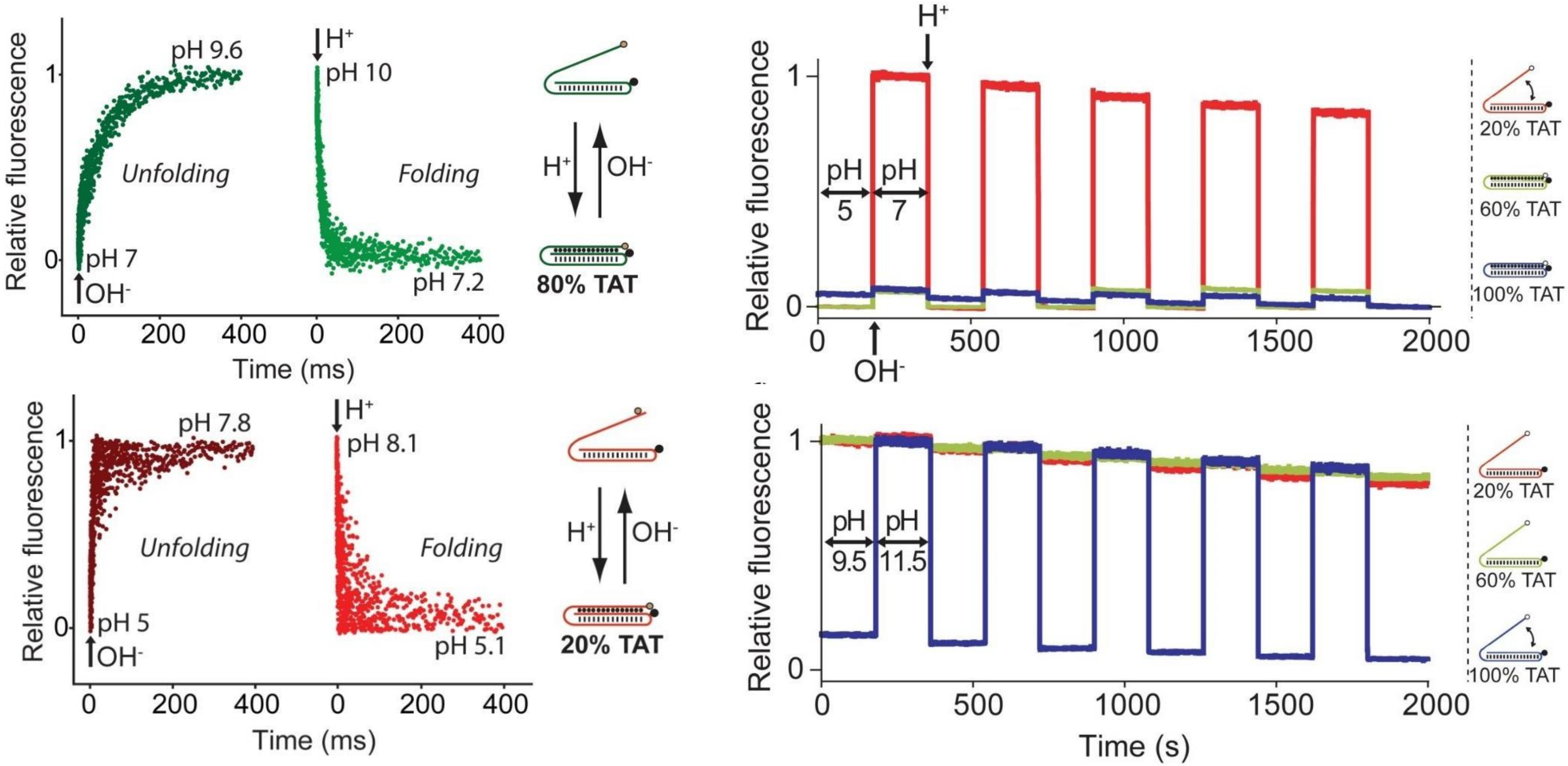
% of TAT in the Triplex

Position	50%	60%	80%	100%
1	TAT	TAT	TAT	TAT
2	TAT	TAT	TAT	TAT
3	TAT	*CGC*	*CGC*	TAT
4	TAT	*CGC*	TAT	TAT
5	TAT	TAT	TAT	TAT
6	*CGC*	TAT	TAT	TAT
7	*CGC*	*CGC*	TAT	TAT
8	*CGC*	TAT	*CGC*	TAT
9	*CGC*	TAT	TAT	TAT
10	*CGC*	*CGC*	TAT	TAT

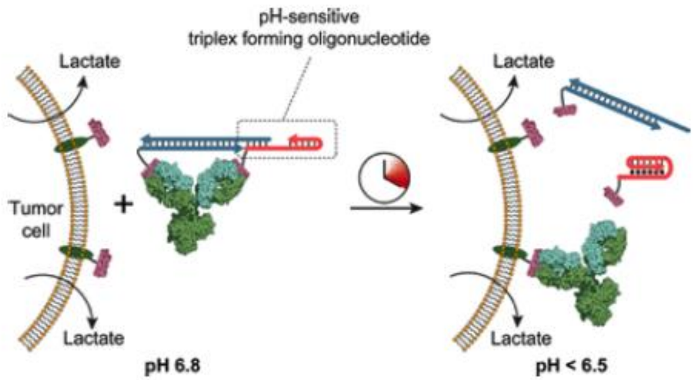


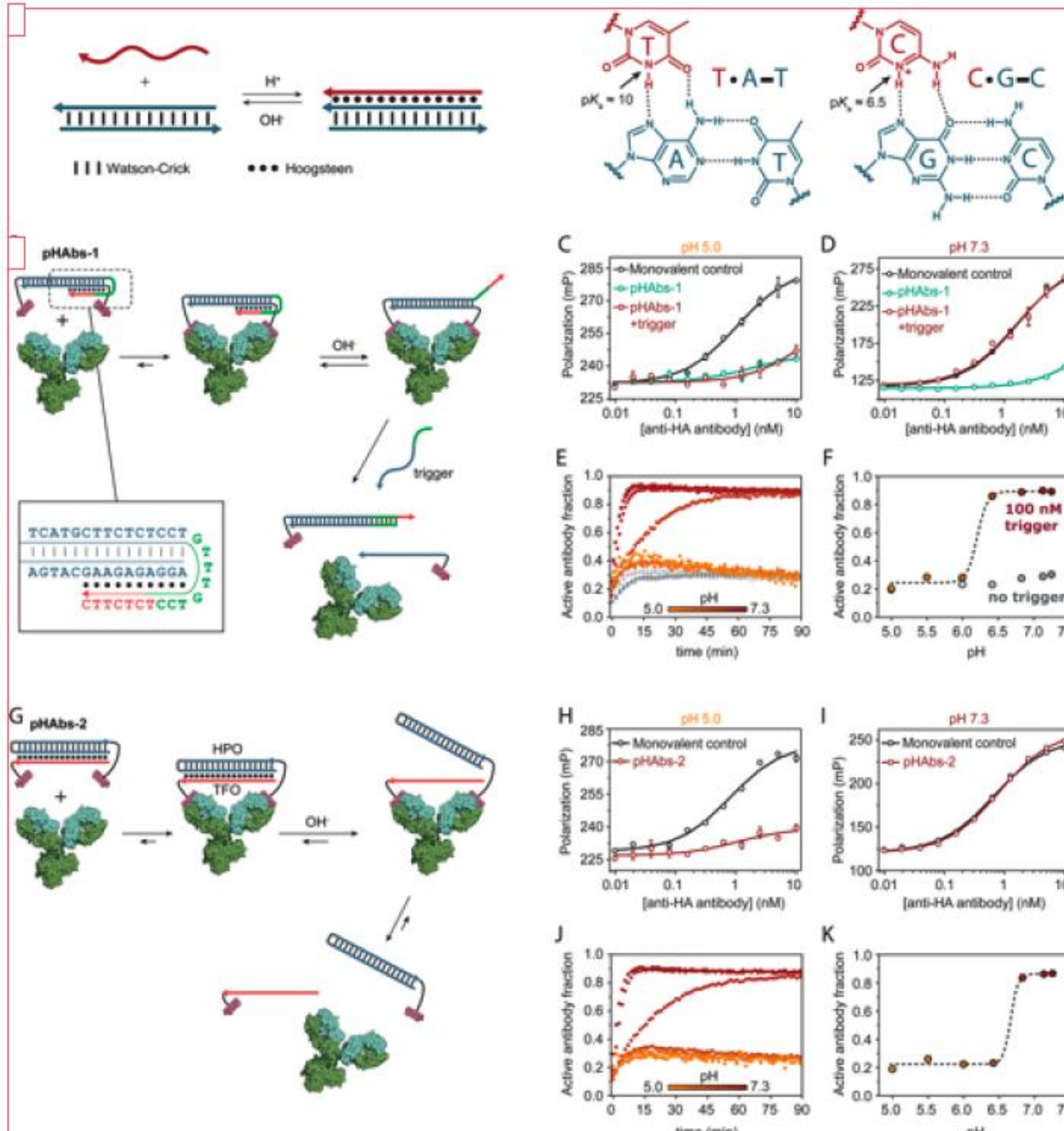
Idili A, Vallée-Bélisle A, Ricci F. **J Am Chem Soc.** 2014, 136, 5836

