DNA-based nanomachines powered by biological inputs for diagnostic and drug delivery applications

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University of Teramo 3rd May 2022

About me

2012
Visiting research period
University of California Santa Barbara (UCSB)
EU Marie Curie FP7 IRSES

2013
Master Degree (Chemistry)

2015
Visiting periods
University of California Santa Barbara (UCSB)
Universitè de Montrèal (UDEM)
EU Marie Curie FP7 IRSES

2017
PhD Chemical Science
University of Rome Tor Vergata

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Post-Doc Fellowship
Fondazione Umberto Veronesi
University of Rome Tor Vergata

2020-2023
Marie Curie Post-Doc Fellow
MSCA Individual Global Fellowship
University of California Santa Barbara (UCSB)
University of RomeTor Vergata

Outline

DNA Nanotechnology

Structural DNA-based systems

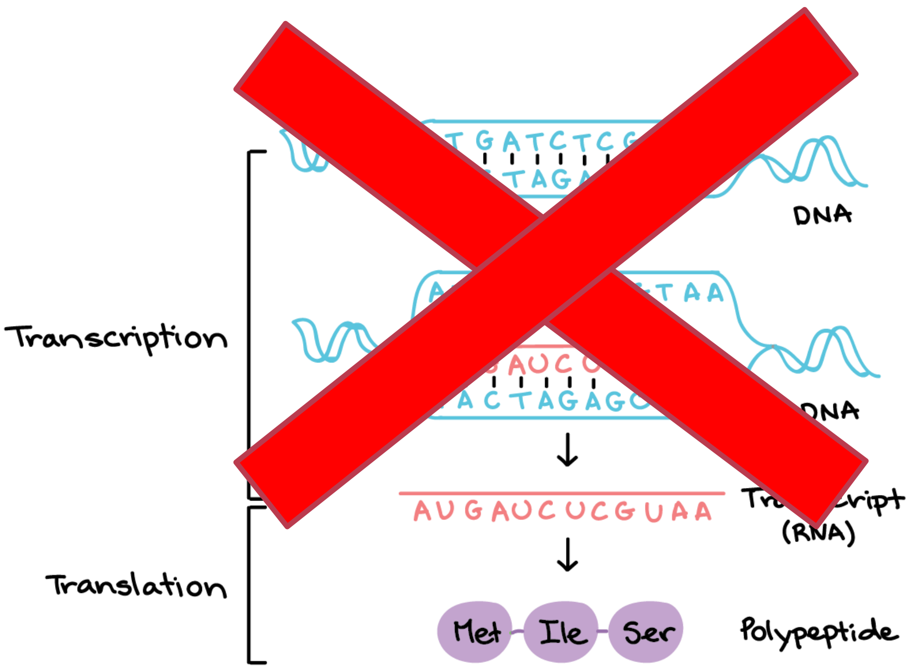
Nanomachines for diagnostic applications

Nanomachines for drug-delivery applications

Nanomachines as molecular tools
to mediate protein-protein communication

DNA in Biological Organism

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.

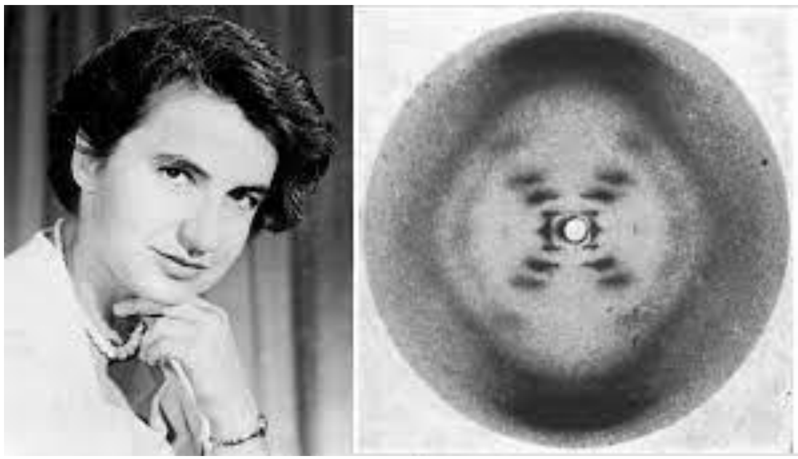
Why DNA as nanomaterial building block?

- 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 Double Helix:
Rosalind Franklin *vs* Watson and Crick

Rosalind Franklin:
in 1951-1952: Photograph 51



Watson and Crick:
in 1953: publication in Nature

In 1962 Nobel Prize to Watson and Crick for the discovery of DNA structure. NO mention to Rosalind Franklin.

What was wrong?

DNA Nanotechnology

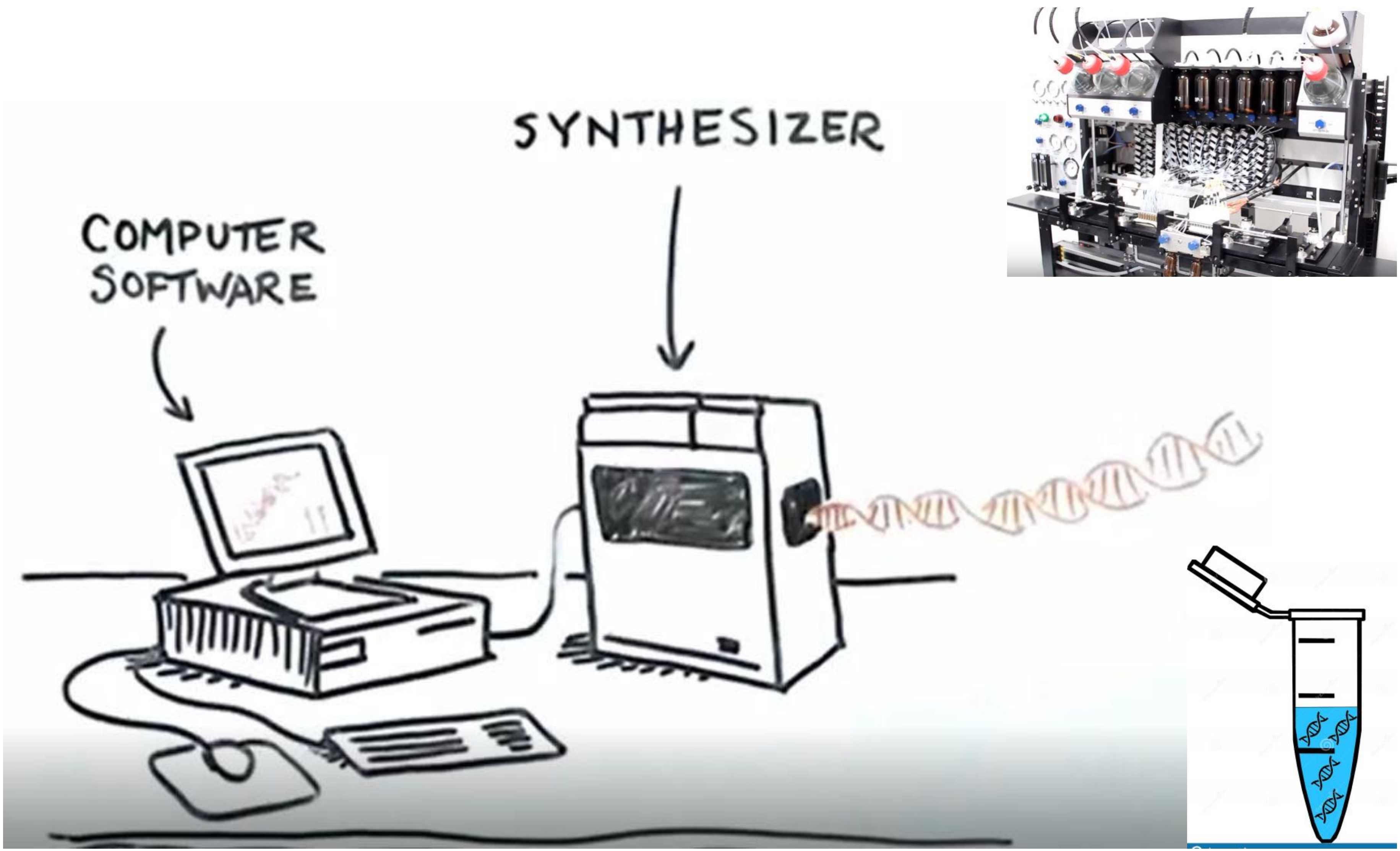
01 Structural DNA Nanotechnology: 2D

02 Structural DNA Nanotechnology: 3D

03 Functional DNA Nanotechnology

04 Structural + Functional DNA Nanotechnology

Solid-phase synthesis of DNA



Structural DNA-Nanotechnology

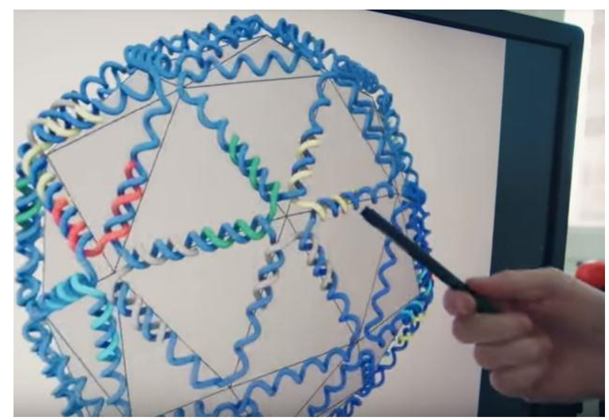
To build object of any shape at the nanometer scale through self assembly of DNA strands.

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

Structural DNA-Nanotechnology

1. Draw the raw shape of the structure!

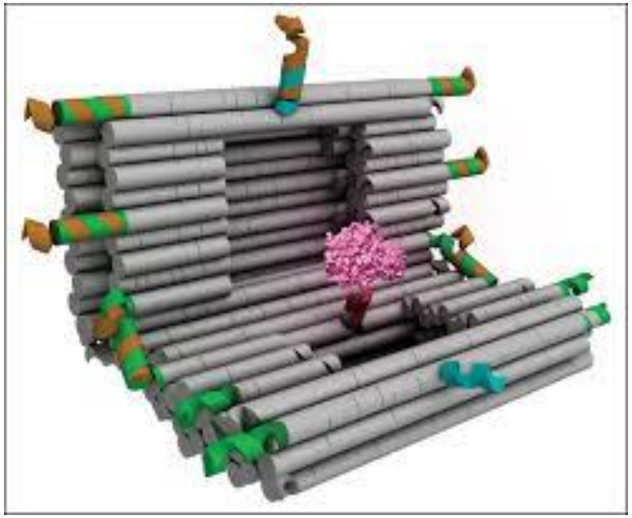
2. An algorithm calculates how the DNA scaffold strand will arrange itself on the surface of the structure and will also calculate all the short DNA strands.



Structural DNA-Nanotechnology

3. Order the oligonucleotides

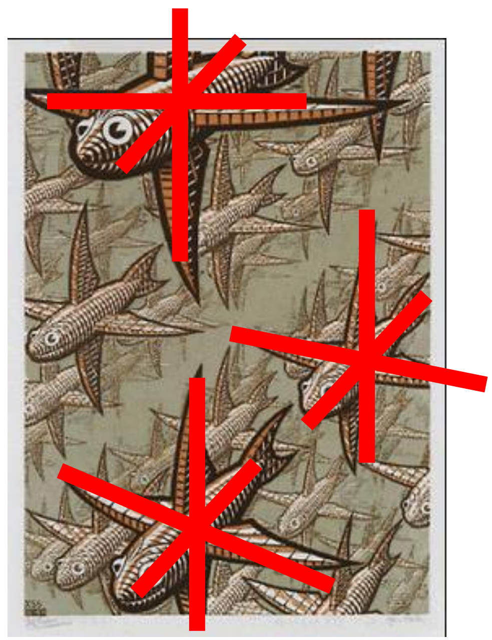
4. Mixing, annealing over night and the DNA nanostructure is done!



The early years: the Ned Seeman's dream

"I went to the campus pub to think about 6-arm DNA junctions"

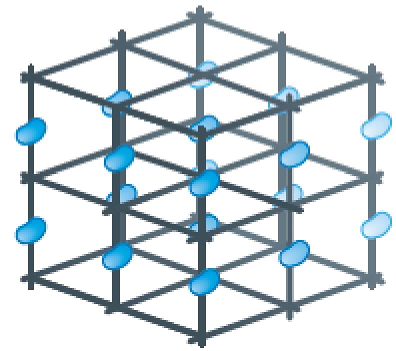
The fish are organized periodically in 3D, just like the molecules in a molecular crystal

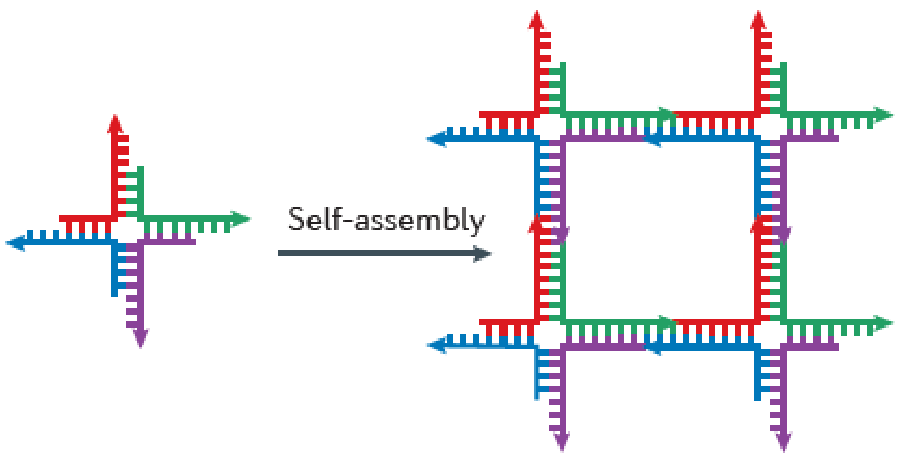


Escher's woodcut "Depth"

The early years: the Ned Seeman's dream

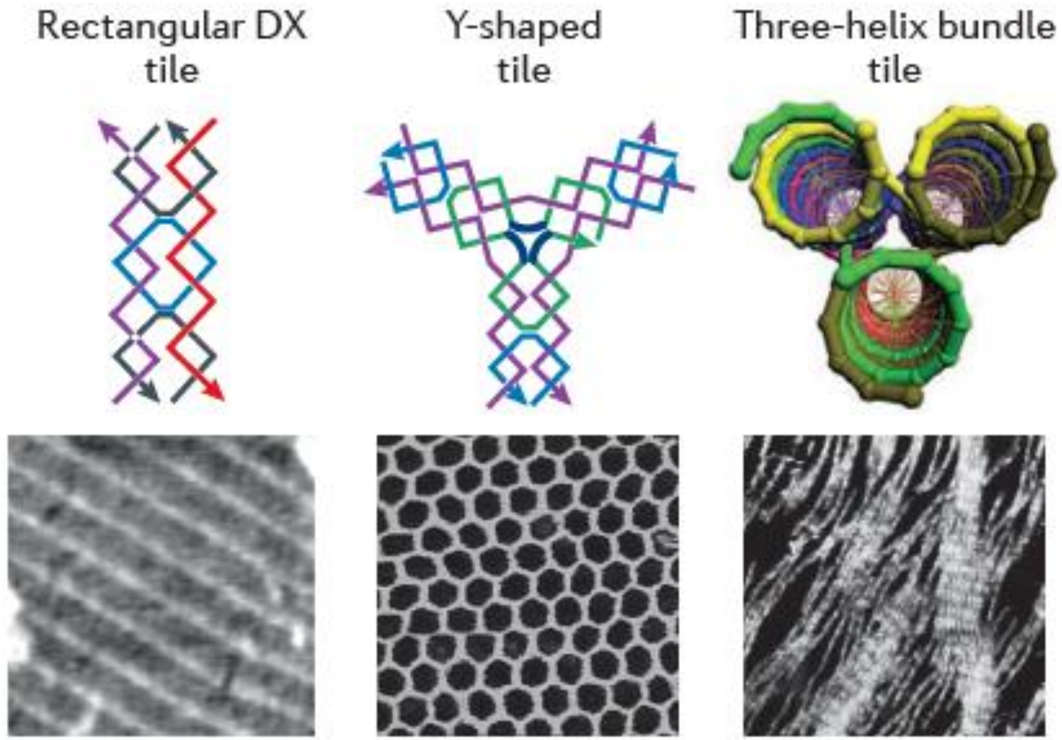
Rather than relying on trial and error to crystallize biological macromolecules, the idea was that crystals could be assembled using WC-interactions





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

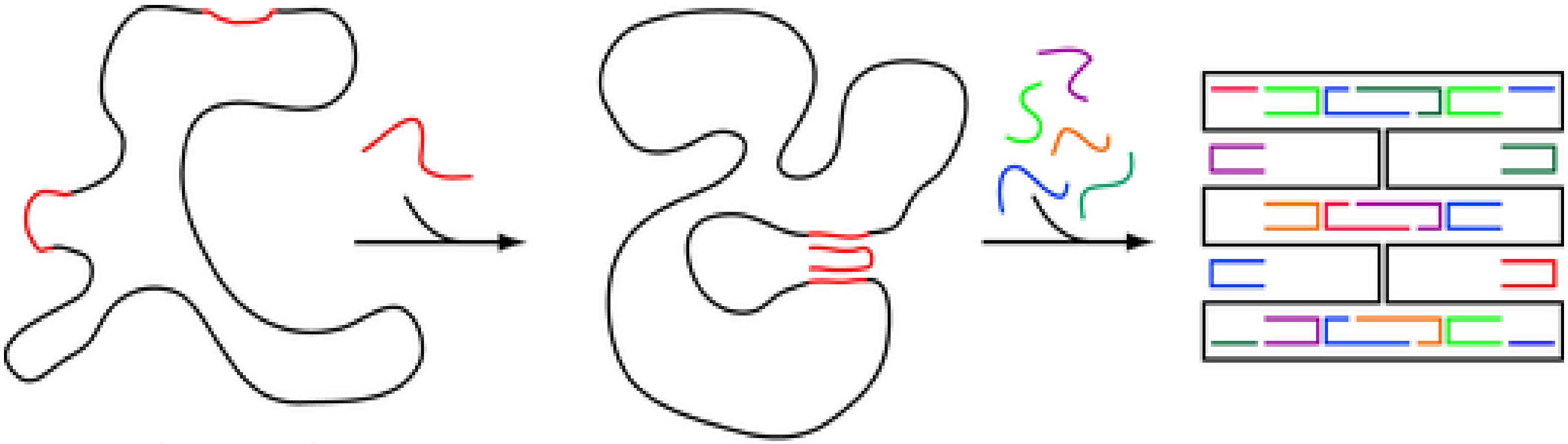
DNA tile self-assembly: 2D DNA crystals by design