Programmable pH-triggeíed DNA-based nanomachines



pH-Based Control of Antibody Activity using programmable bivalent peptide–DNA locks

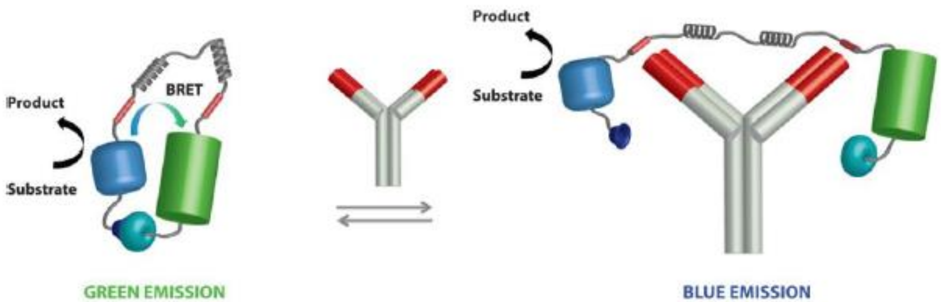




- ➢ pH-dependent DNA lock
- ➢ Detection of the specific antibody
- ➢ Targeting of tumor cells

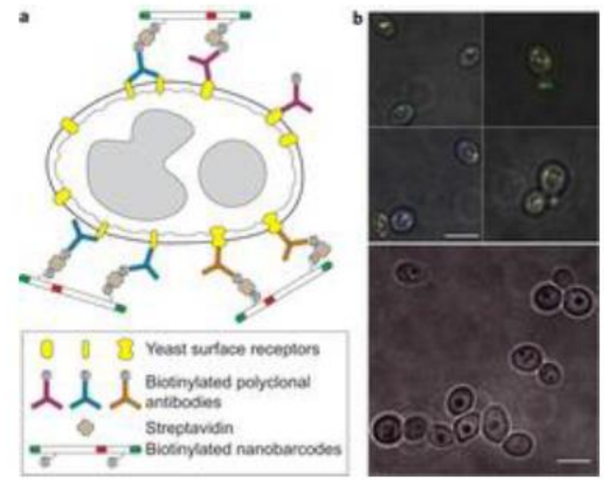
Merkx *et al.*, ***ACS Cent. Sci.*** 2020, 6, 1, 22-31

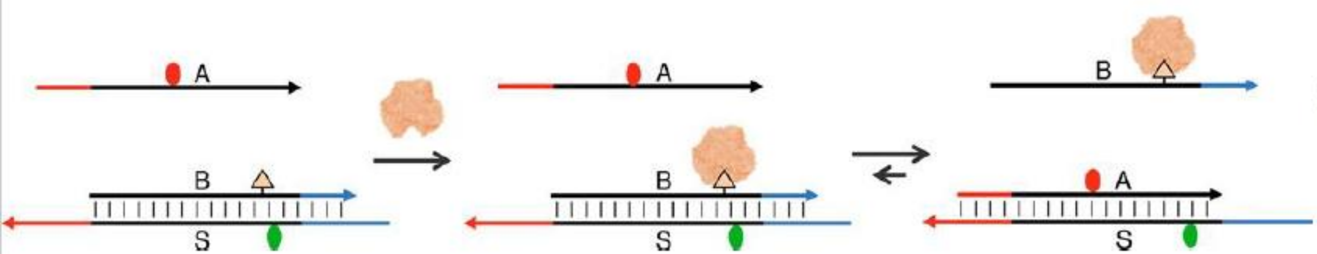
Antibodies as triggering input for DNA-based nanomachines



Arts R. *et al.*, *Anal. Chem.*, 2016, 88, 4525–4532

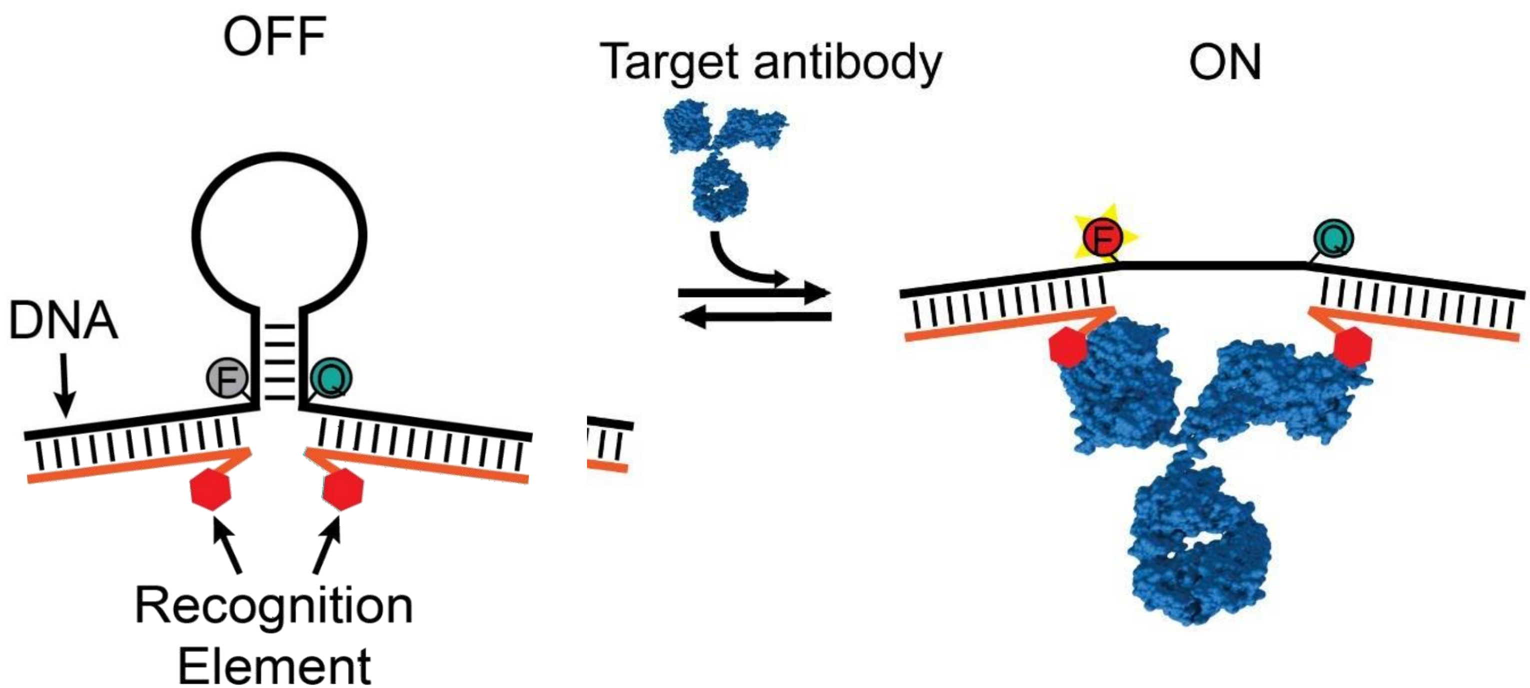
Chenxiang L. *et al.*, *Nature Chemistry, 2012, 4, 832–839*





Zhang Z. *et al.*, *J. Am. Chem. Soc.*, 2014, 136, 11115–11120

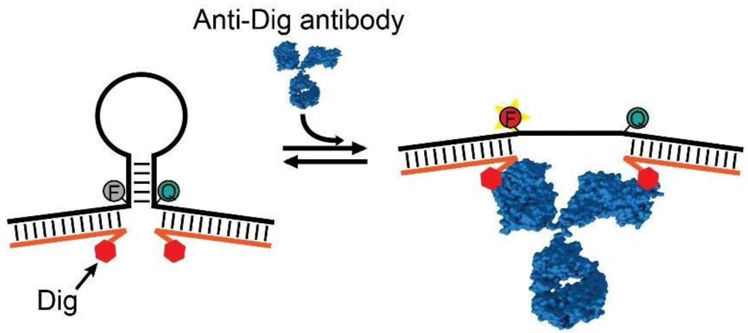
Antibody-switch:
A DNA-based biomolecular switch that, through a binding-induced conformational change, detects antibodies



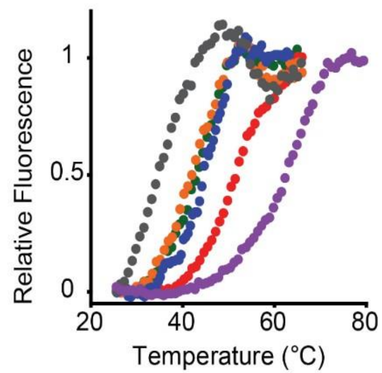
Woíking píinciple

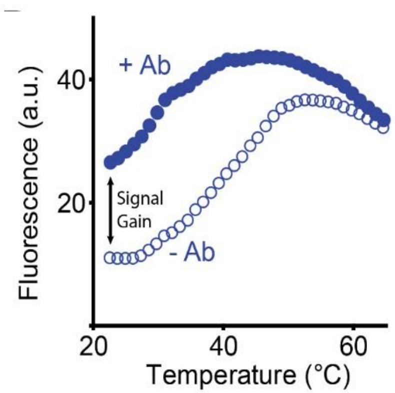
Ranallo S. *eī al., Angew. Chem. Inī. Ed*. **54, 13214–13218 (2015).**