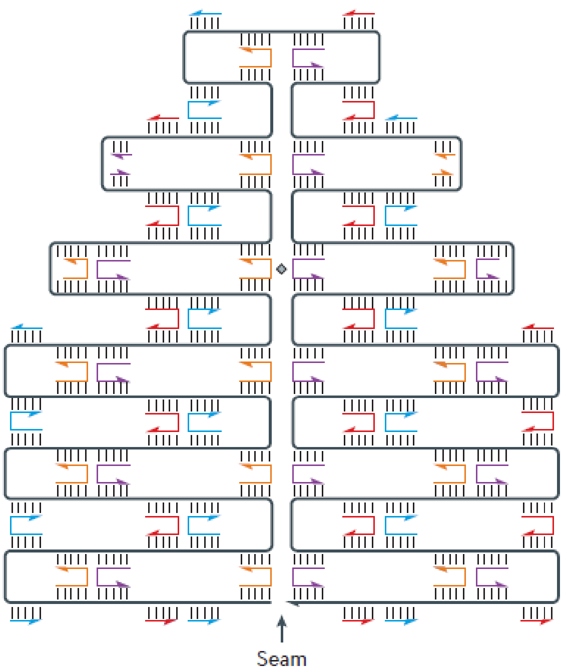


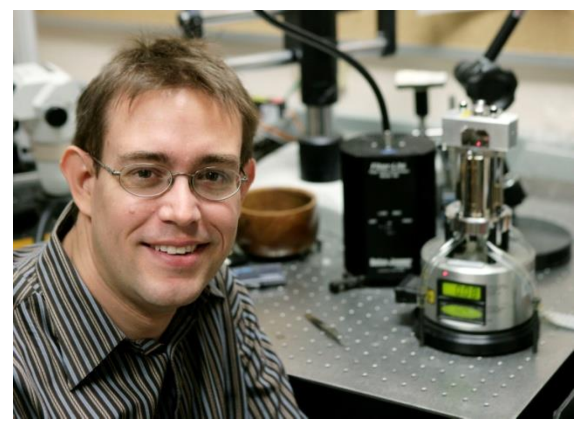
1) Making arms with unique sequences (limited branch migration)
2) Three-arm junctions , and double-crossover (DX) molecules comprising two DNA double helices linked together (geometric rigidity, proper stability and topology)

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

DNA Origami



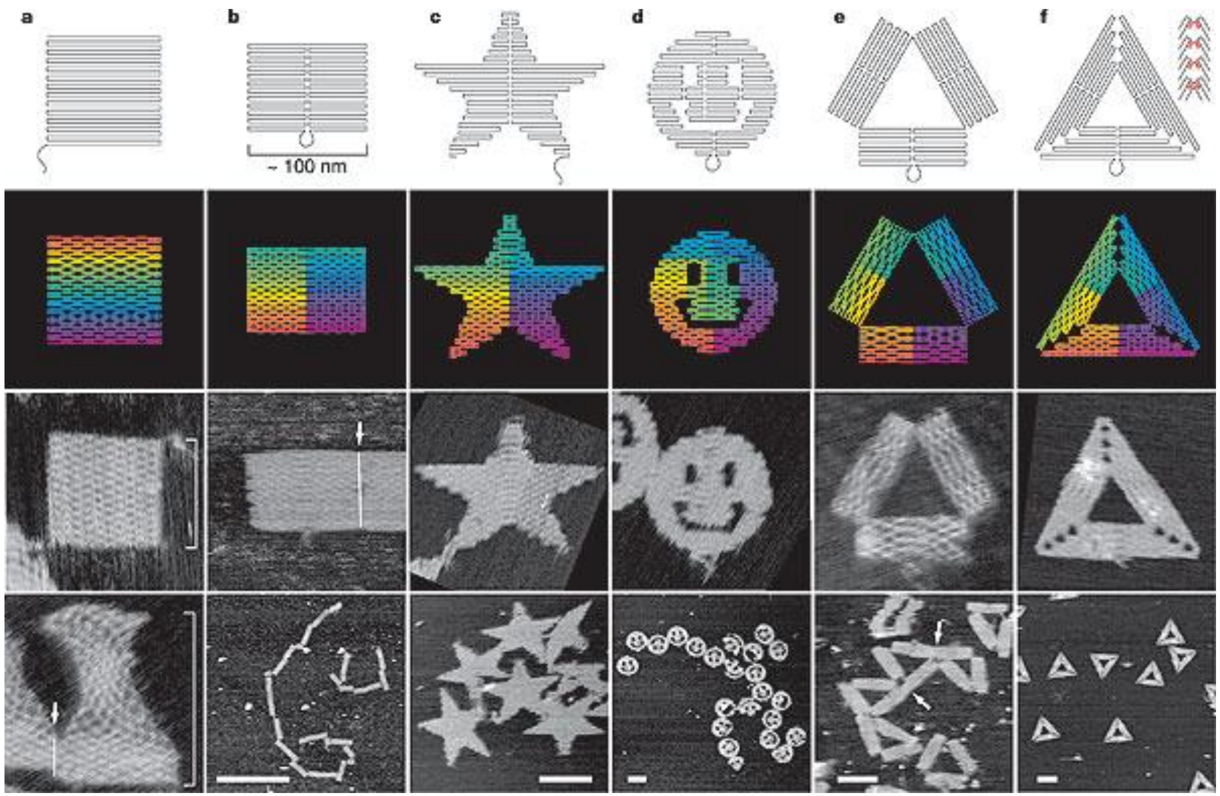




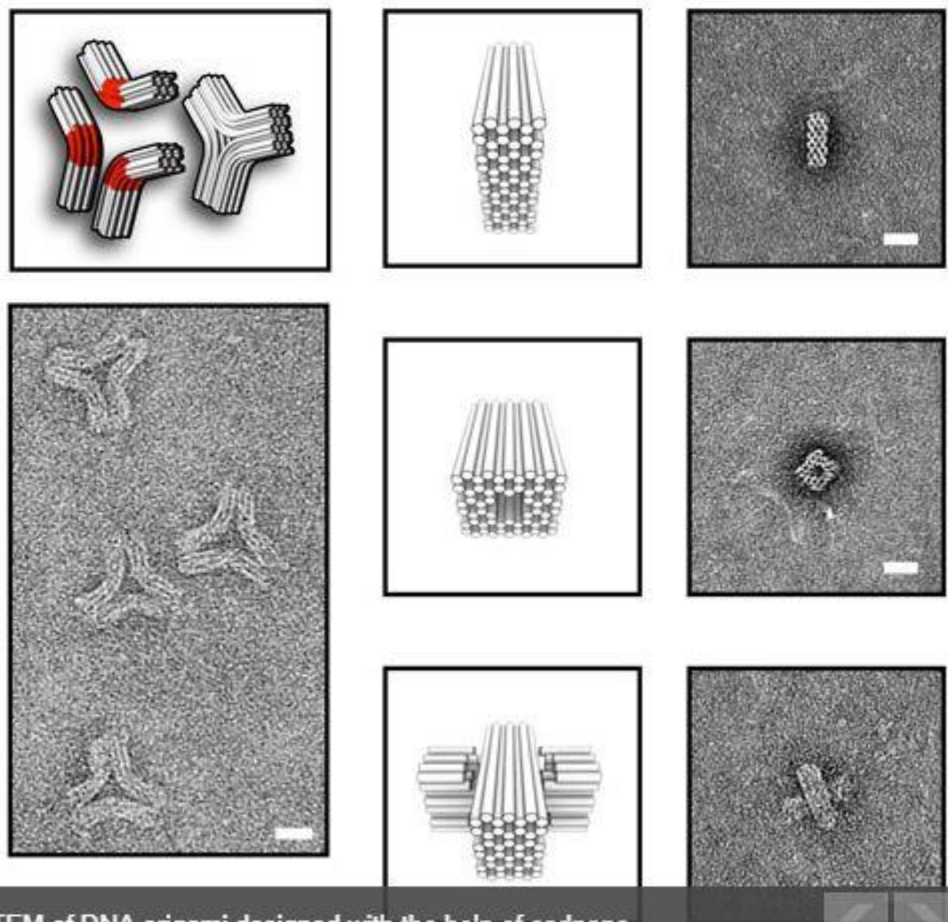
Rothemund, P. W. Folding DNA to create nanoscale shapes and patterns. *Nature* **440**, 297–302 (2006).

DNA Origami: 2D Structure

Non-periodic 2D structures of arbitrary 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 create nanoscale shapes and patterns. *Nature* **440**, 297–302 (2006).



TEM of DNA origami designed with the help of cadnano.

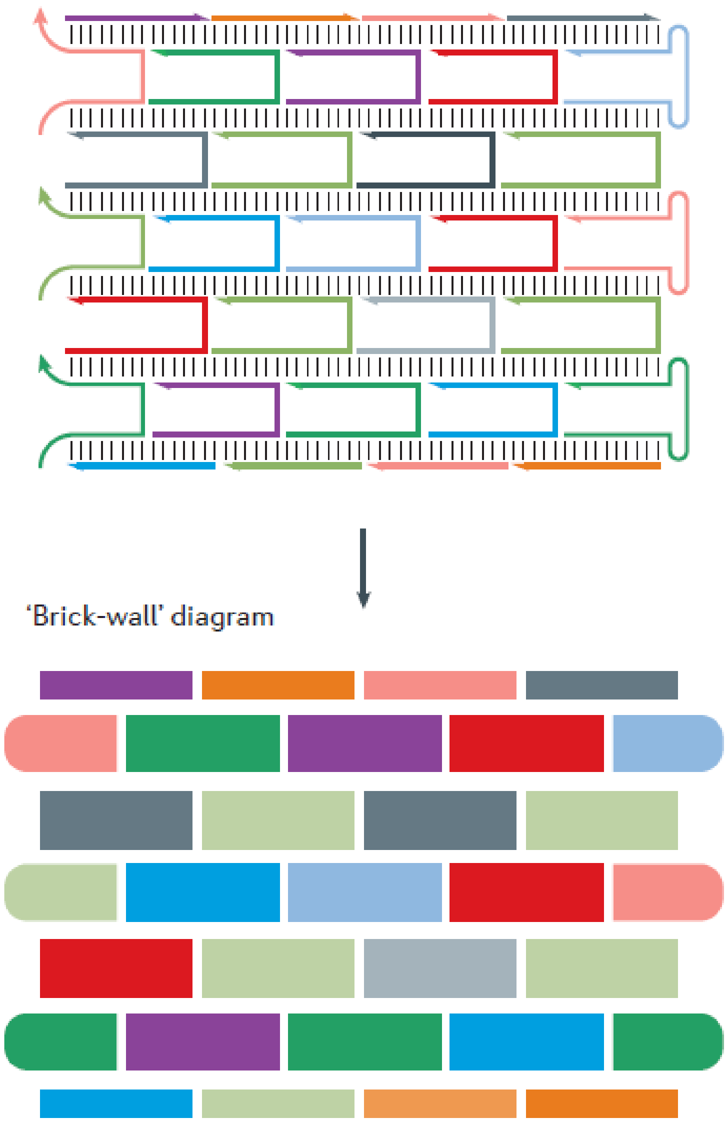
cadnano simplifies and enhances the process of designing three-dimensional DNA origami nanostructures. Through its user-friendly 2D and 3D interfaces it accelerates the creation of arbitrary designs. The embedded rules within **cadnano** paired with the finite element analysis performed by cando, provide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

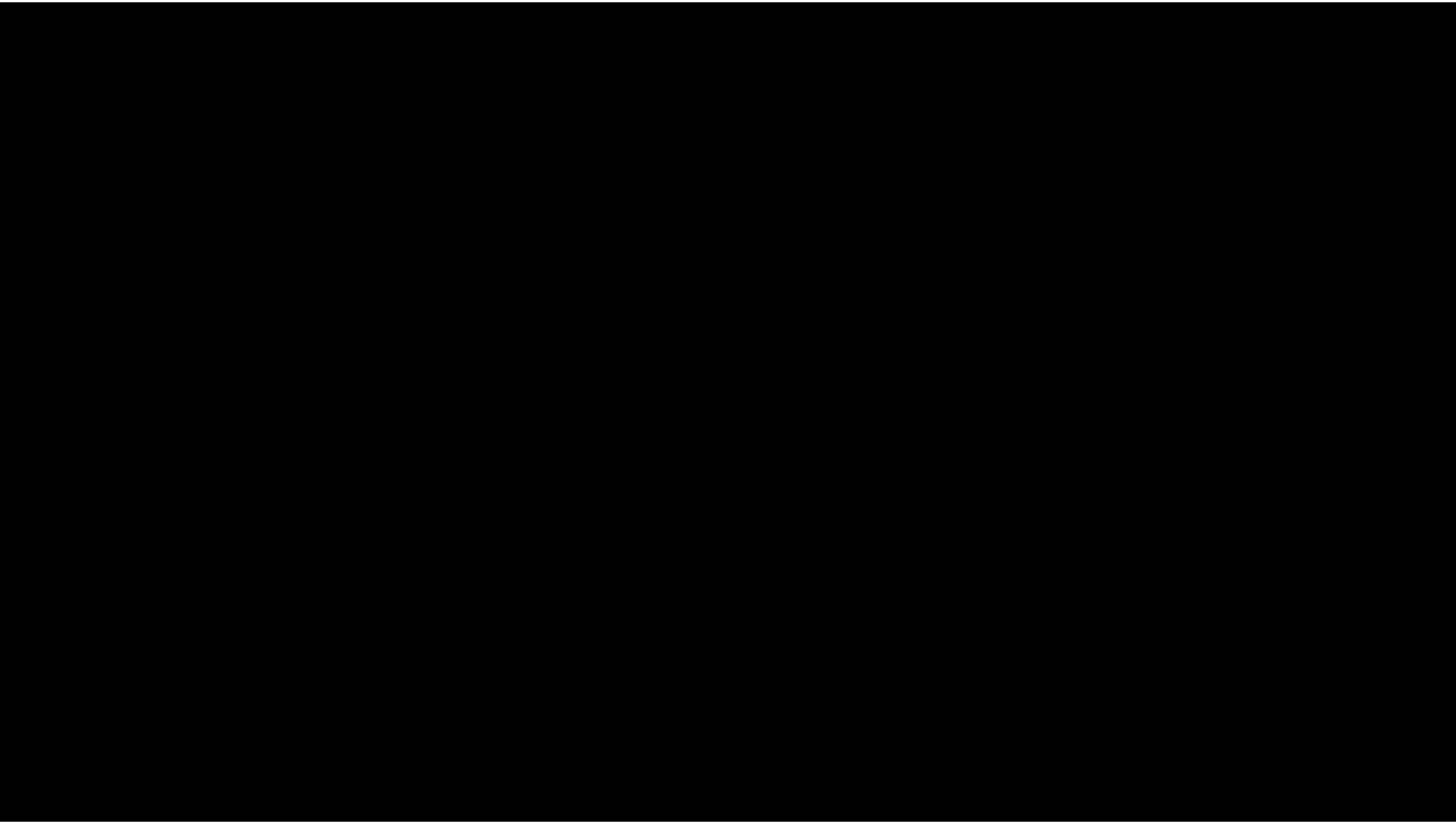


The building blocks are single strands of DNA containing four modular domains, which are designed to form interconnected staggered duplexes with one another, resulting in DNA lattices.

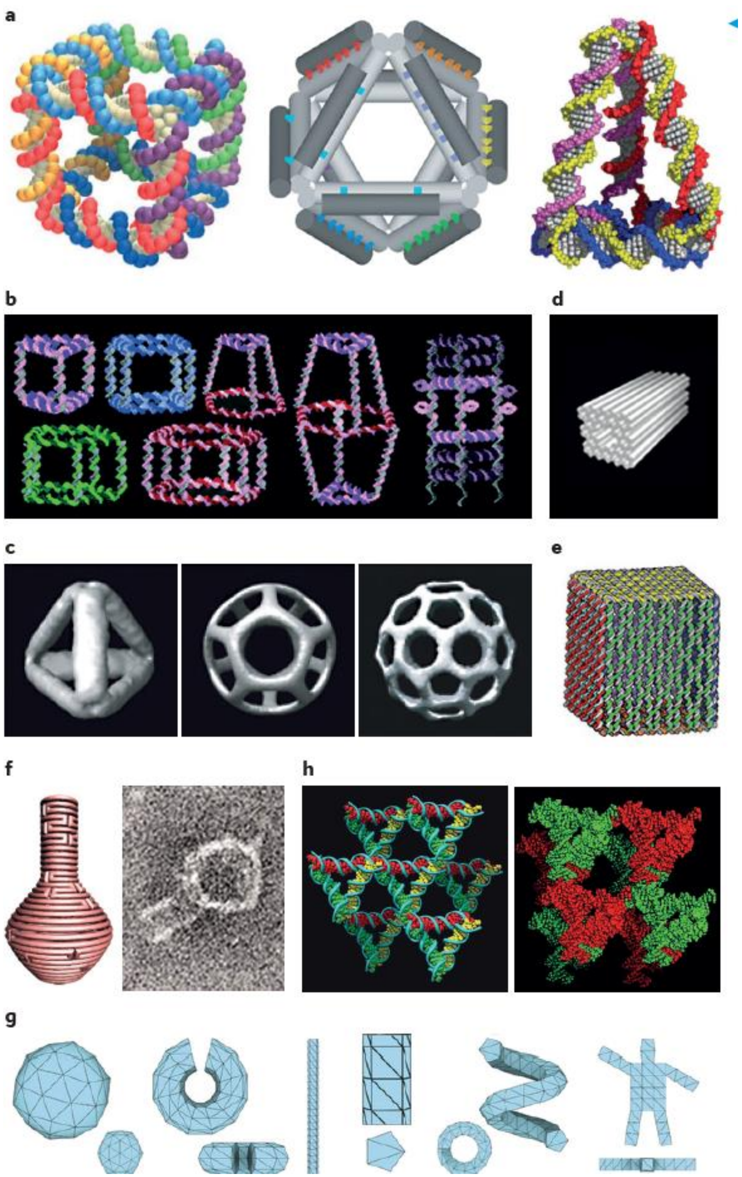
- The sequences are all unique.
- Any arbitrary shape by selecting the set of strands that defines the structure.

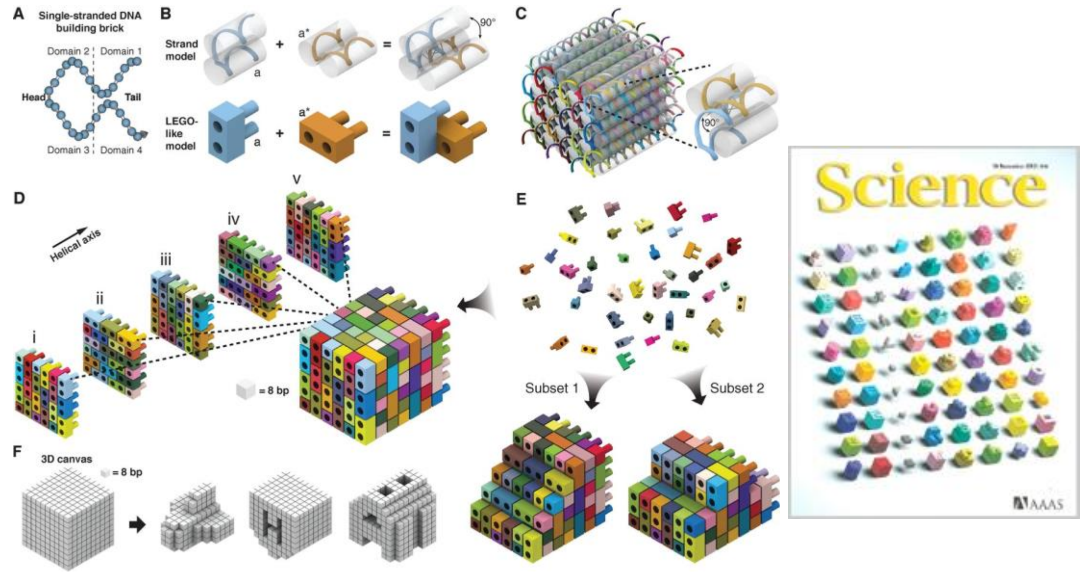
Wei, B. et al. Nature 485, 623–626 (2012).

Building 3D Structures with DNA Bricks



3D Nanostructure



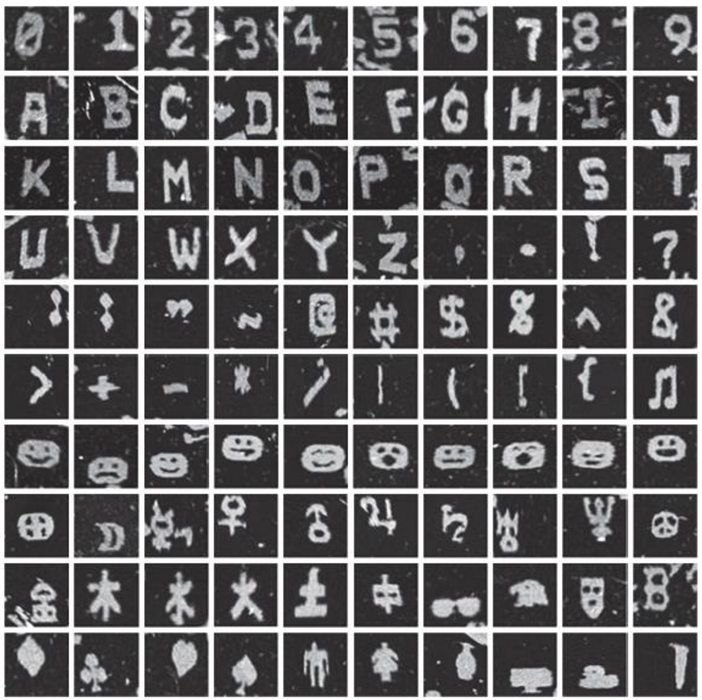


A number of approaches to produce 3D structures:

- 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



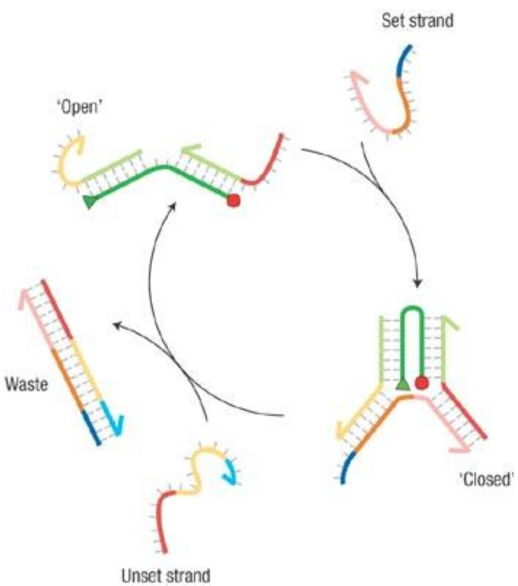
- Their need for hundreds of DNA strands
- The synthesis cost is rapidly decreasing but it is still a issue.
- **Scalability,** limitation to application that requires few micrograms of material

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

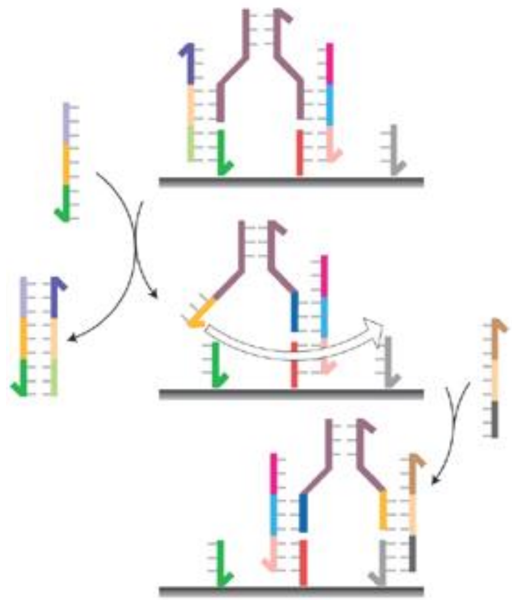
Functional DNA Nanotechnology: DNA Nanomachines

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.

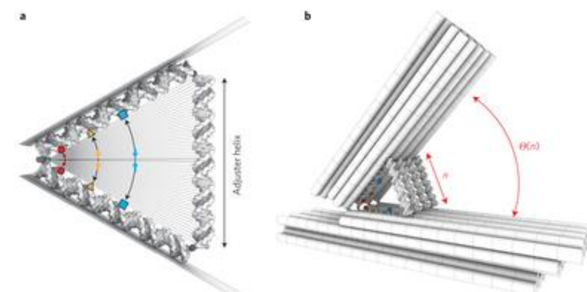
DNA Tweezers



DNA Walkers

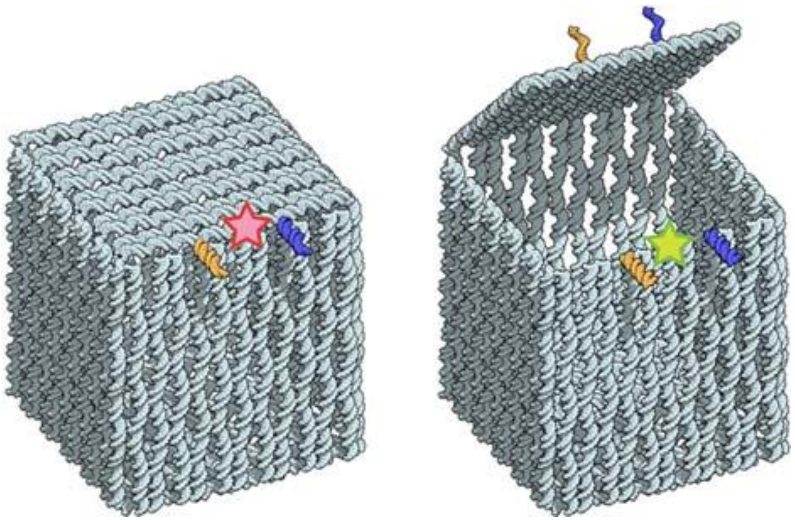


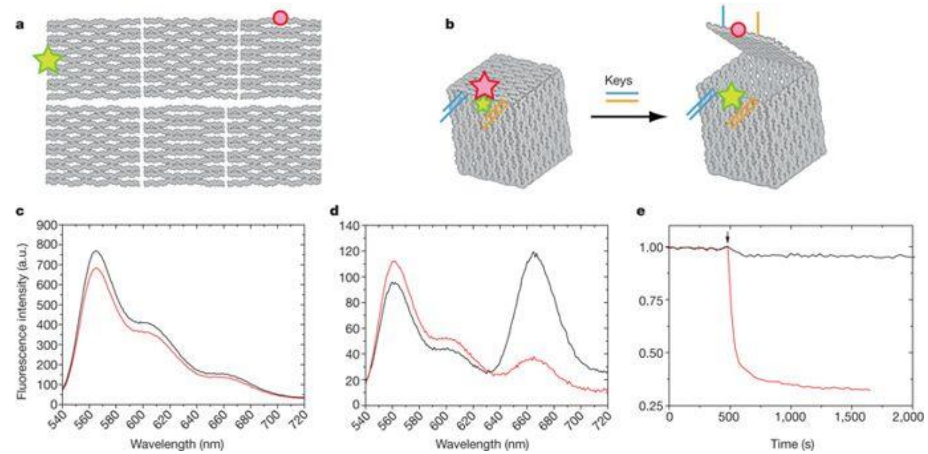
DNA Switches



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



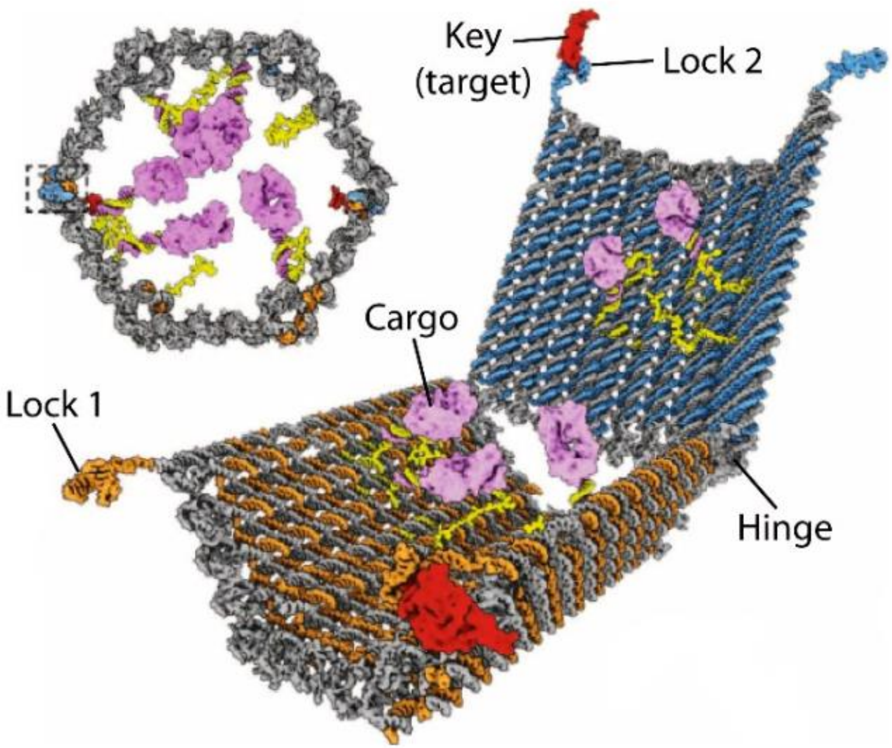


DNA box with a controllable lid that can be opened in the presence of externally supplied DNA ‘keys’.

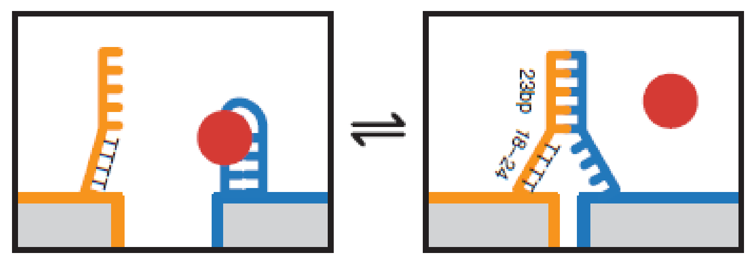
The lids of the DNA box have the potential to be uniquely programmed to respond to complex combinations of oligonucleotide sequences

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

DNA Nanorobot for Targeted Transport of Molecular Payloads

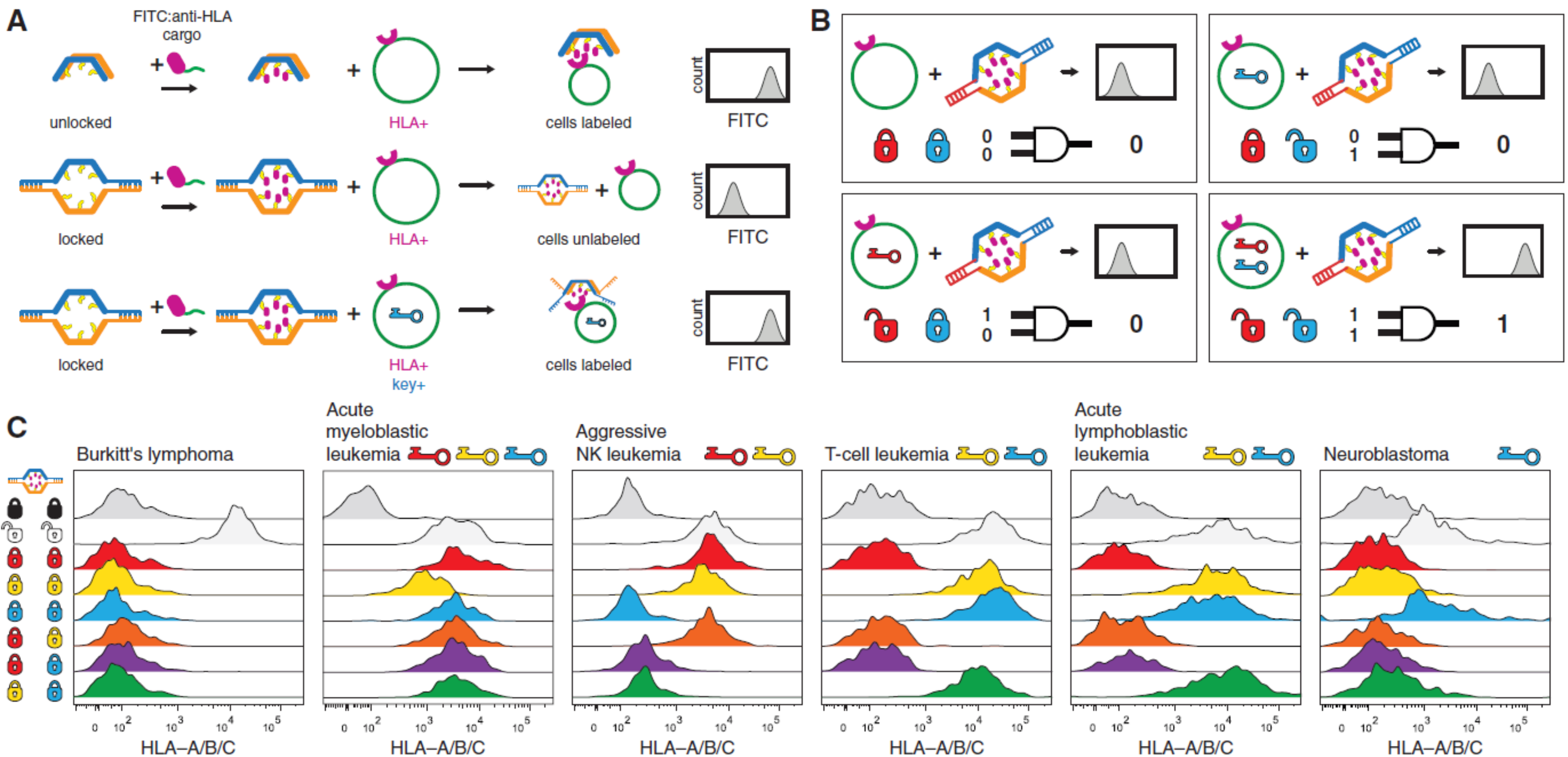


- Transport of molecular payload
- Sensing cell surface inputs and trigger activation
- Reconfiguration of its structure for payload delivery

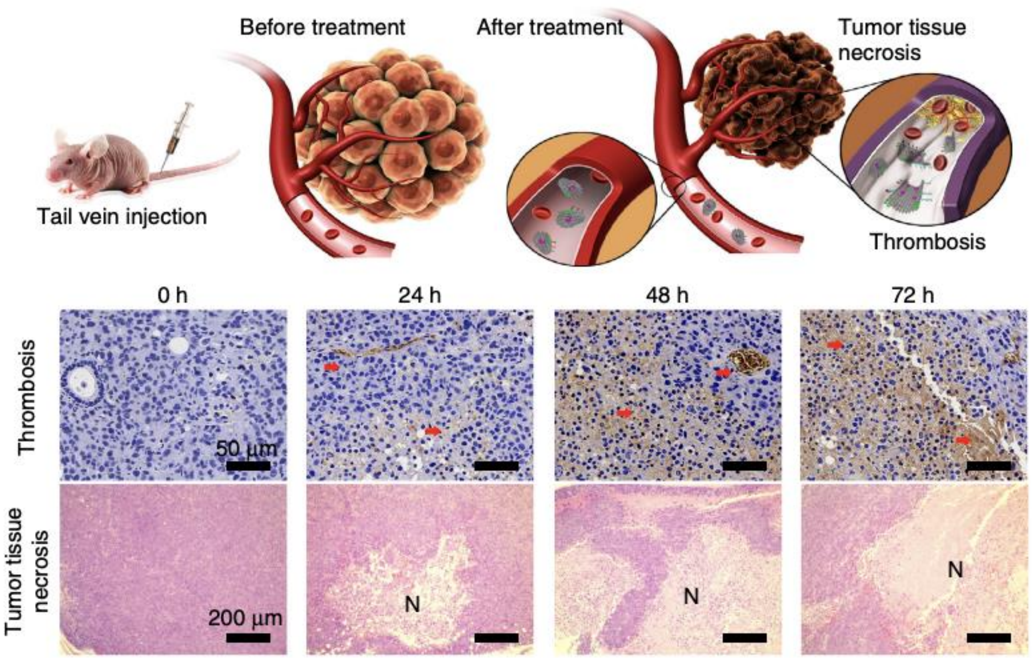


Douglas, Shawn M.; Bachelet, Ido; Church, George M. 2012 *Science* 335 (6070): 831–834.