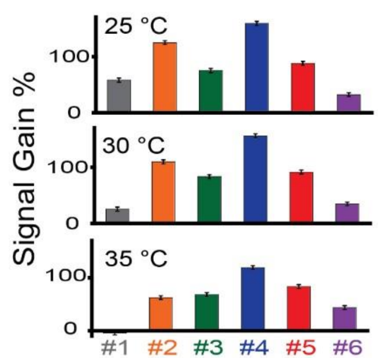
Proof of principle of optical antibody-binding switch

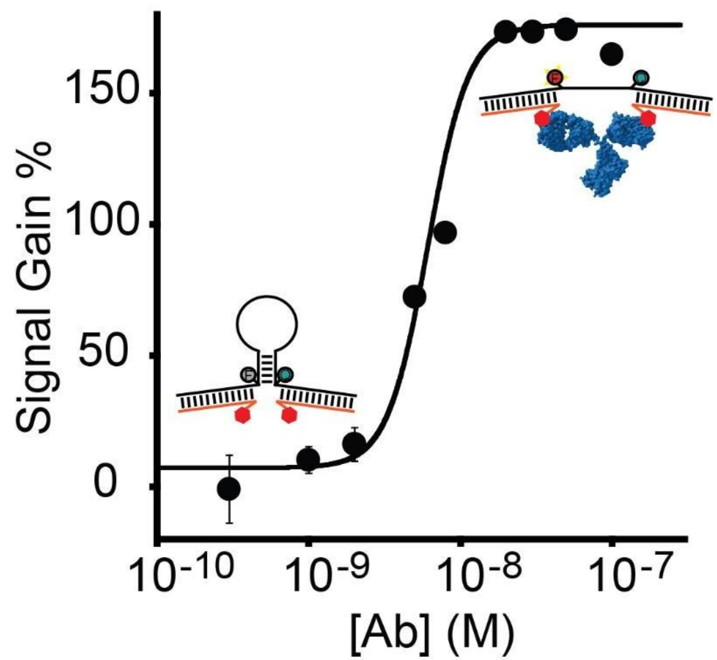


Variant	Stem	Loop
#1	3GC+2AT	poly-T (20mer)
#2	3GC+2AT	poly-T (13mer)
#3	2GC+2AT	Random (15mer)
#4	3GC+2AT	poly-T (15mer)
#5	3GC+2AT	Random (15mer)
#6	4GC+1AT	Random (15mer)







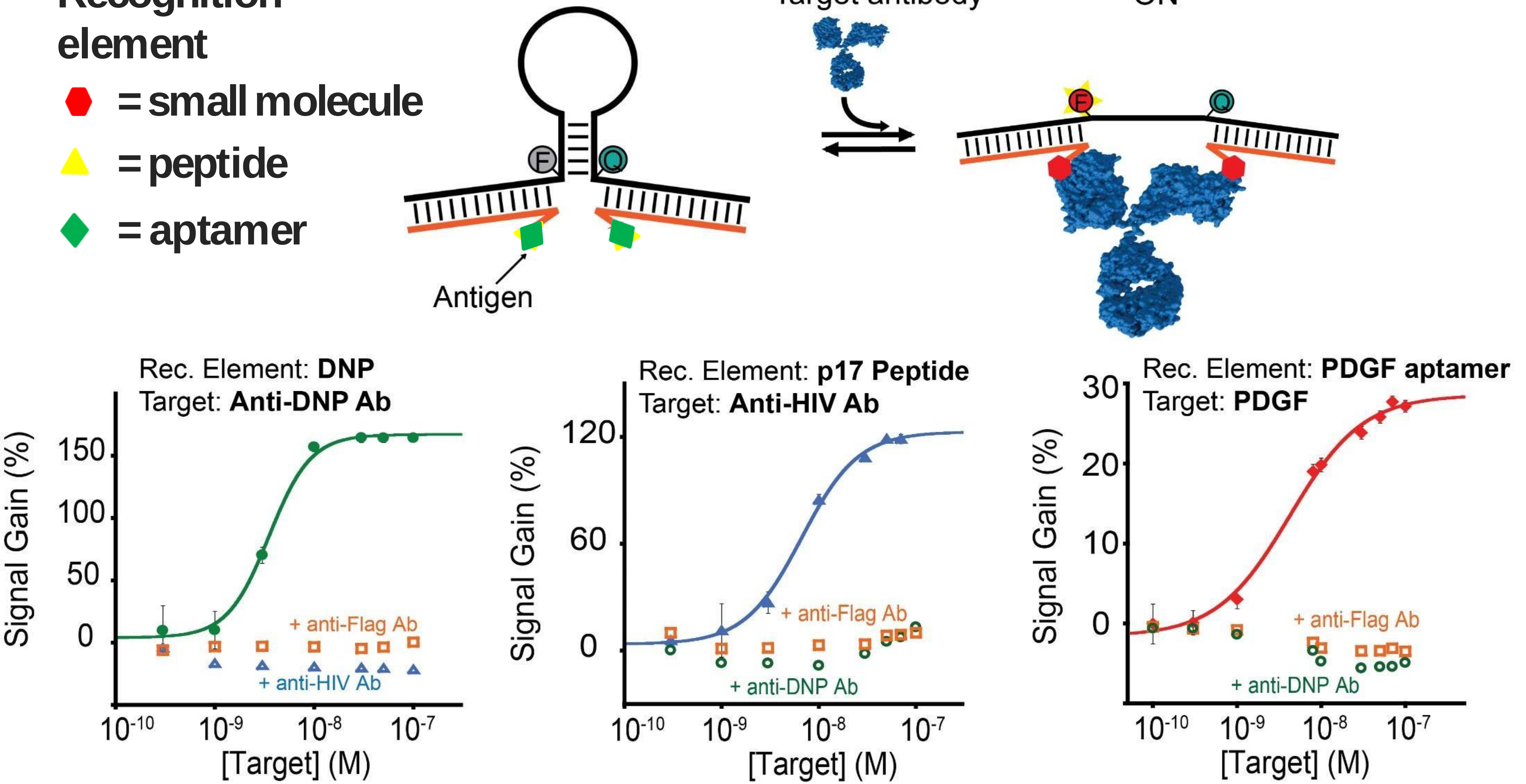


Ranallo S. *et al., Angew. Chem. Int. Ed*. **54, 13214–13218 (2015).**

A versatile platform

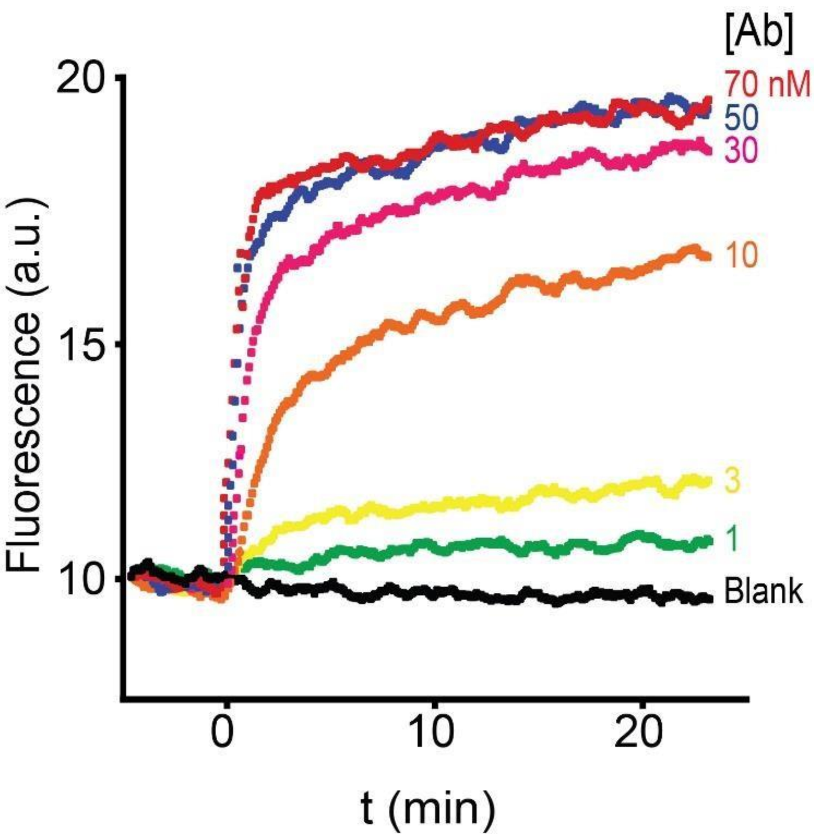
Recognition element

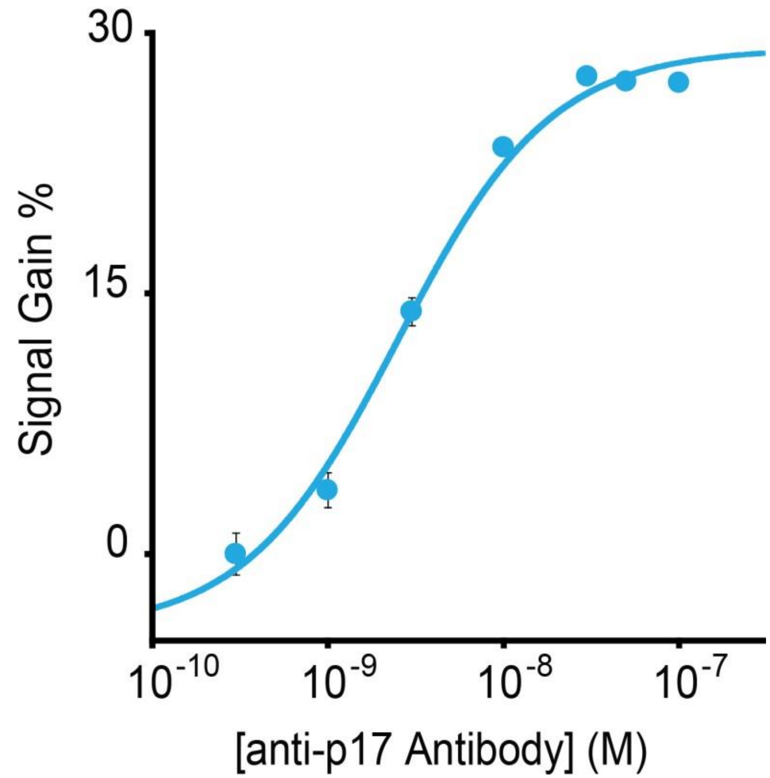
- = **small molecule**
- = **peptide**
- = **aptamer**