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.



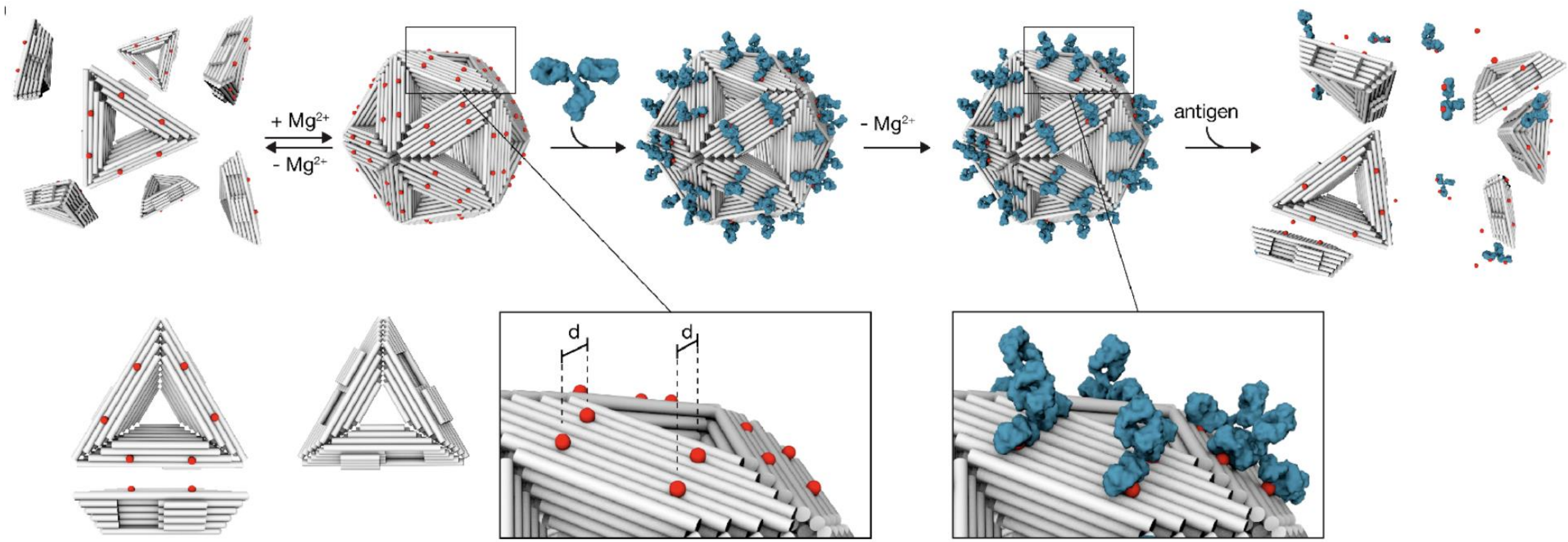
DNA nanorobot functions as a cancer therapeutic in response to a molecular trigger in vivo



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.

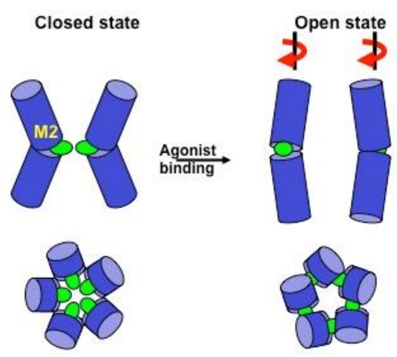
Antigen-Triggered Logic-Gating of DNA Nanodevices

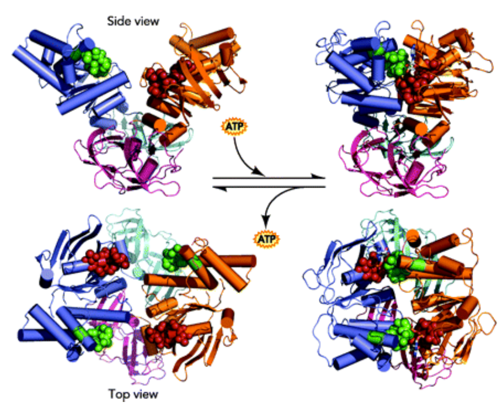


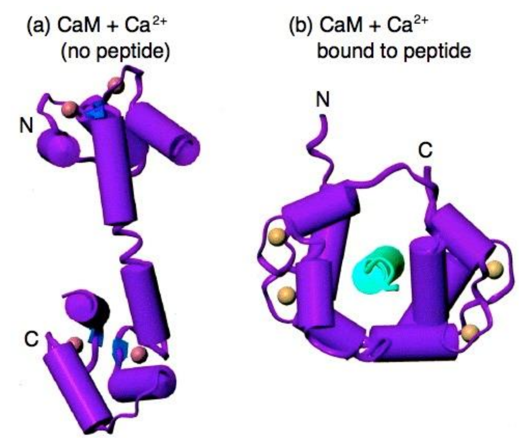
Dietz H., **J. Am. Chem. Soc.** 2021, 143, 51, 21630–21636

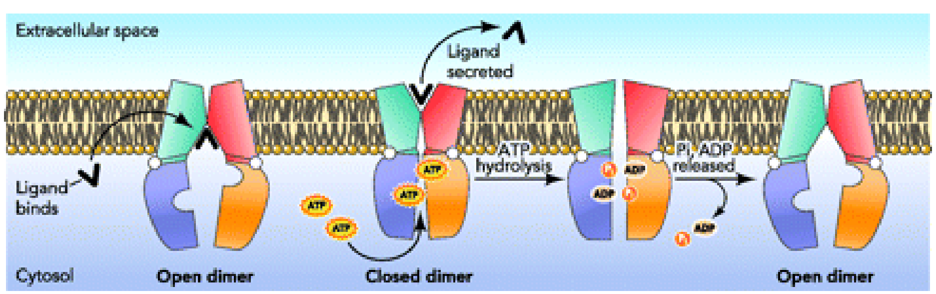
Biomolecular switches

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



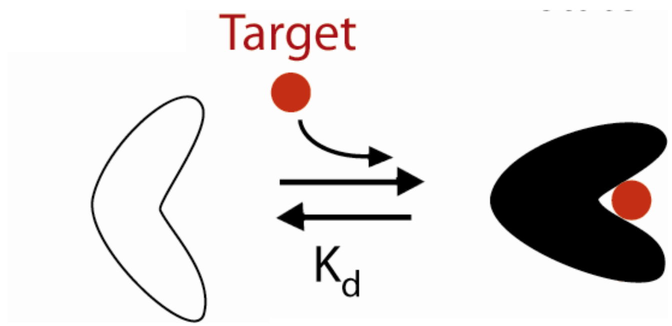






CONCEPT: mimic nature for developing novel diagnostic and drug-delivery tools

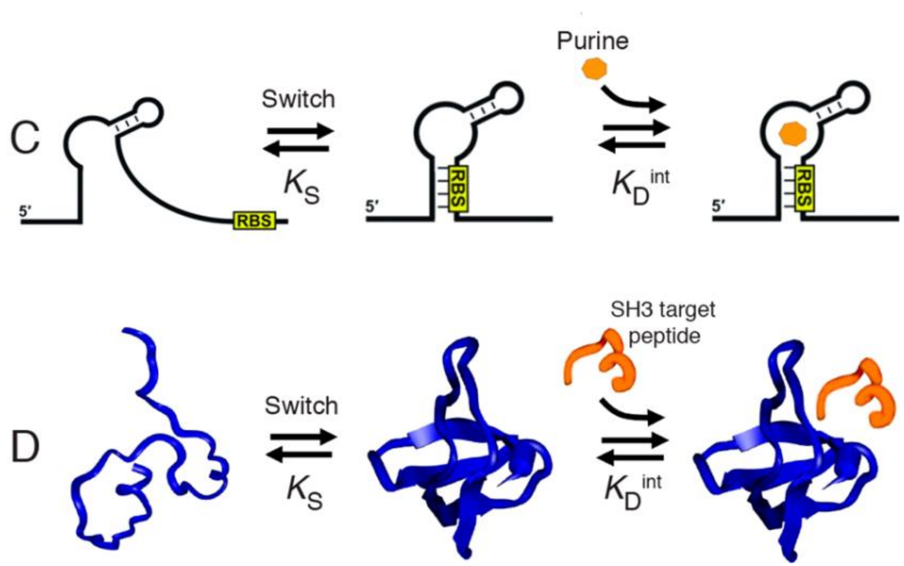
INSPIRATION: Naturally occurring switches



Development of DNA based switches for biotechnology applications

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. Proc. Natl. Acad. Sci. U.S.A.*(2005)

DNA-based nanomachines

Supramolecular nucleic acid assembly that undergoes cyclic, switchable, transitions between two distinct states in the presence of appropriate triggers and counter triggers.

Input

pH (e.g. Triplex, i-motif)

Electronic

Metal Ions

Small molecule, Nucleic ac

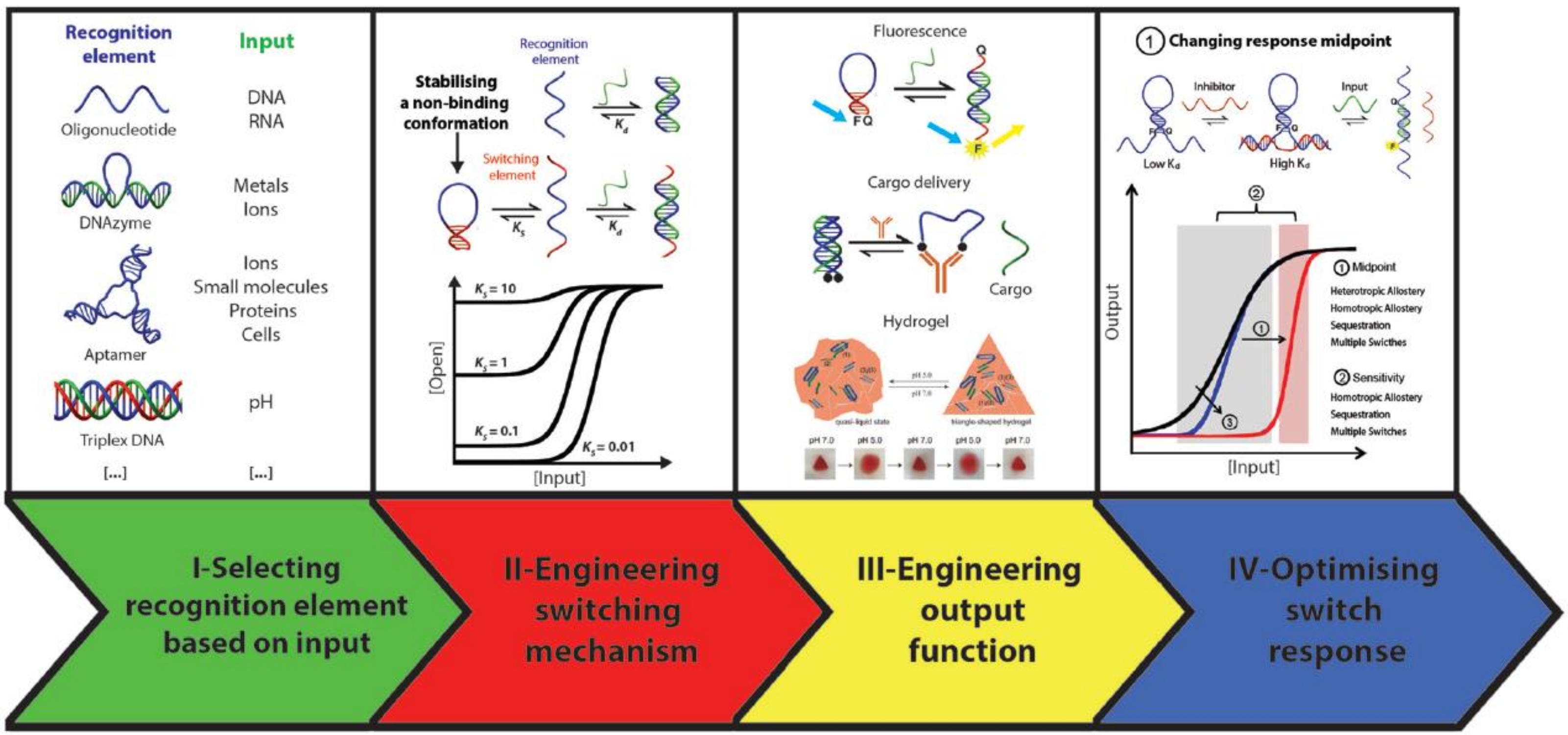
Conformational change

Release of a cargo

Activation of a reaction

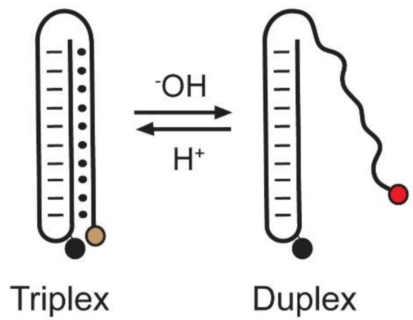
Regulation of DNA nanostructures

DNA-based nanomachines design



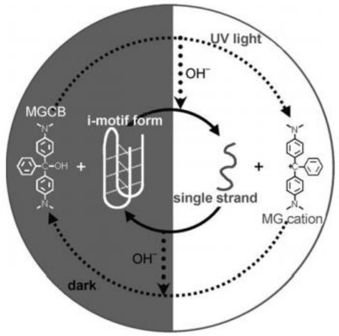
DNA-based nanomachines: Possible inputs

pH



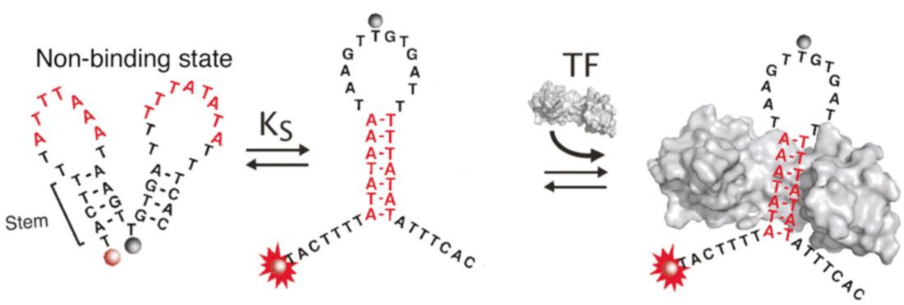
Idili, A. *et al.* JACS 2014, 136, 5836-5839

Light



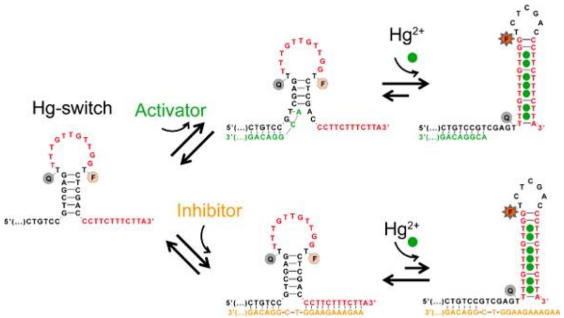
Liu, H. *et al.* Angew. Chem. Int. Ed. 2007, 46, 2515-2517

Transcription Factor



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

Small Molecule



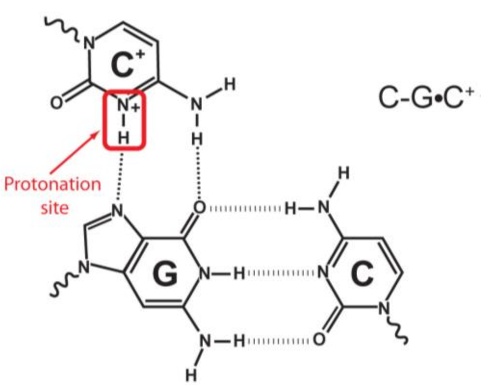
Porchetta, A. *et al.* J. Am. Chem. Soc. 2013, 135, 13238−41