Ranallo S. *et al., Angew. Chem. Int. Ed*. **54, 13214–13218 (2015).**

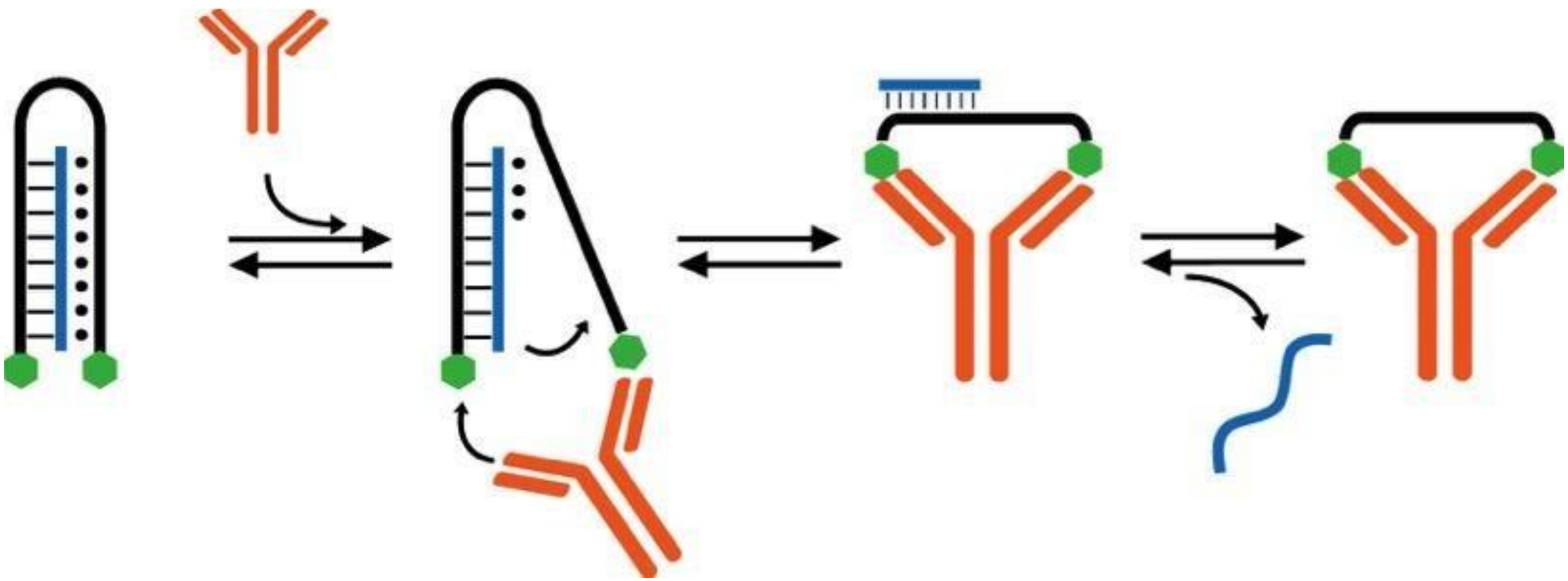
Rapid and quantitative in complex samples (serum)





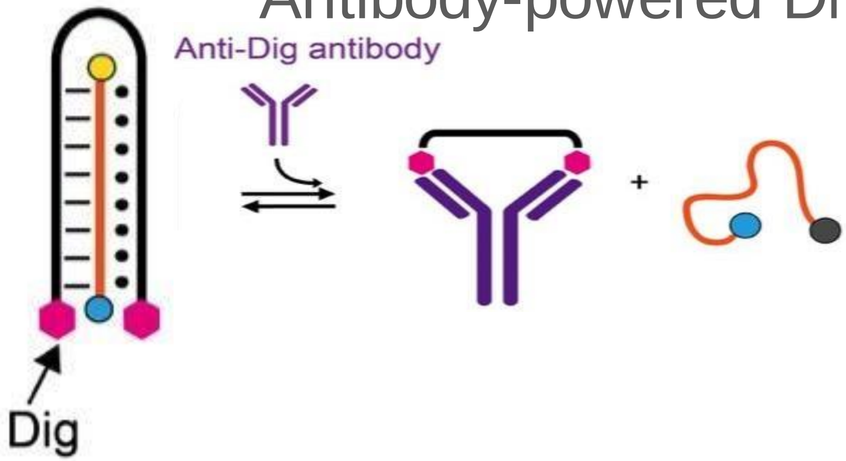
Ranallo S. *et al., Angew. Chem. Int. Ed*. **54, 13214–13218 (2015).**

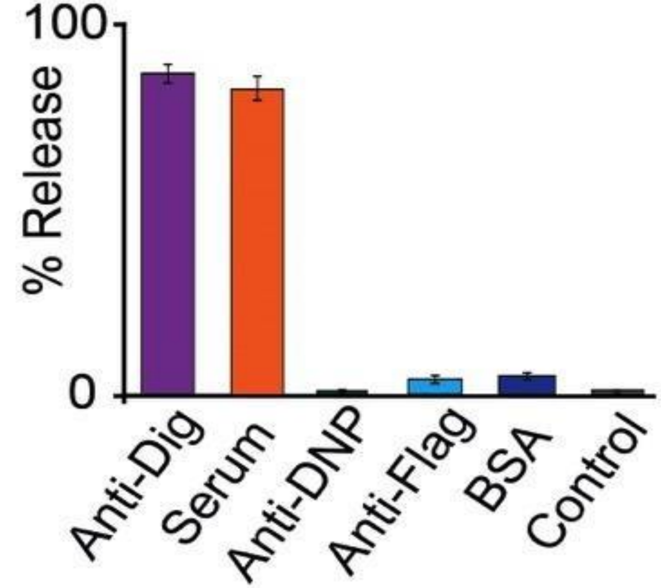
Working principle

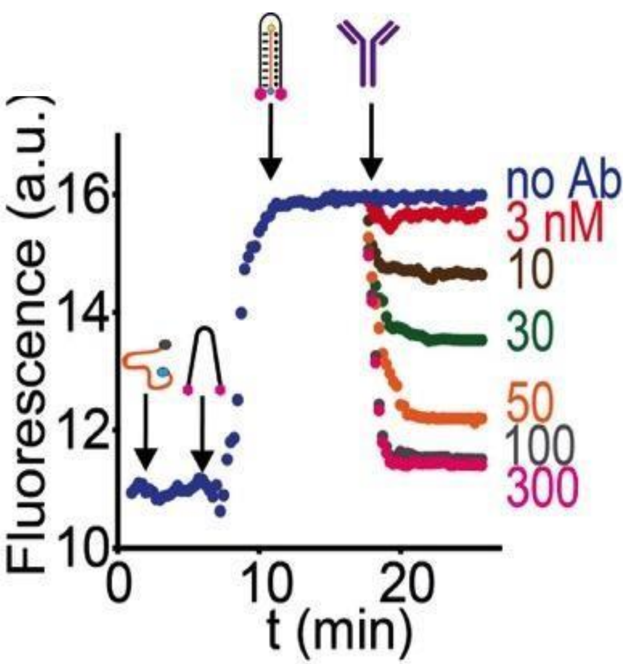


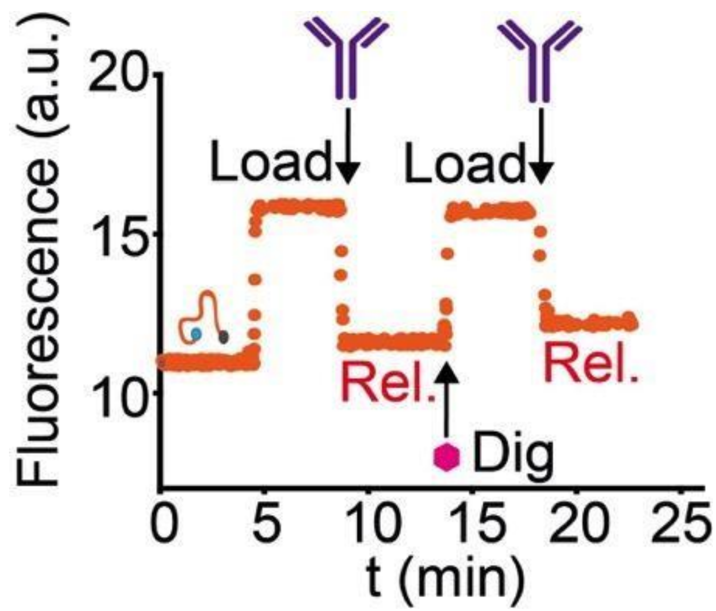
Ra S. *et al.*, ***Nat. Commun.*** **2017, 8, 15150.**

Antibody-powered DNA-based nanomachine



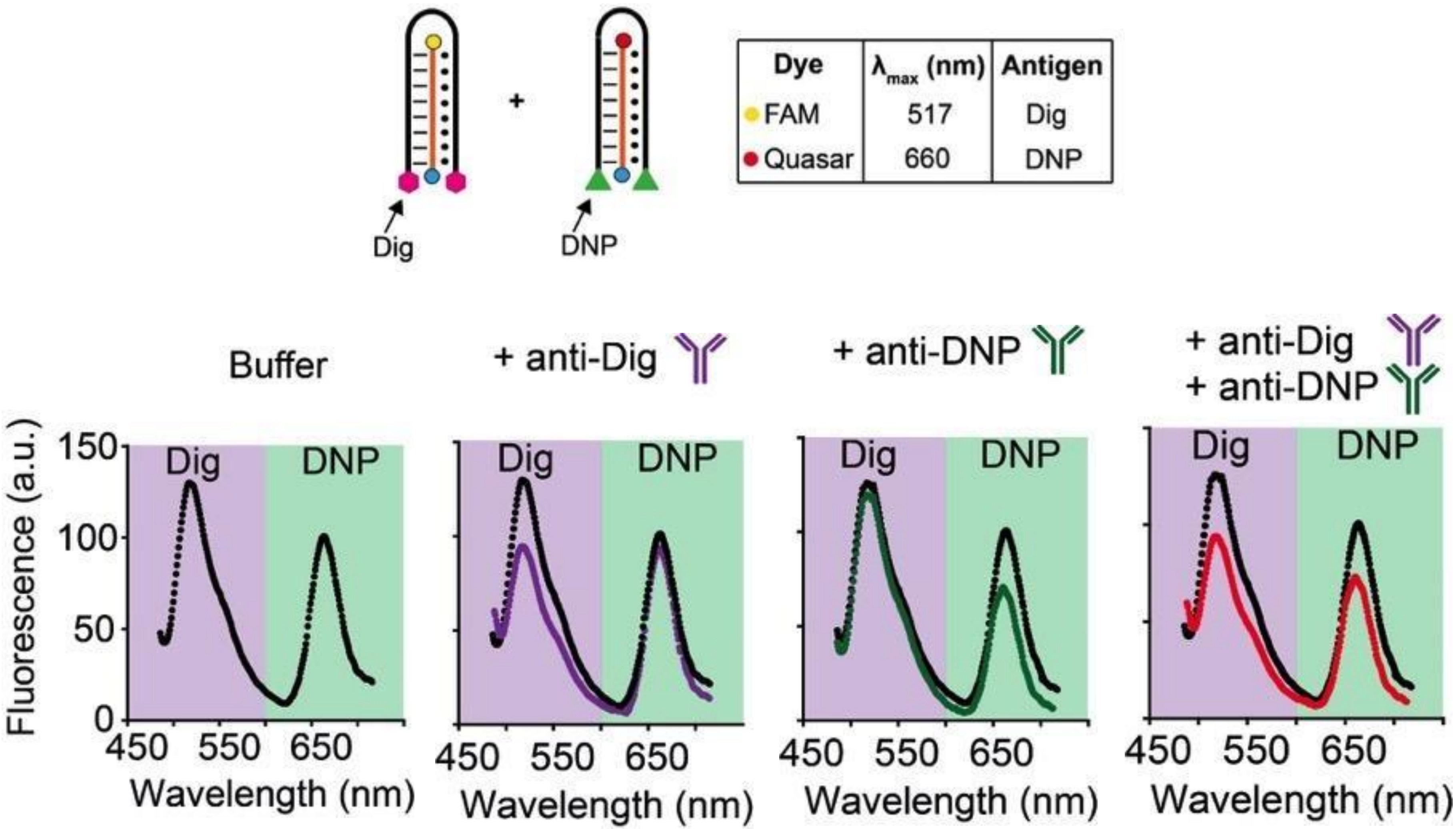






Ranallo S. *et al., **Nat. Commun.*** **2017, 8, 15150.**

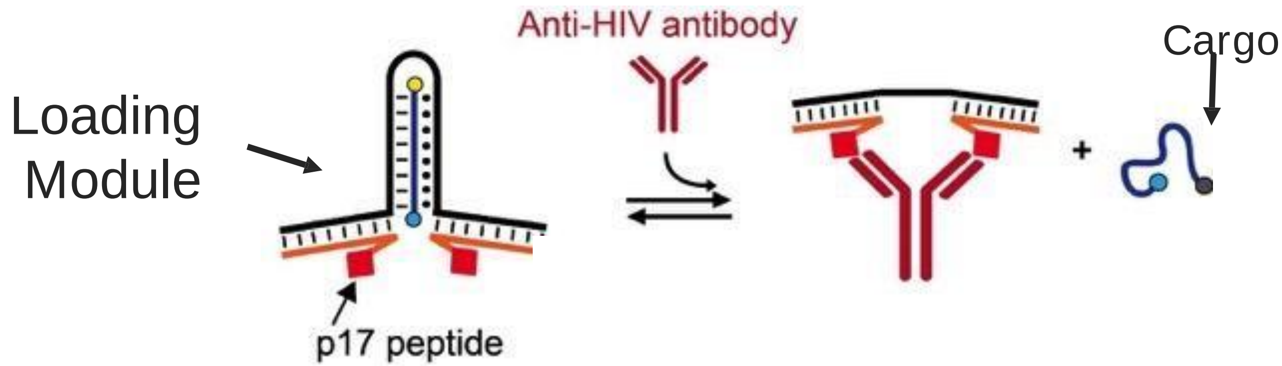
Antibody-poweíed DNA-based nanomachine

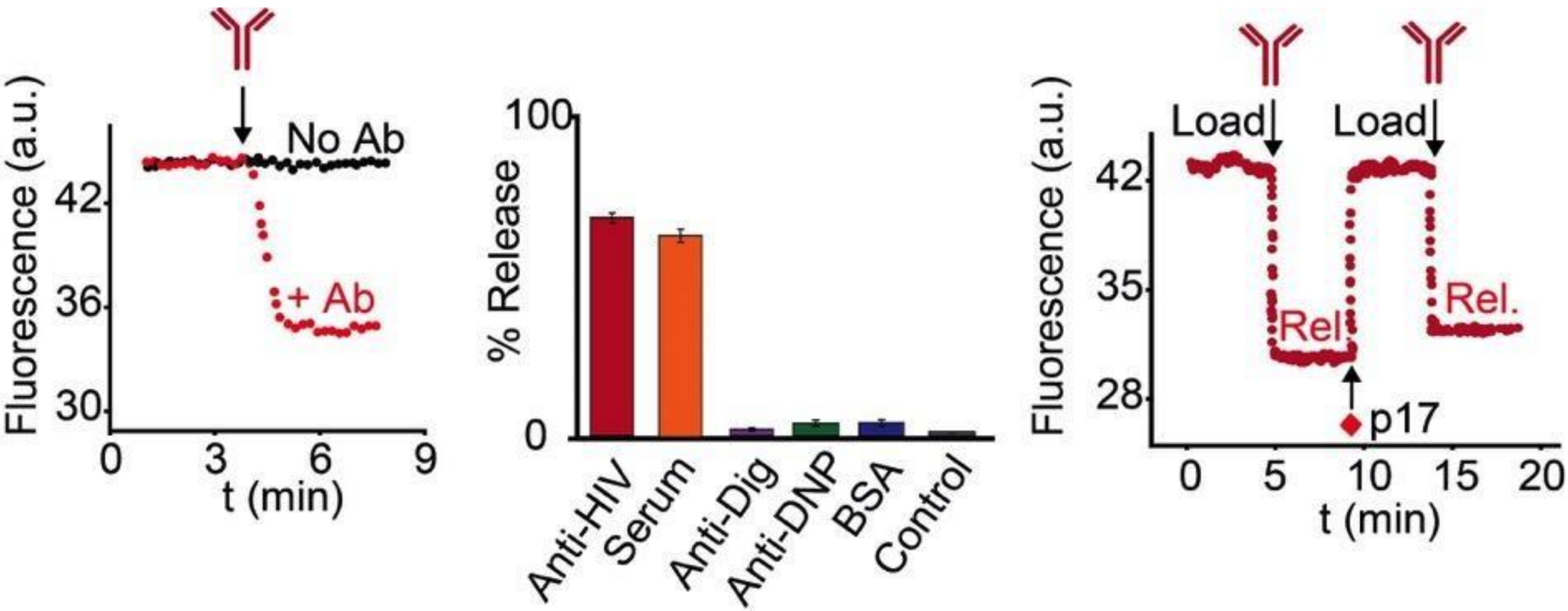


Dye	λ_{max} (nm)	Antigen
FAM	517	Dig
Quasar	660	DNP

Ranallo S. *et al.*, ***Nat. Commun.*** **2017, 8, 15150.**

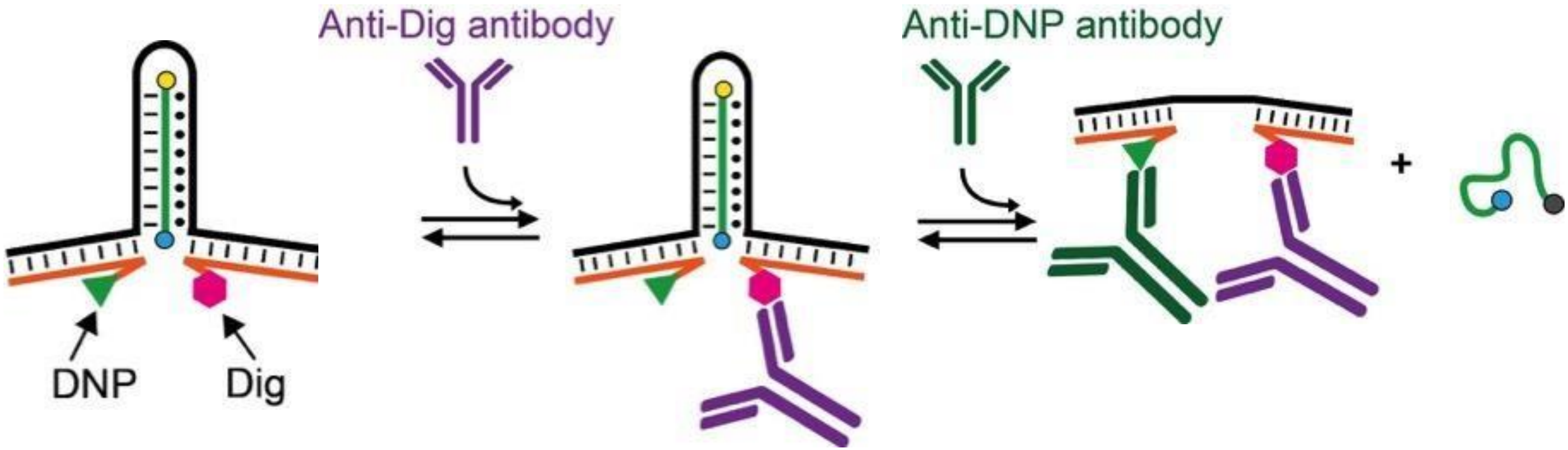
Modular antibody-powered DNA nanomachine

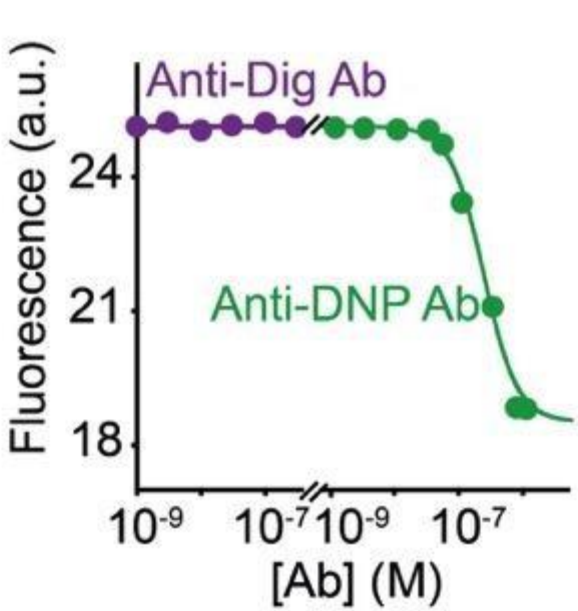


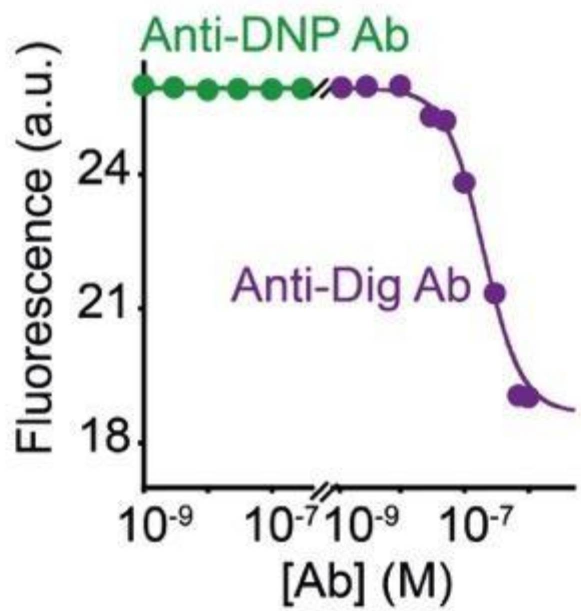


Ranallo S. *et al., **Nat. Commun.*** **2017, 8, 15150.**