Selecting Recognition Element based on Chemical Input

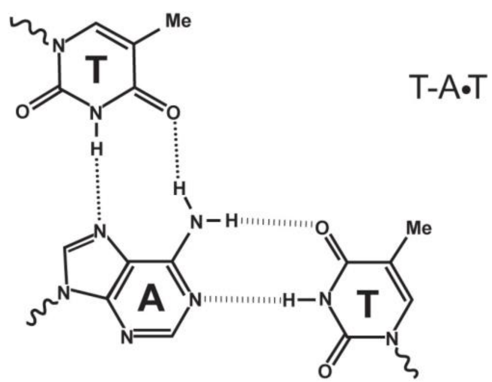
1. pH-controlled switches

Structural information:

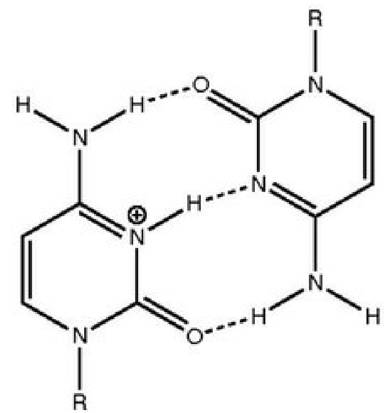
- *Triplex DNA (TAT, CGC triplet)*
- *i-motif (C-rich sequences)*



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

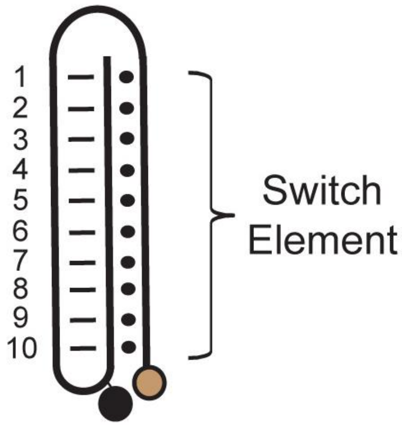


T-A•T Hoogsteen interactions are less pH-dependent (pKa ≈ 10)



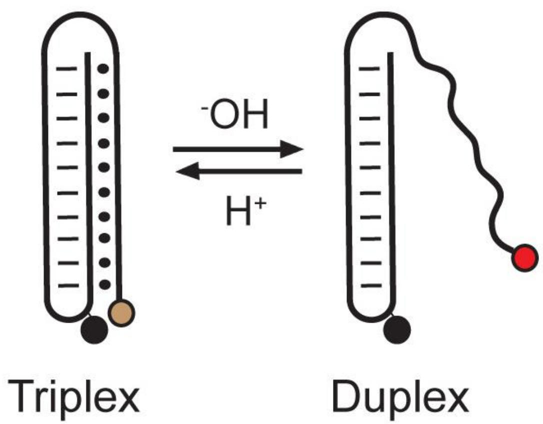
C·C+ base pairing found in i-motif structures. Base-pairing energy=169.7 kJ/mol.

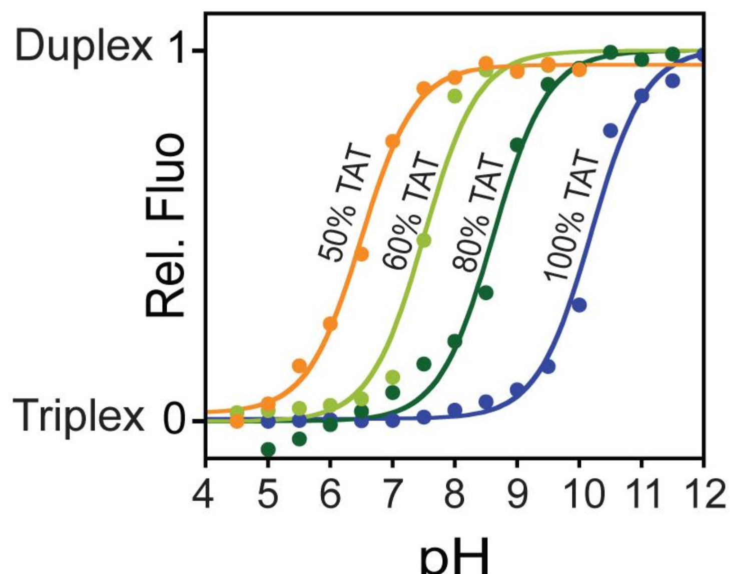
Programmable pH-triggered DNA-based nanomachines



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

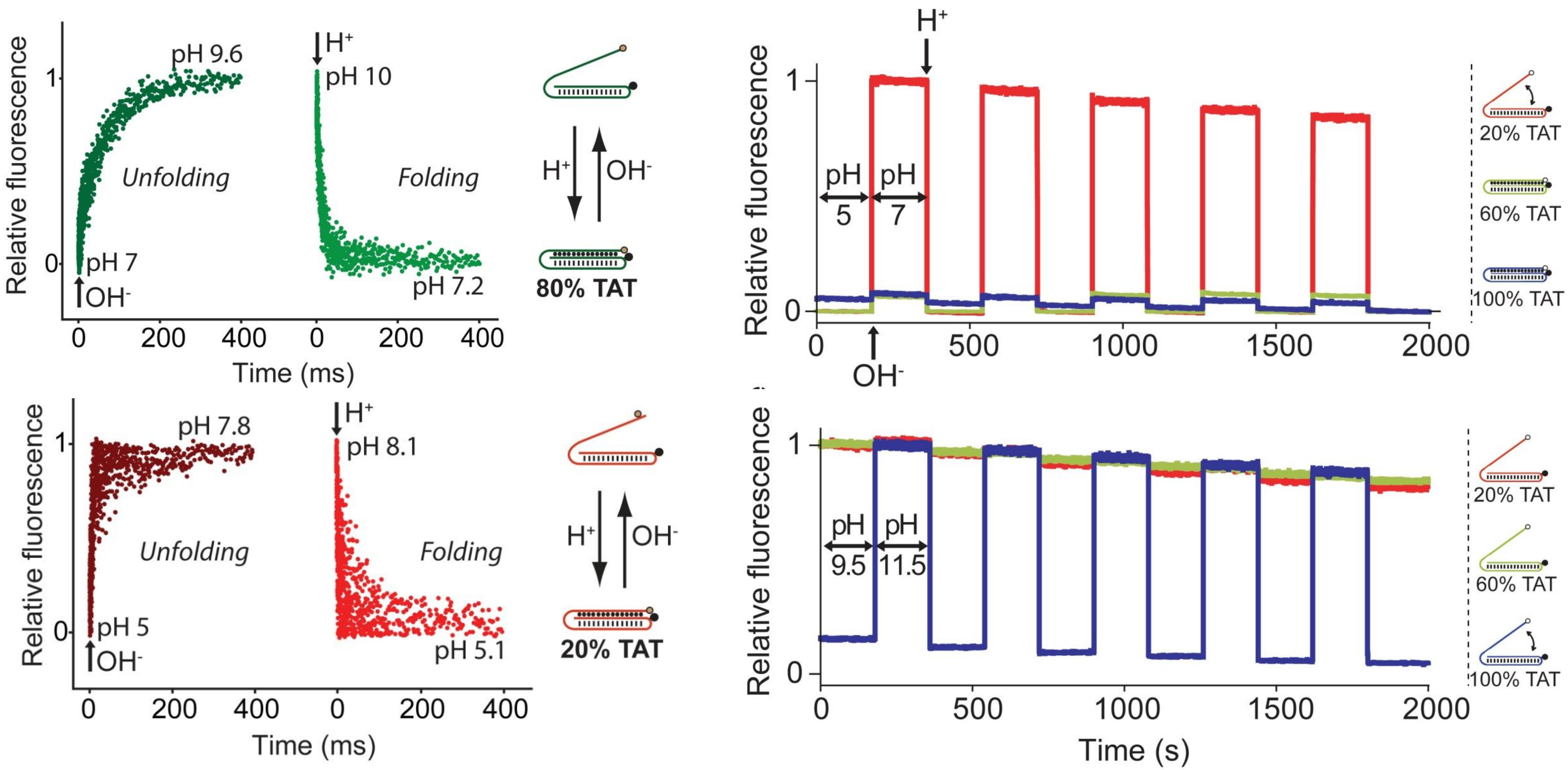
% of TAT in the Triplex



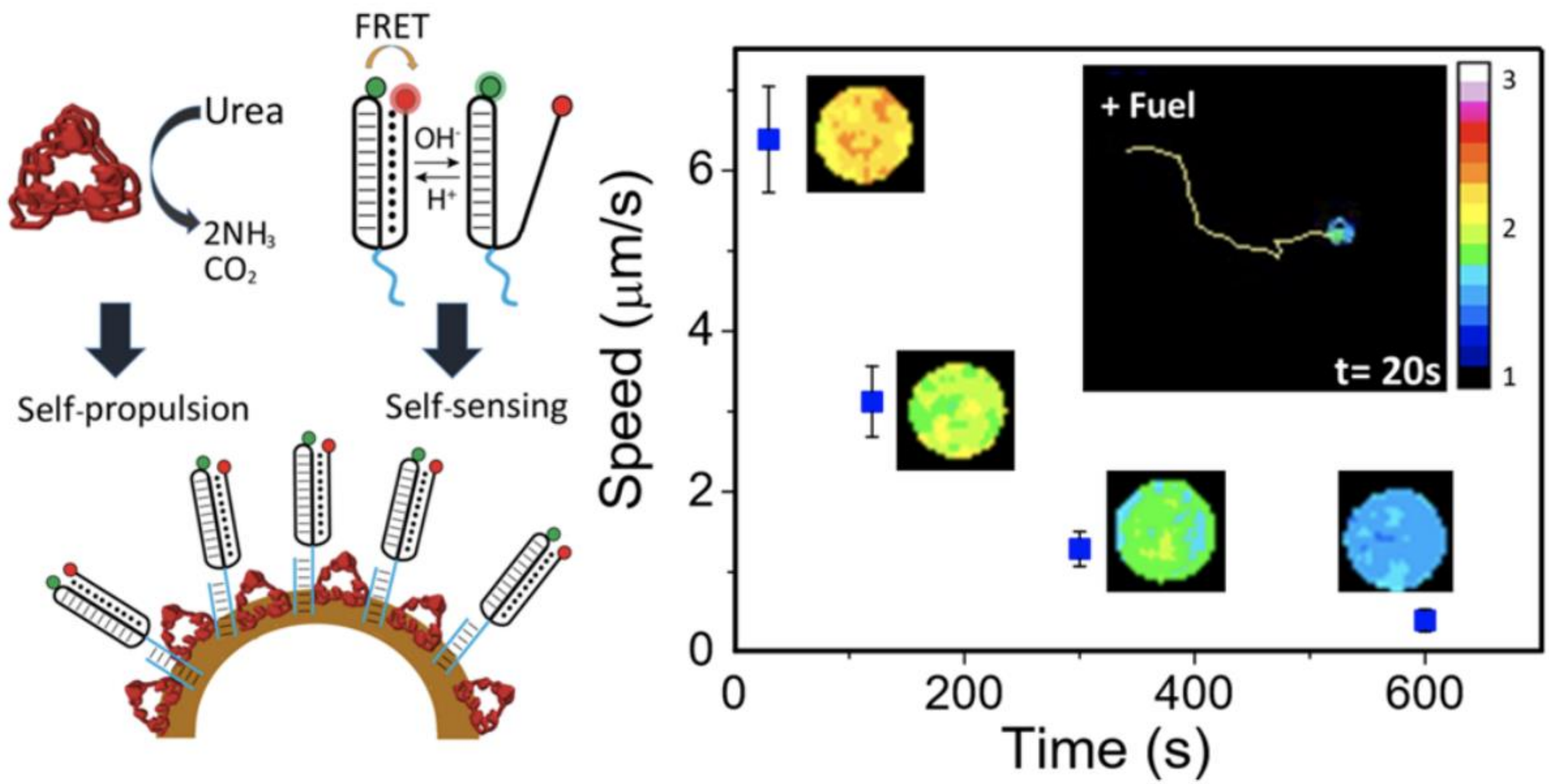


Idili A, Vallée-Bélisle A, Ricci F. **J Am Chem Soc.** 2014, 136, 5836

Programmable pH-triggered DNA-based nanomachines

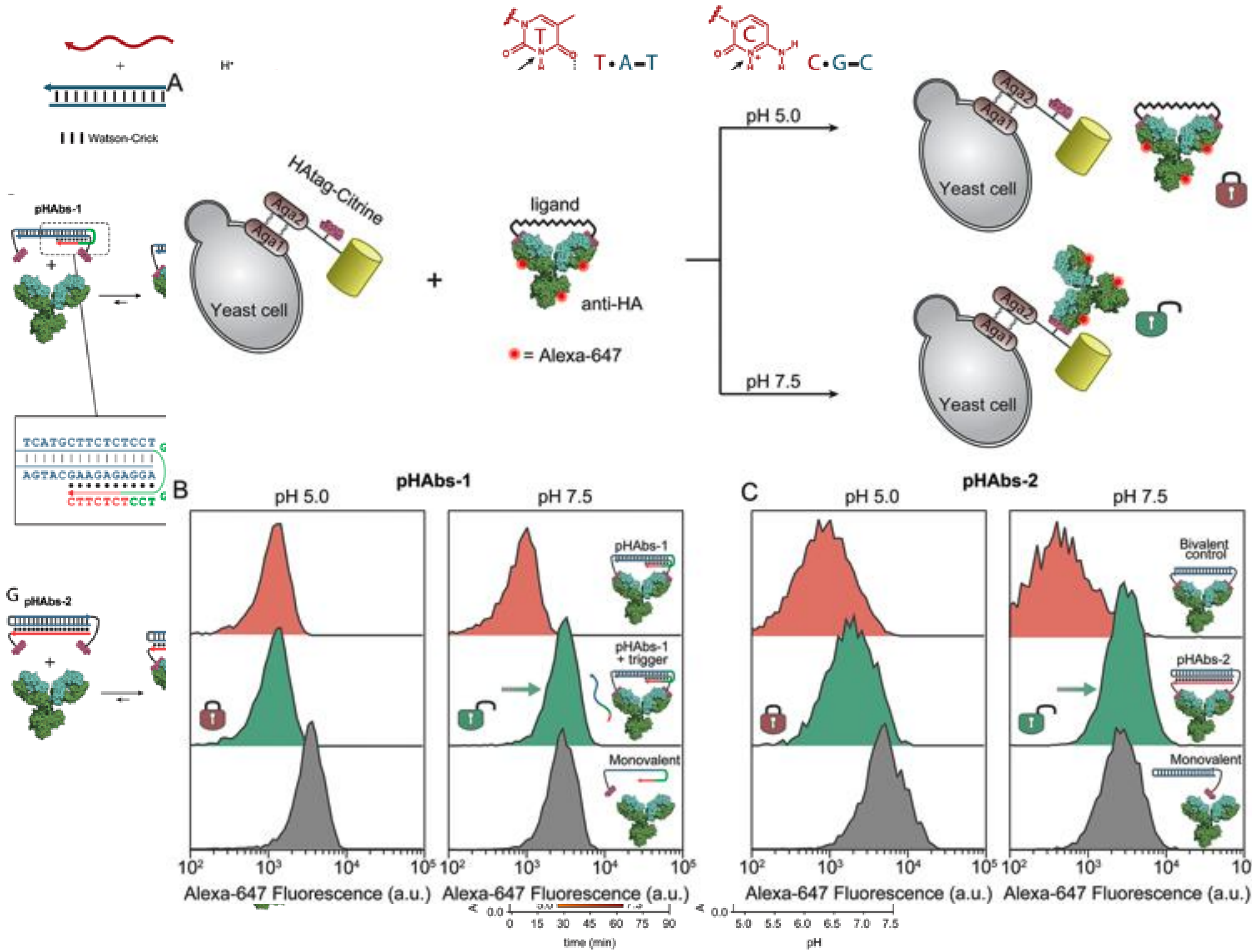


Biocatalytic nanomotors and pH-triggered DNA nanomachine:



Patino Padial T. *et. al*, **Nano Lett.** 2019, 19, 6, 3440–3447

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



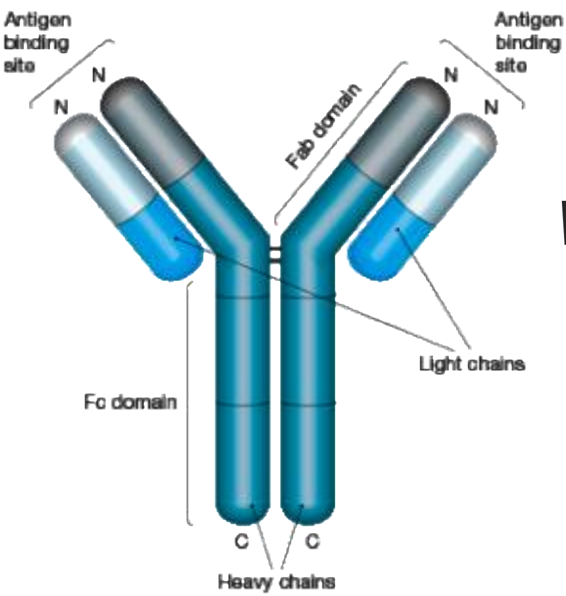
NA lock
specific

nor cells

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

Antibodies

Immunoglobulins produced by the immune system in response to foreign compounds and infectious agents.



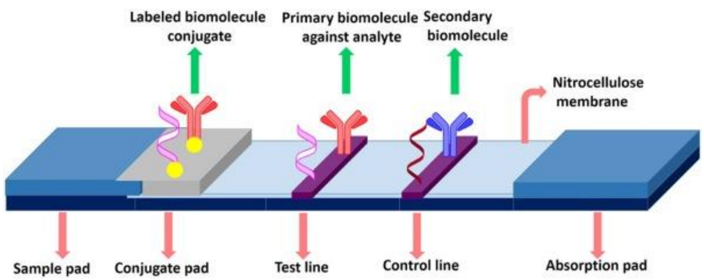
Why their detection plays a crucial role?

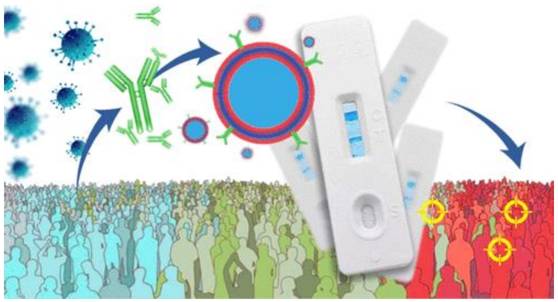
- ➢ Biomarkers for a wide range of pathologies (including infection and autoimmune diseases)
- ➢ Immunotherapy is one of the most promising treatment strategy in oncology

Develop new “POINT-OF-CARE” methods is urgent!

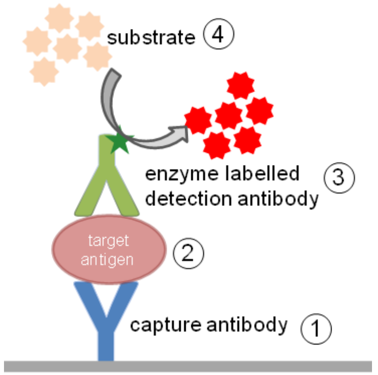
Antibody detection tests

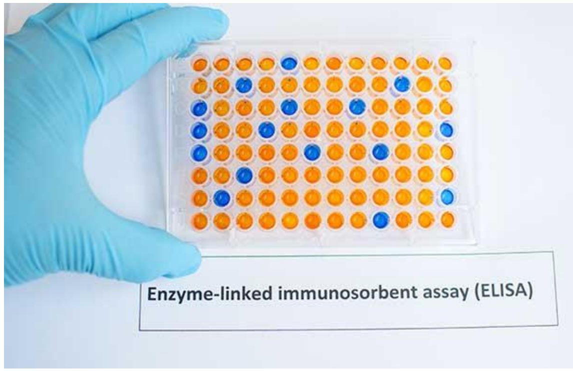
Lateral flow immunoassay



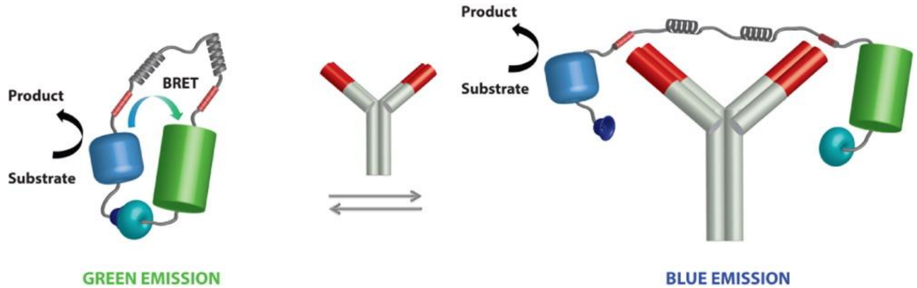


Enzyme-linked immunosorbent assay (ELISA)

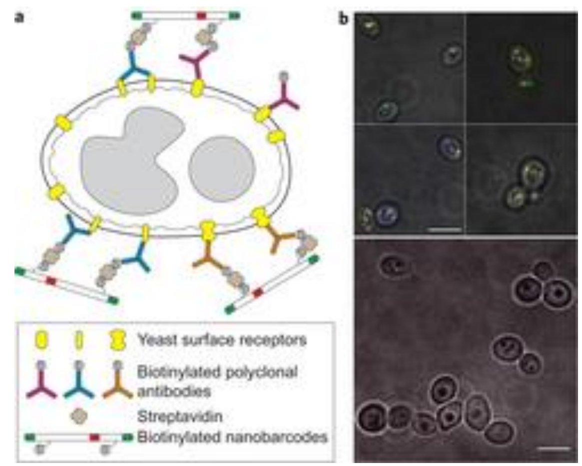




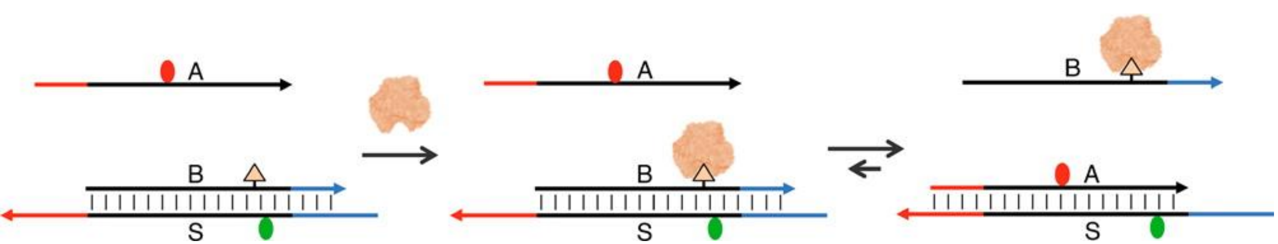
Antibodies as triggering input for DNA-based nanomachines



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



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

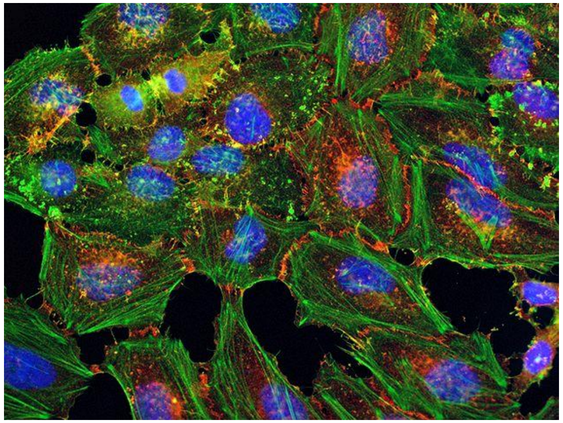


Zhang Z. *et al., J. Am. Chem. Soc.*, 2014, 136, 11115−11120

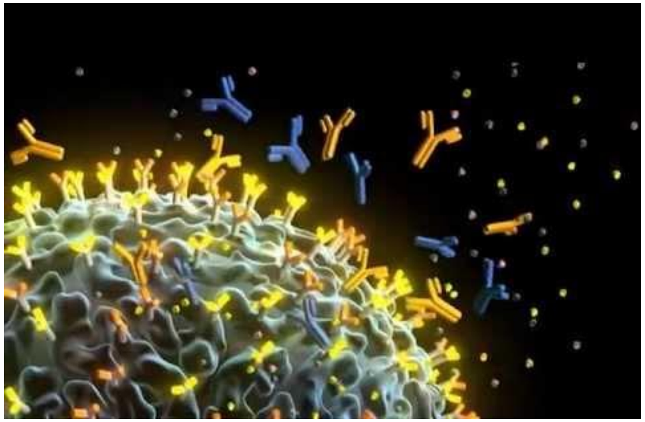
Antibodies

Diagnostic

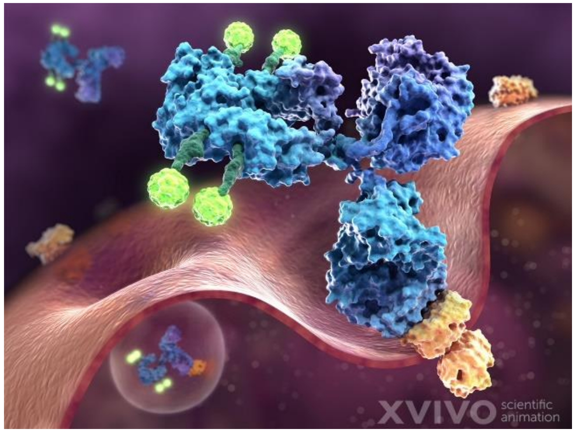
Molecular Imaging

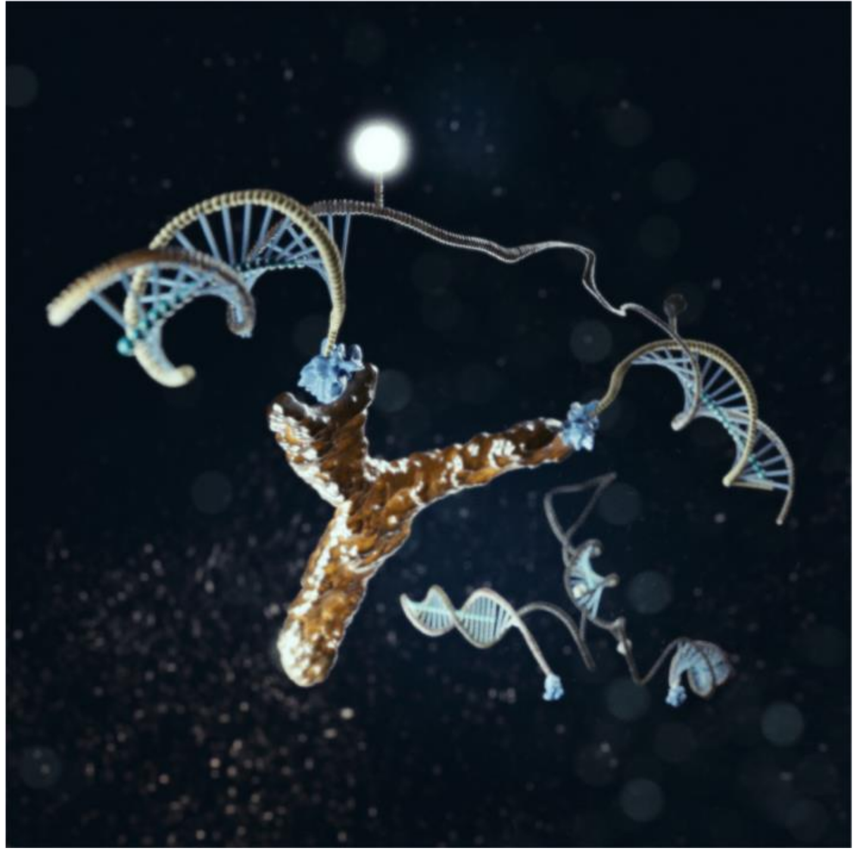


Antibody-based Therapy



Targeted drug delivery

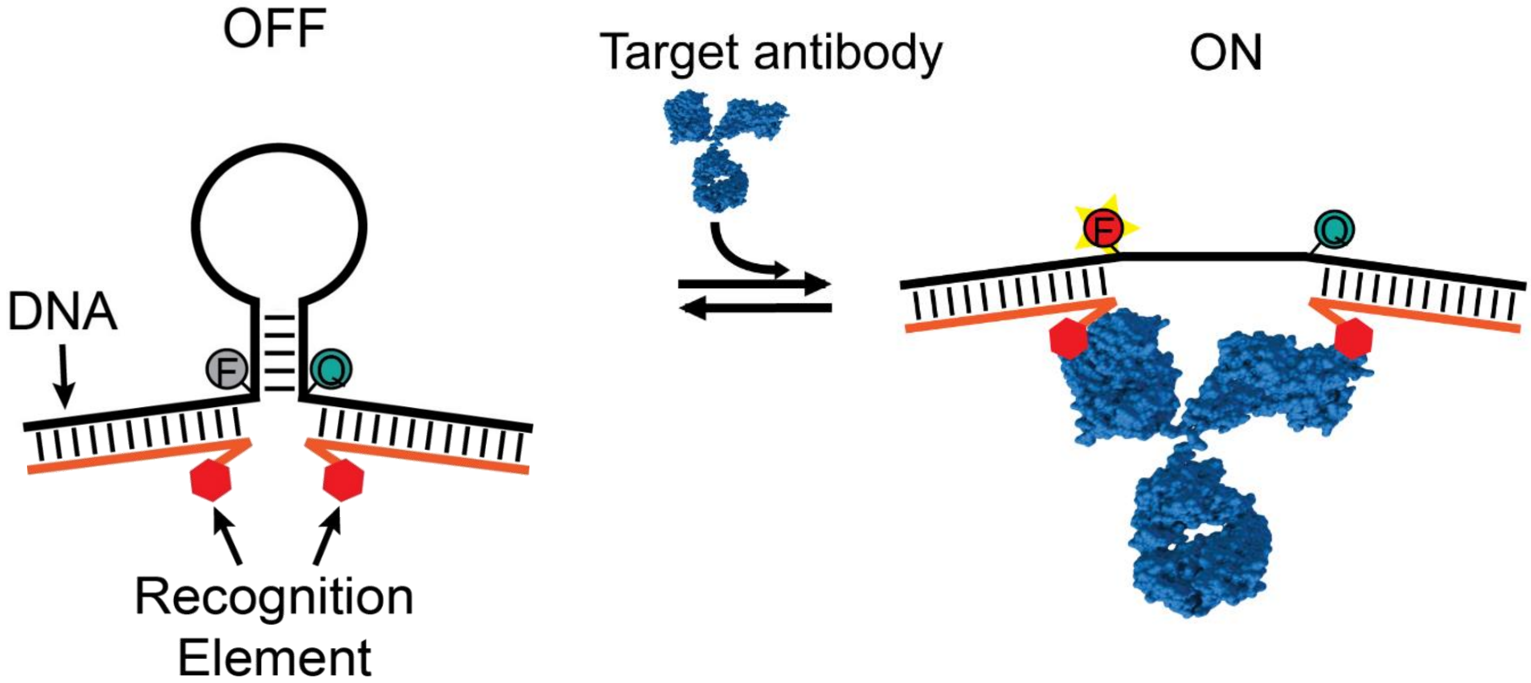




Antibody-switch:
A DNA-based biomolecular switch that, through a binding-induced conformational change, detects antibodies

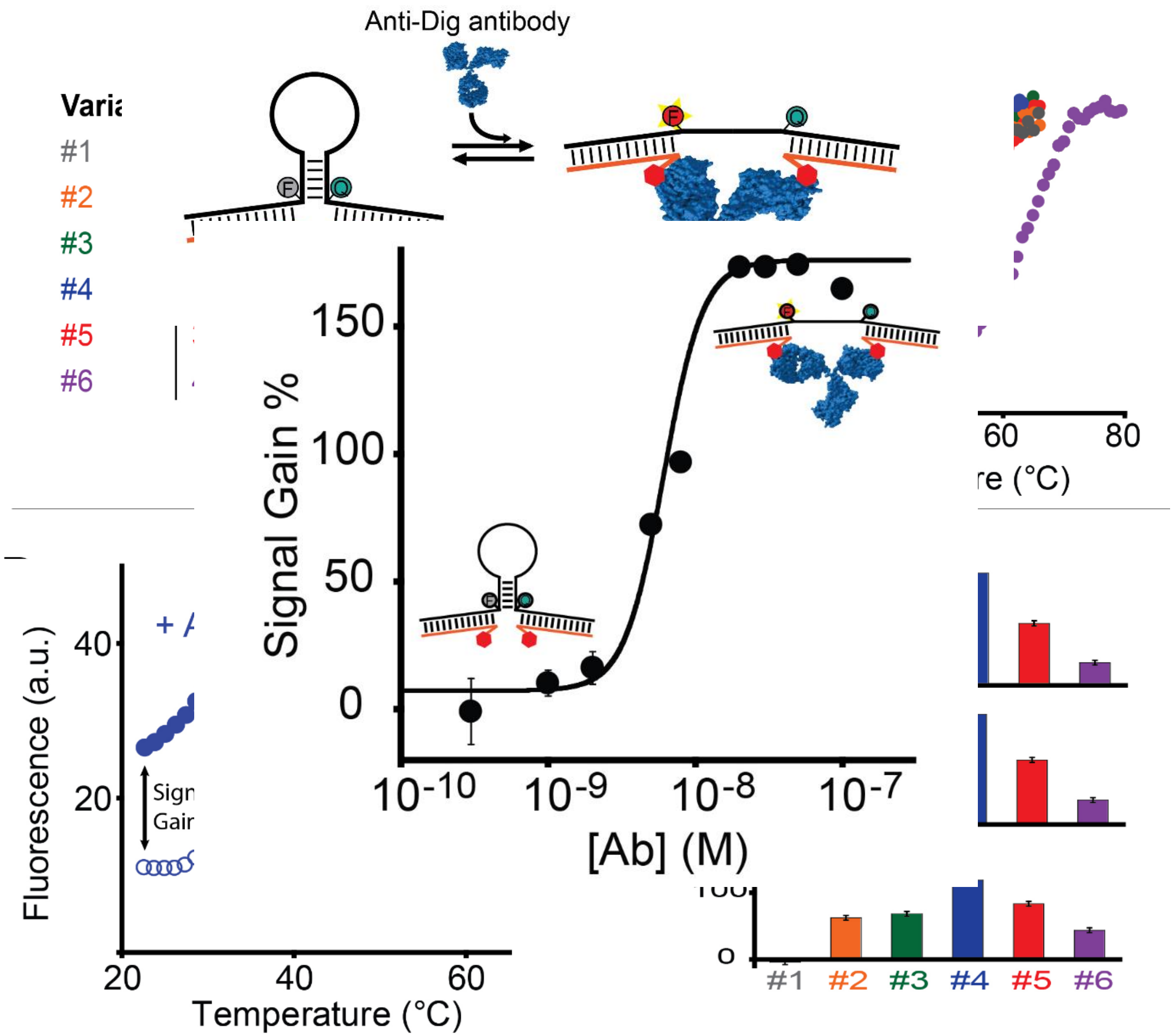
Ranallo S. *et al.*, ***Angew. Chem. Int. Ed.*** 54, 13214–13218 (**2015**).