AND Logic Gate DNA nanomachine







Ranallo S. *e al.,* ***Nat. Commun*****. 2017, 8, 15150**

Antibody-powered nanomachine

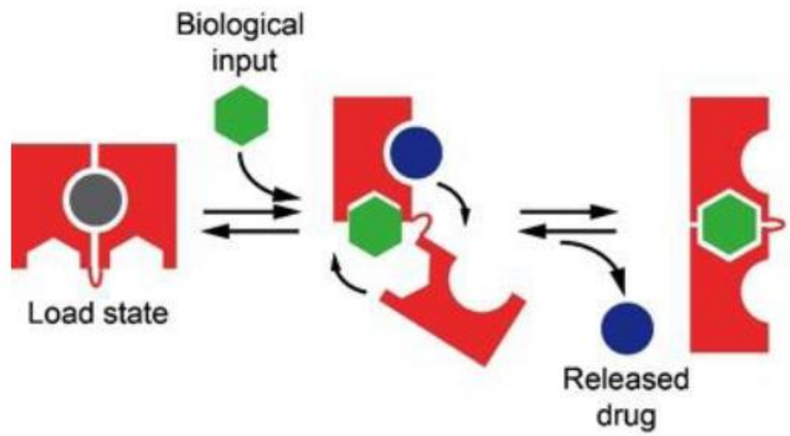
- ✓ Antibody-powered DNA-based nanomachines able to load and release a cargo in a controlled fashion.

- ✓ These nanomachines may prove of utility in a range of applications, including point-of-care diagnostics, controlled drug-release and in-vivo imaging.

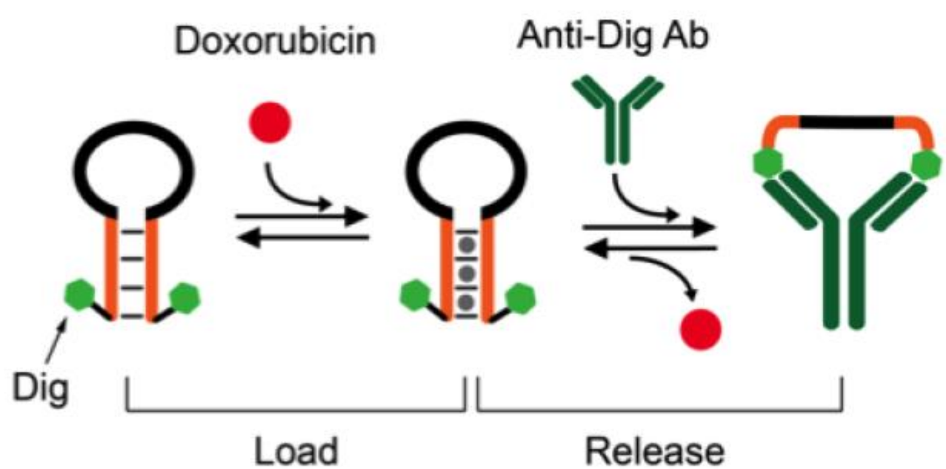
Antibody triggers the release of Doxorubicin loaded in a DNA nanomachine

Rossetti M. *et al.*, ***Chem. Scie.*** 2017, 8, 914-920.