Working principle



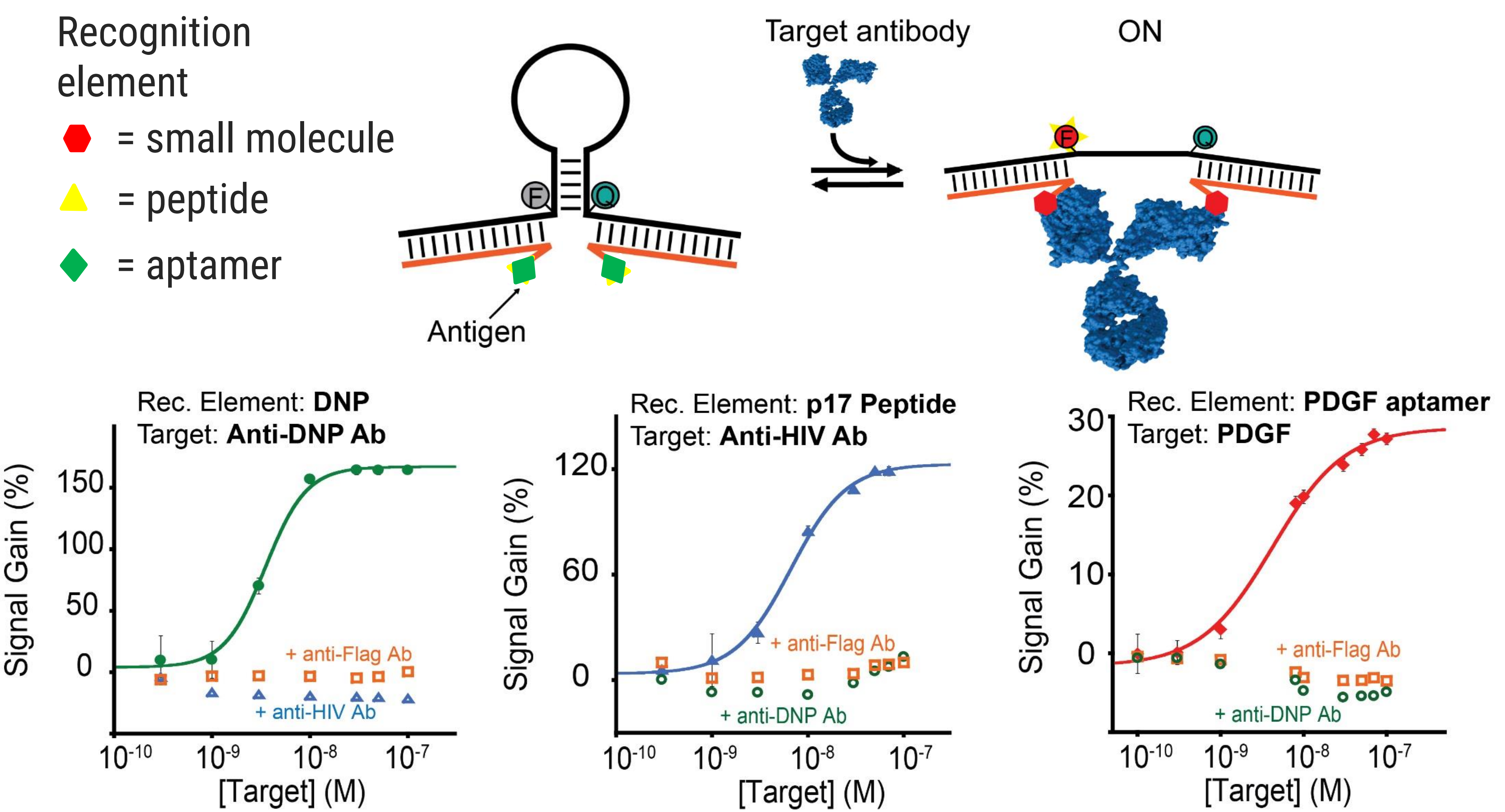
Ranallo S. *et al., Angew. Chem. Int. Ed.* 54, 13214–13218 **(2015)**.

Proof of principle
of optical antibody-binding switch



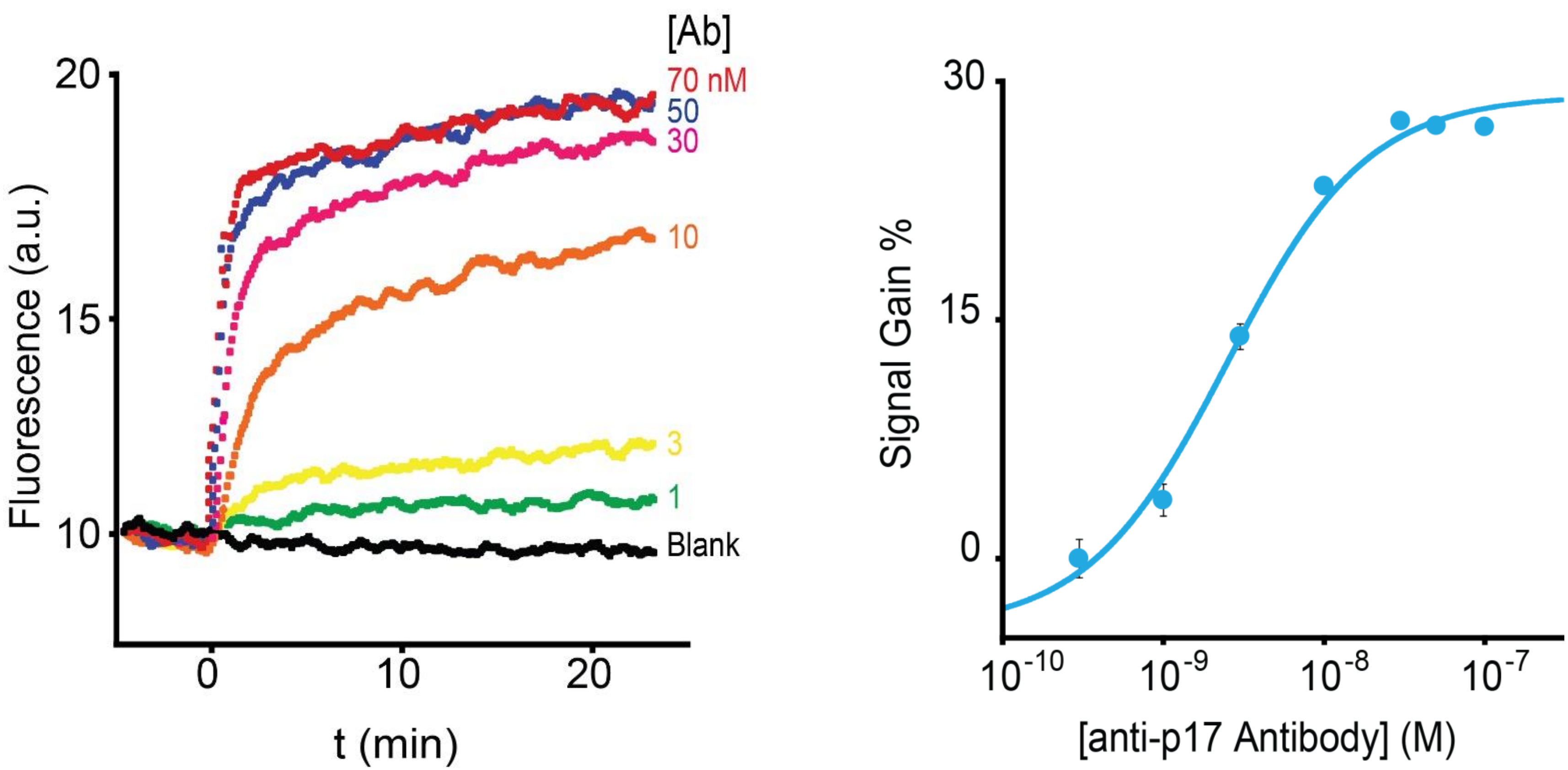
Ranallo S. *et al., Angew. Chem. Int. Ed.* 54, 13214–13218 **(2015)**.

A versatile platform



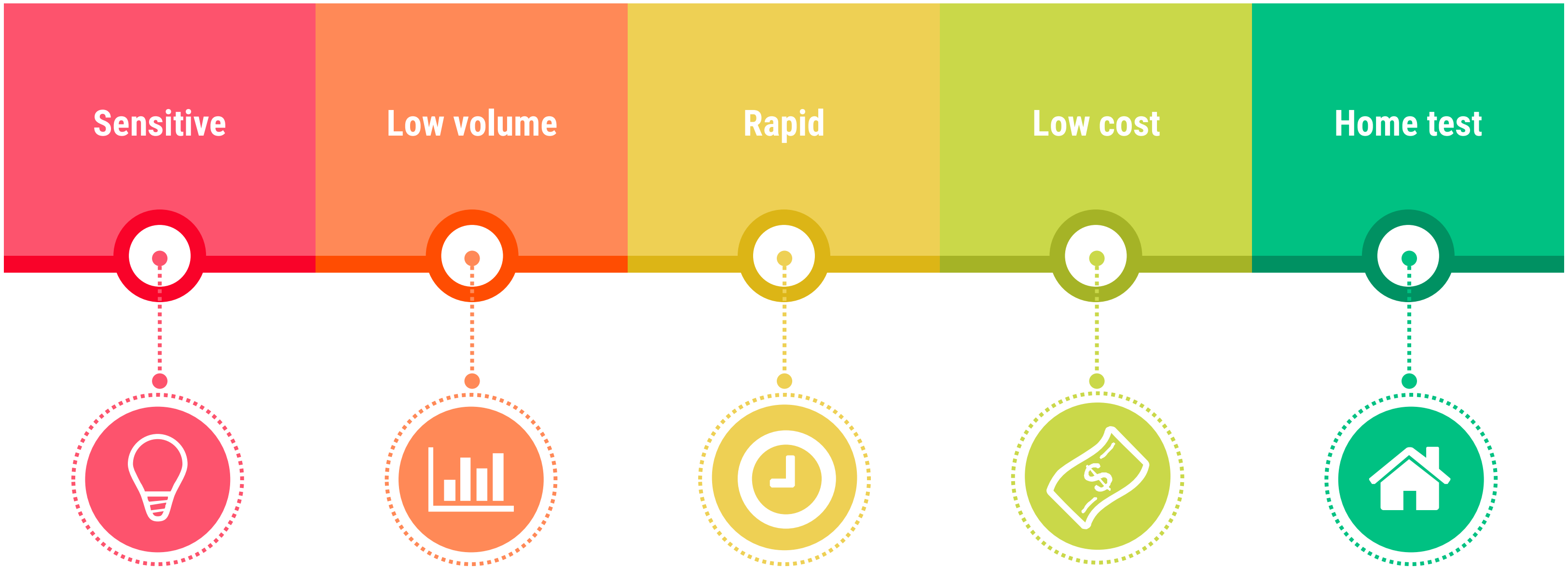
Ranallo S. *et al., Angew. Chem. Int. Ed.* 54, 13214–13218 **(2015)**.

Rapid and quantitative in complex samples



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

Antibody-switch

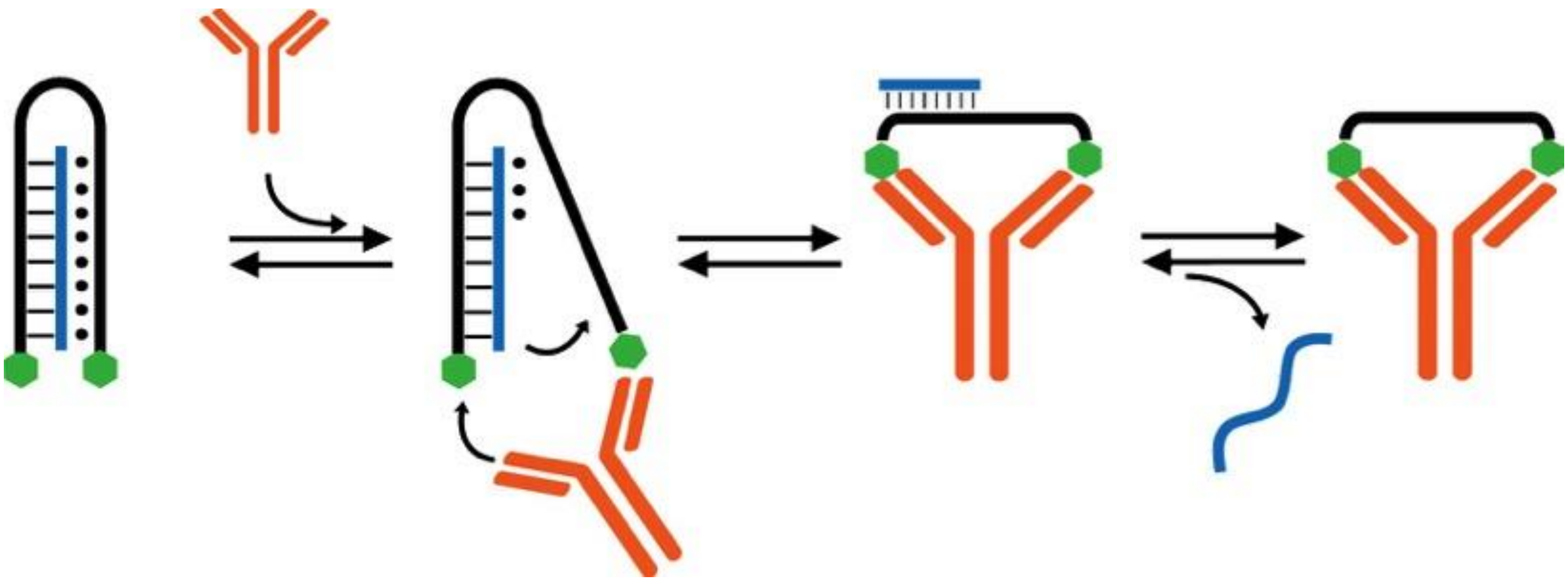


Ranallo S. *et al., Angew. Chem. Int. Ed.* 54, 13214–13218 **(2015)**.

Molecular Slingshot:
DNA-based nanomachine that, upon the binding of a specific antibody, release a cargo strand

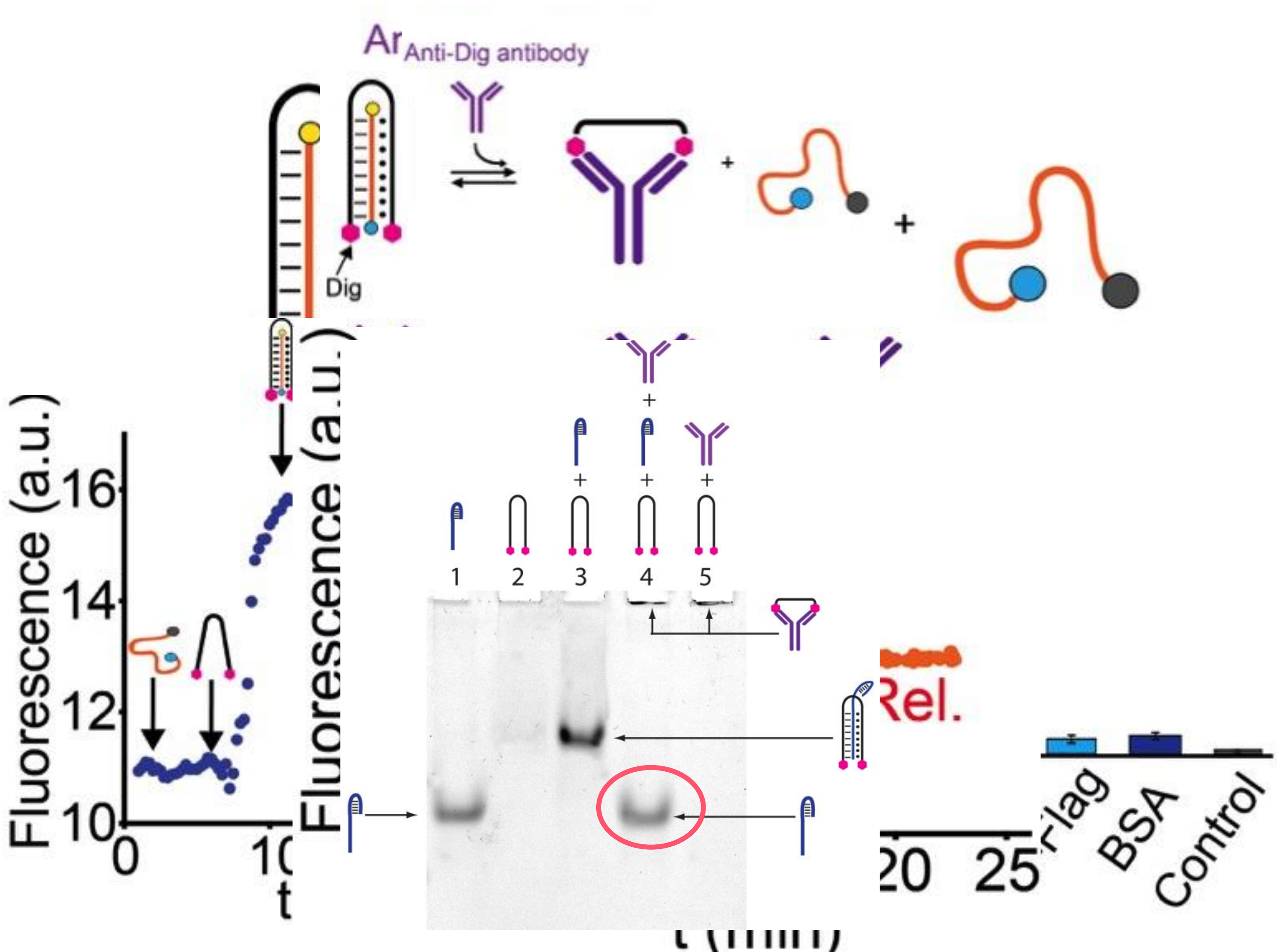
Ranallo S. *et al.*, ***Nat. Commun.*** 2017, 8, 15150.