Working principle

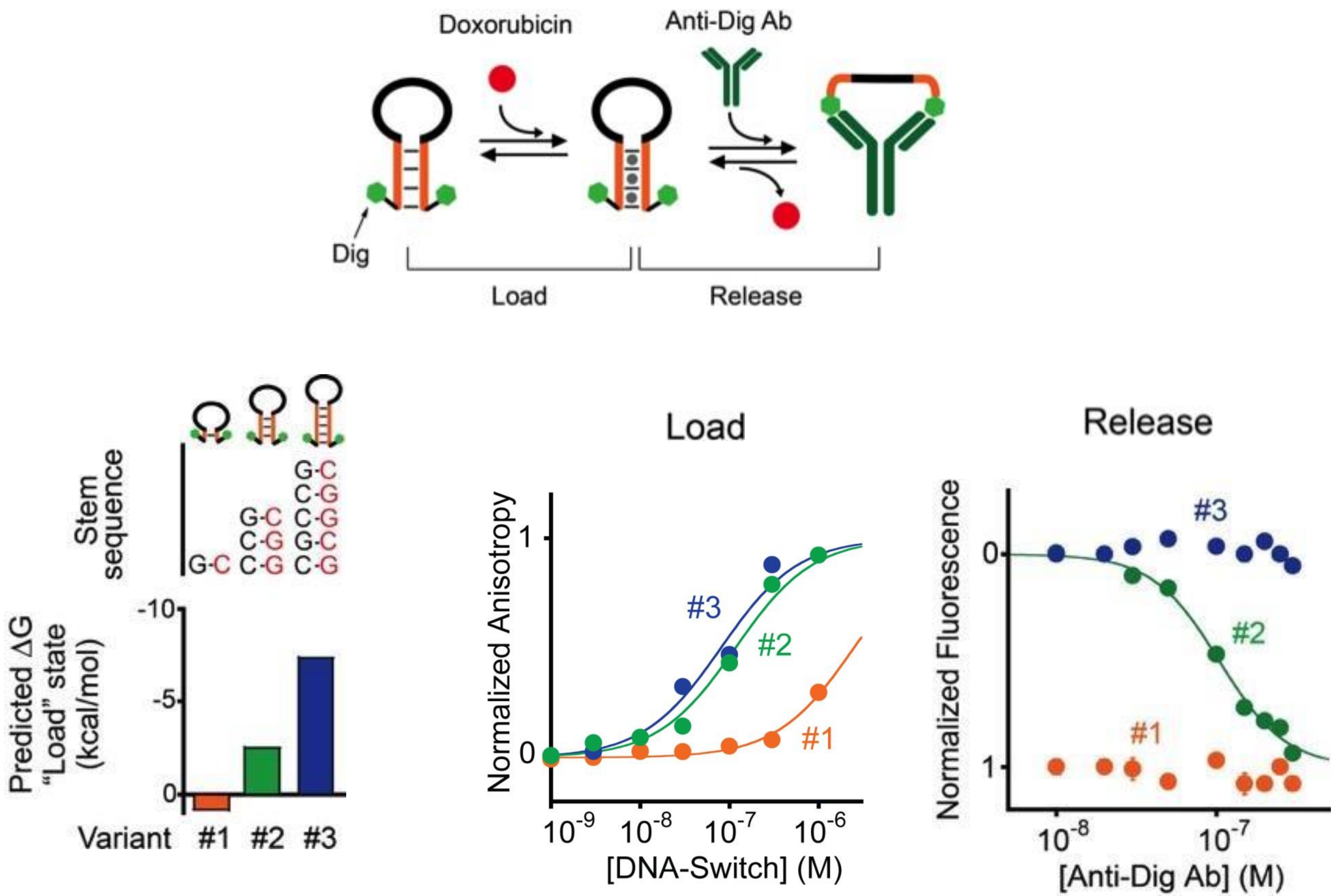


Rational design of a new class of DNA-based nanomachines *allosterically regulated* by specific biological targets, able to release a molecular cargo in a controlled fashion.



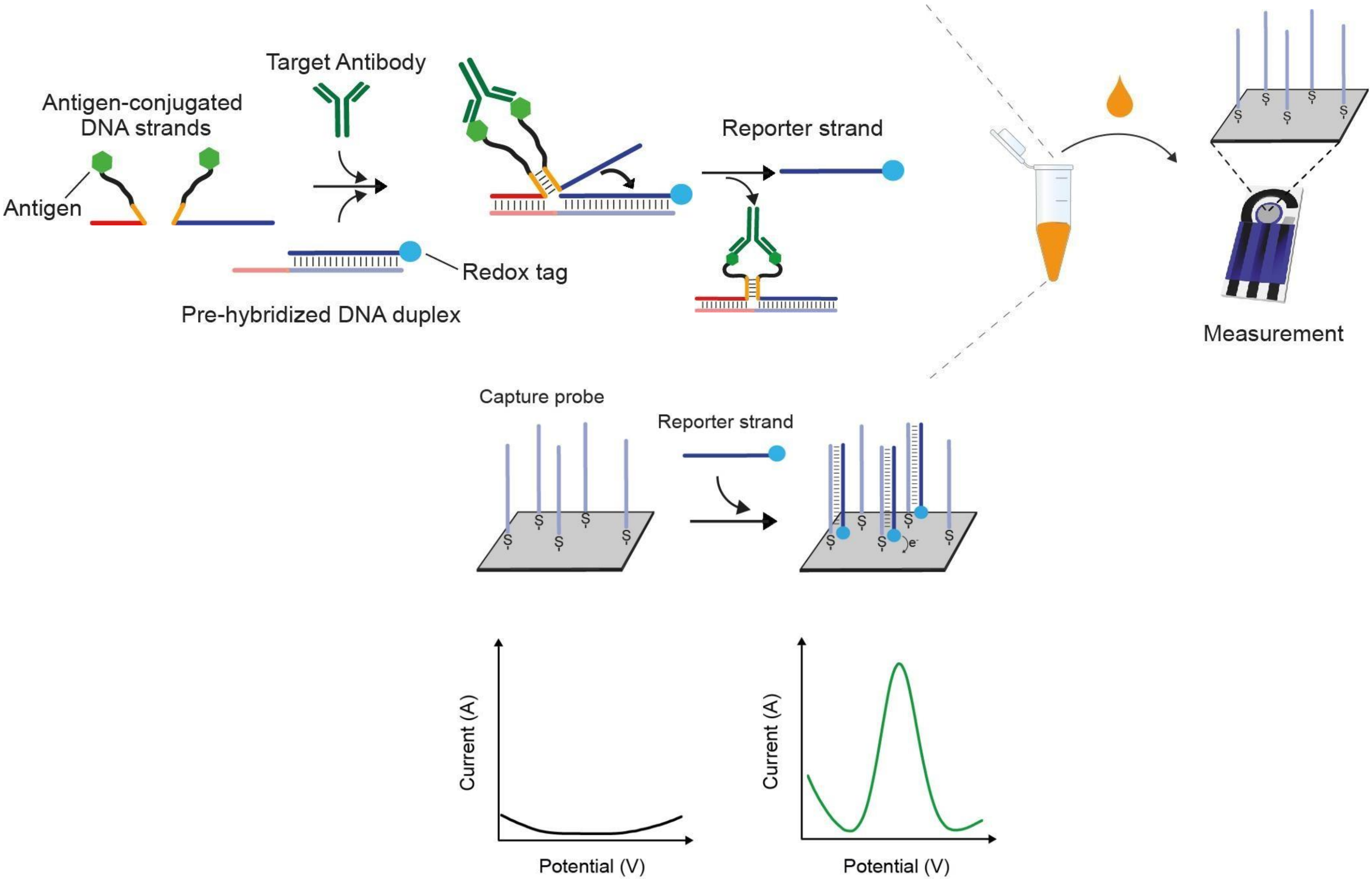
Rossetti M. *et al.*, ***Chem. Scie.*** 2017, 8, 914-920.

DNA-cargo switches activated by Antibodies



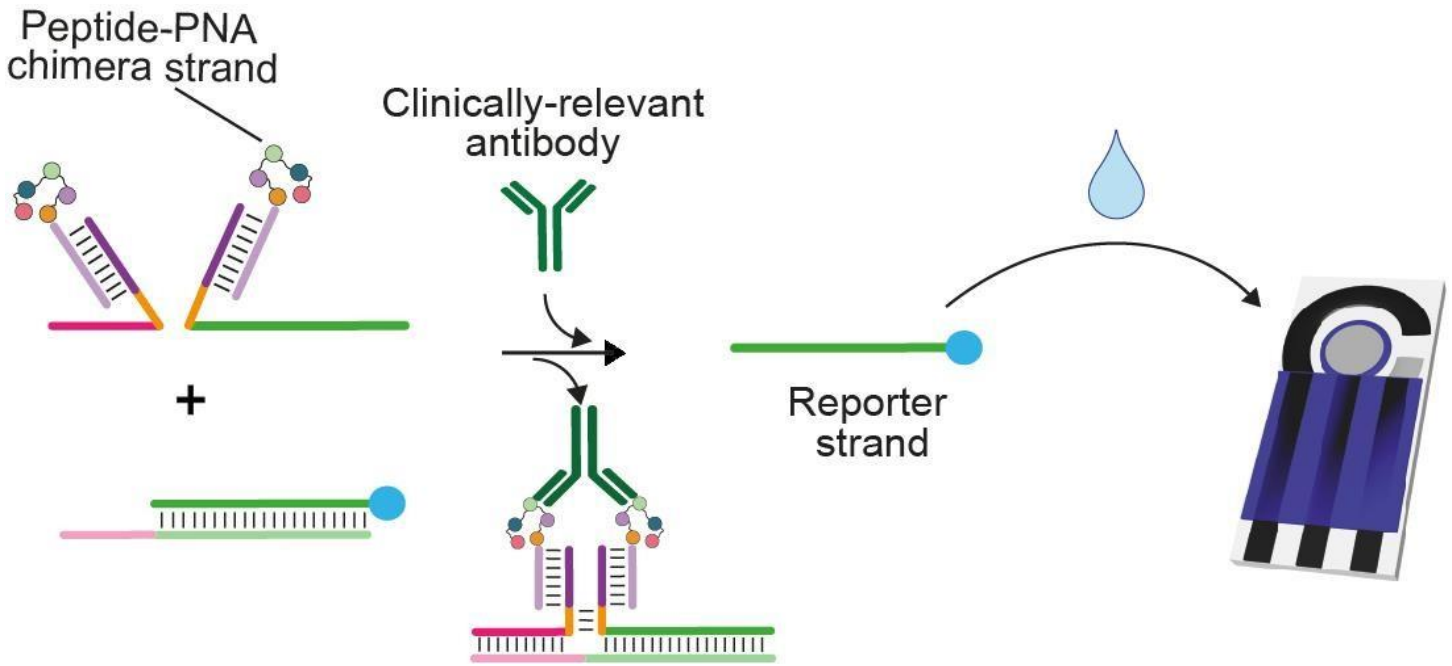
Rossetti M. *et al.*, ***Chem. Sci.*** **2017,** *8*, **914-920.**

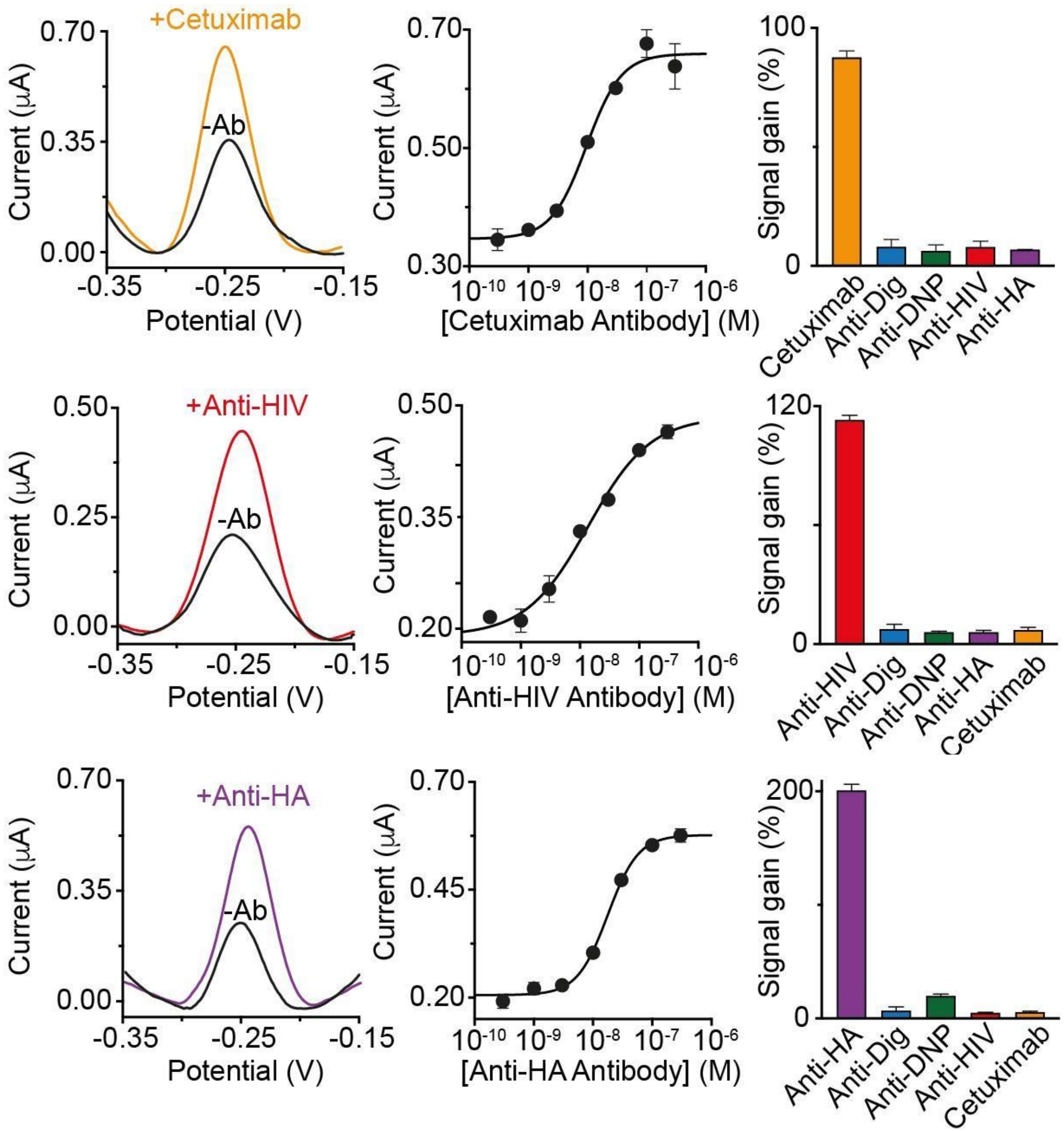
Electrochemical DNA-based Platform foí antibody detection



Bíacaglia ***S.,*** Ranallo S. ***et al.,*** ACS Sens. 2021, 6, 2442–2448

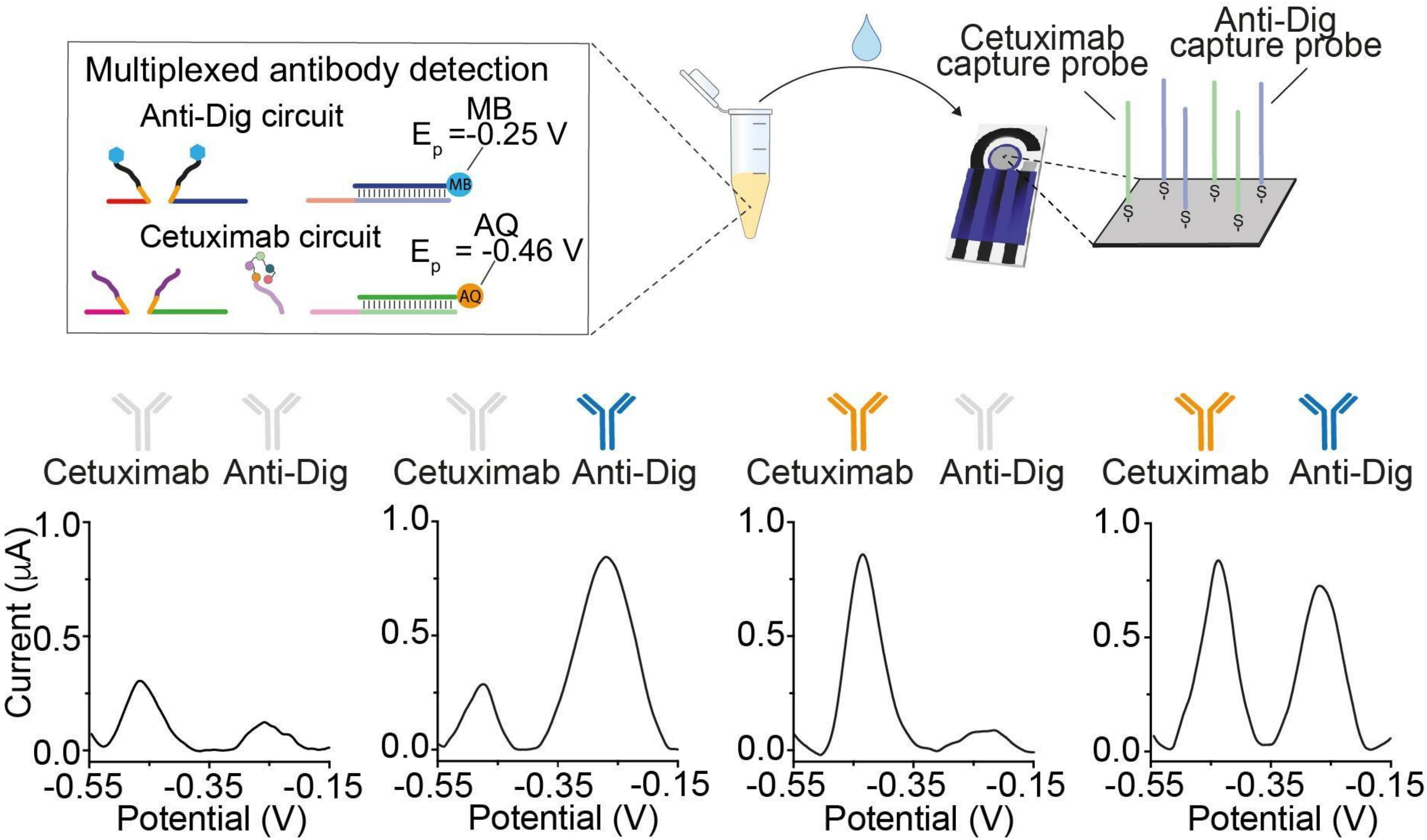
Electrochemical platform:
detection of clinically-relevant antibodies





+Cetuximab
-Ab
Current (μA)
Potential (V)
[Cetuximab Antibody] (M)
Signal gain (%)
Cetuximab
Anti-Dig
Anti-DNP
Anti-HIV
Anti-HA
+Anti-HIV
[Anti-HIV Antibody] (M)
+Anti-HA
[Anti-HA Antibody] (M)

Orthogonal platform: multiplexed antibodies detection



Electíochemical DNA-based platfoím foí antibody detection

Sensiti ve

Low volume

Veísatil e

Low cost

Home test