Working principle



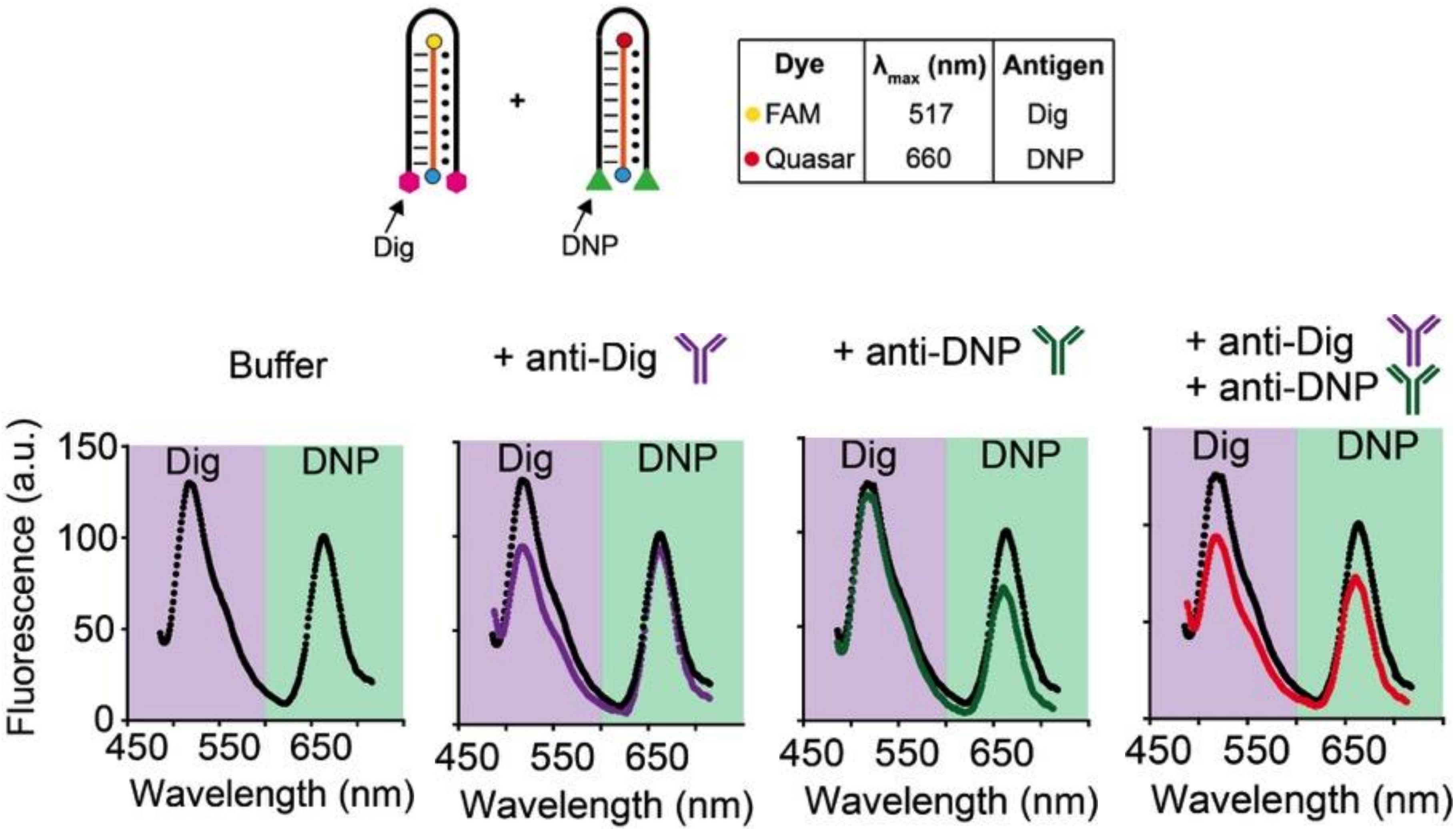
Ranallo S. *et al., **Nat. Commun.*** 2017, 8, 15150.

Antibody-powered DNA-based nanomachine



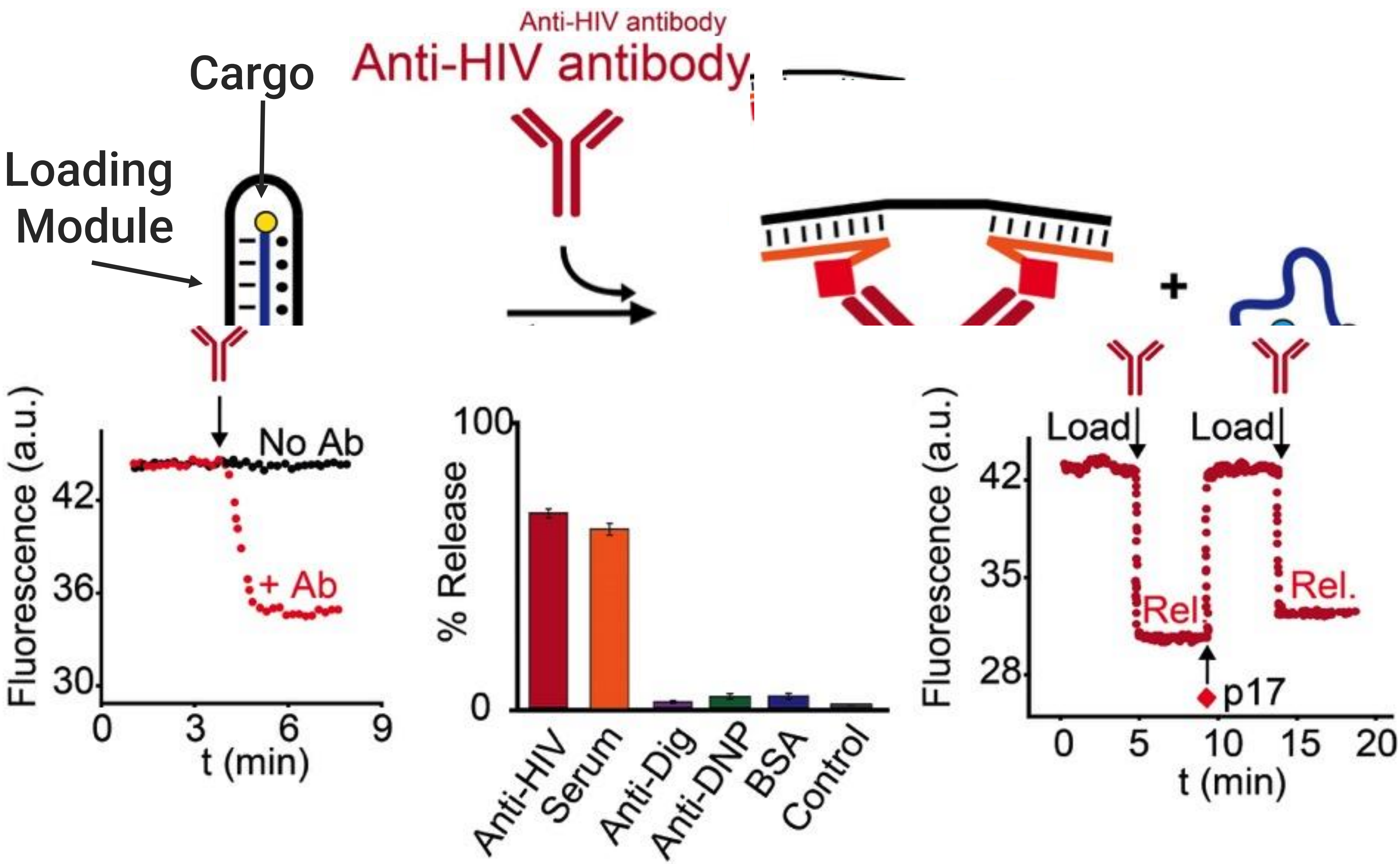
Ranallo S. *et al.*, ***Nat. Commun.*** 2017, 8, 15150.

Antibody-powered DNA-based nanomachine



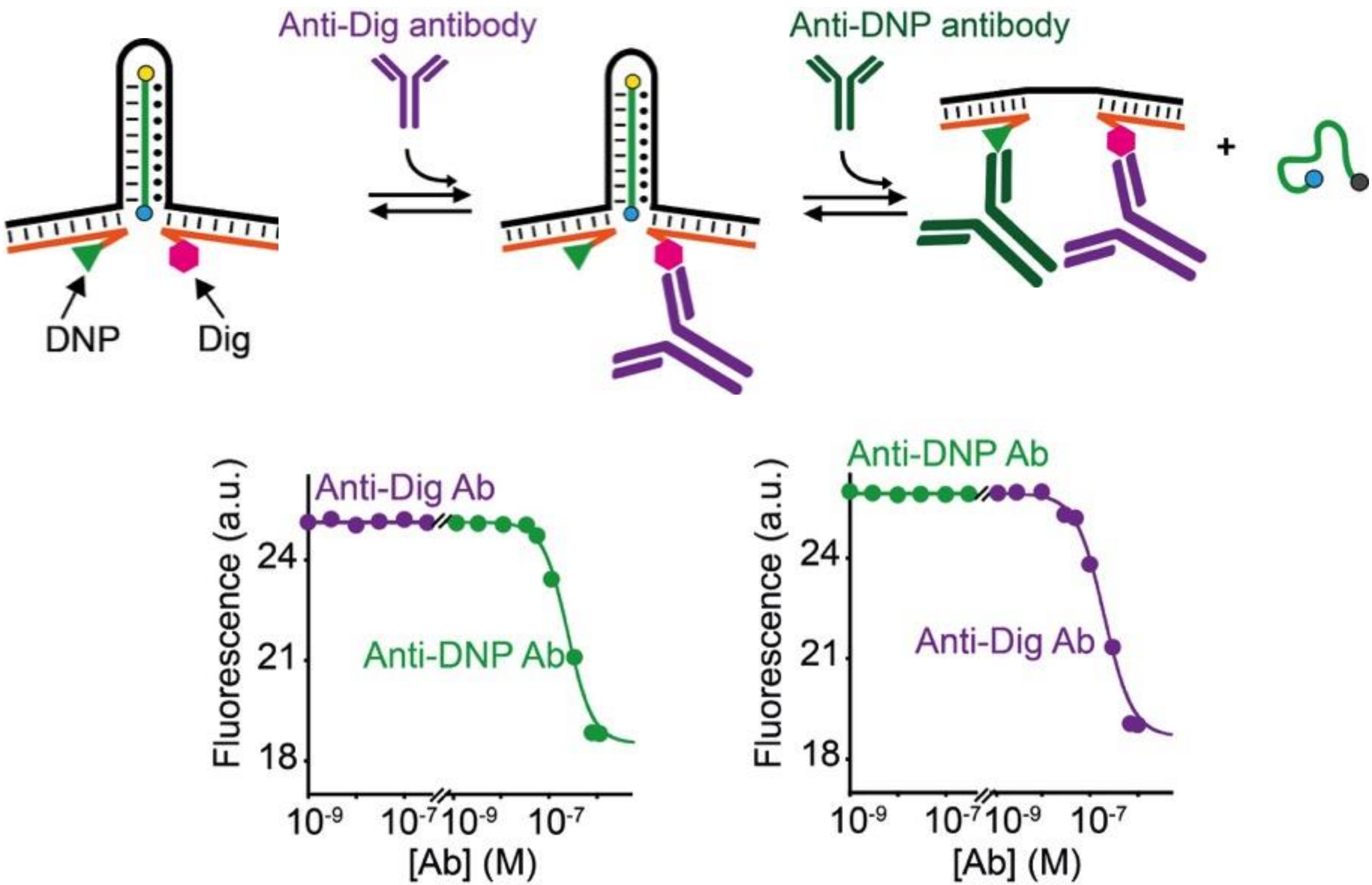
Ranallo S. *et al.*, ***Nat. Commun.*** 2017, 8, 15150.

Modular antibody-powered DNA nanomachine



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

AND Logic Gate DNA nanomachine



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

Antibody-powered nanomachine

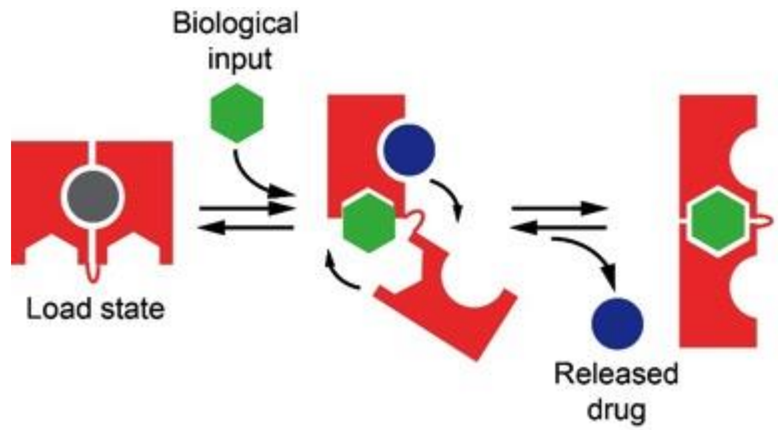
- ✓ Antibody-powered DNA-based nanomachines able to load and release a cargo in a controlled fashion.

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

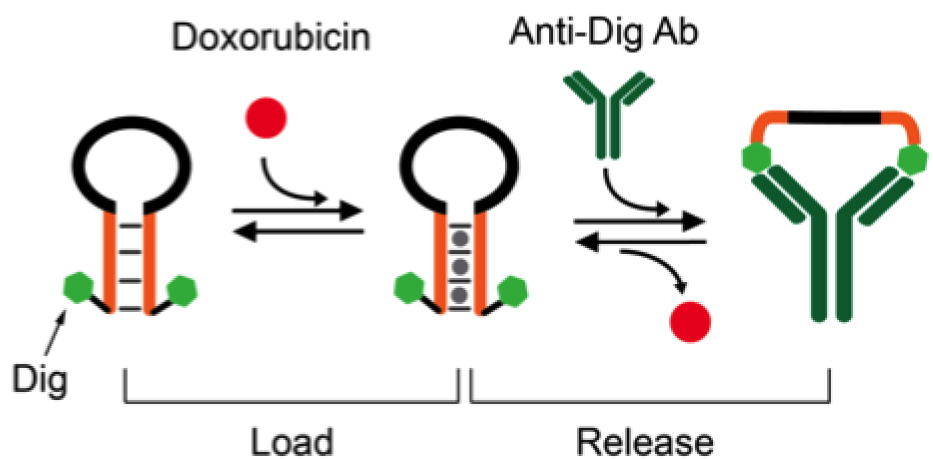
Antibody triggers the release of Doxorubicin loaded in a DNA nanomachine

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

Working principle

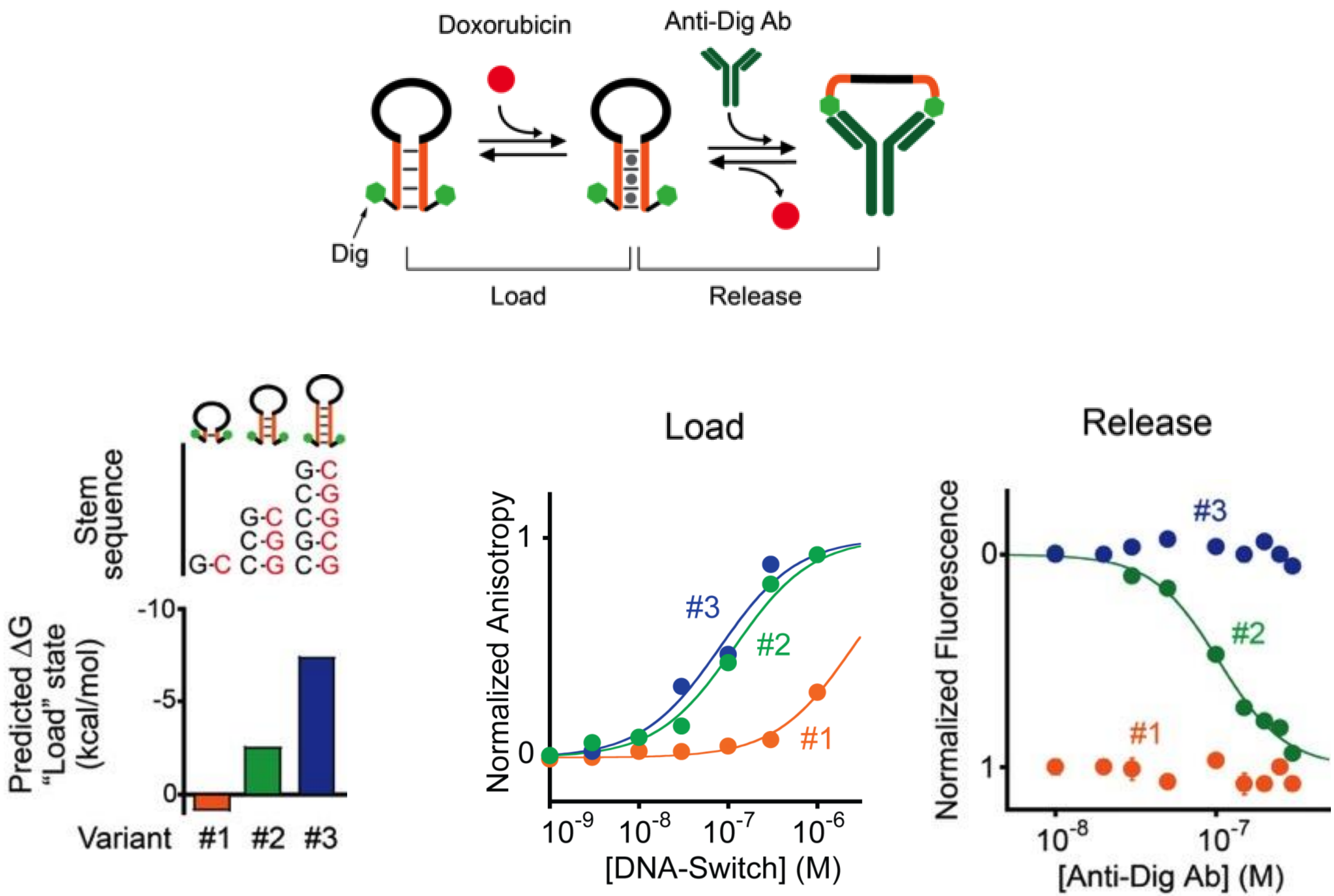


Rational design of a new class of DNA-based nanomachines *allosterically regulated* by specific biological targets, able to release a molecular cargo in a controlled fashion.



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.

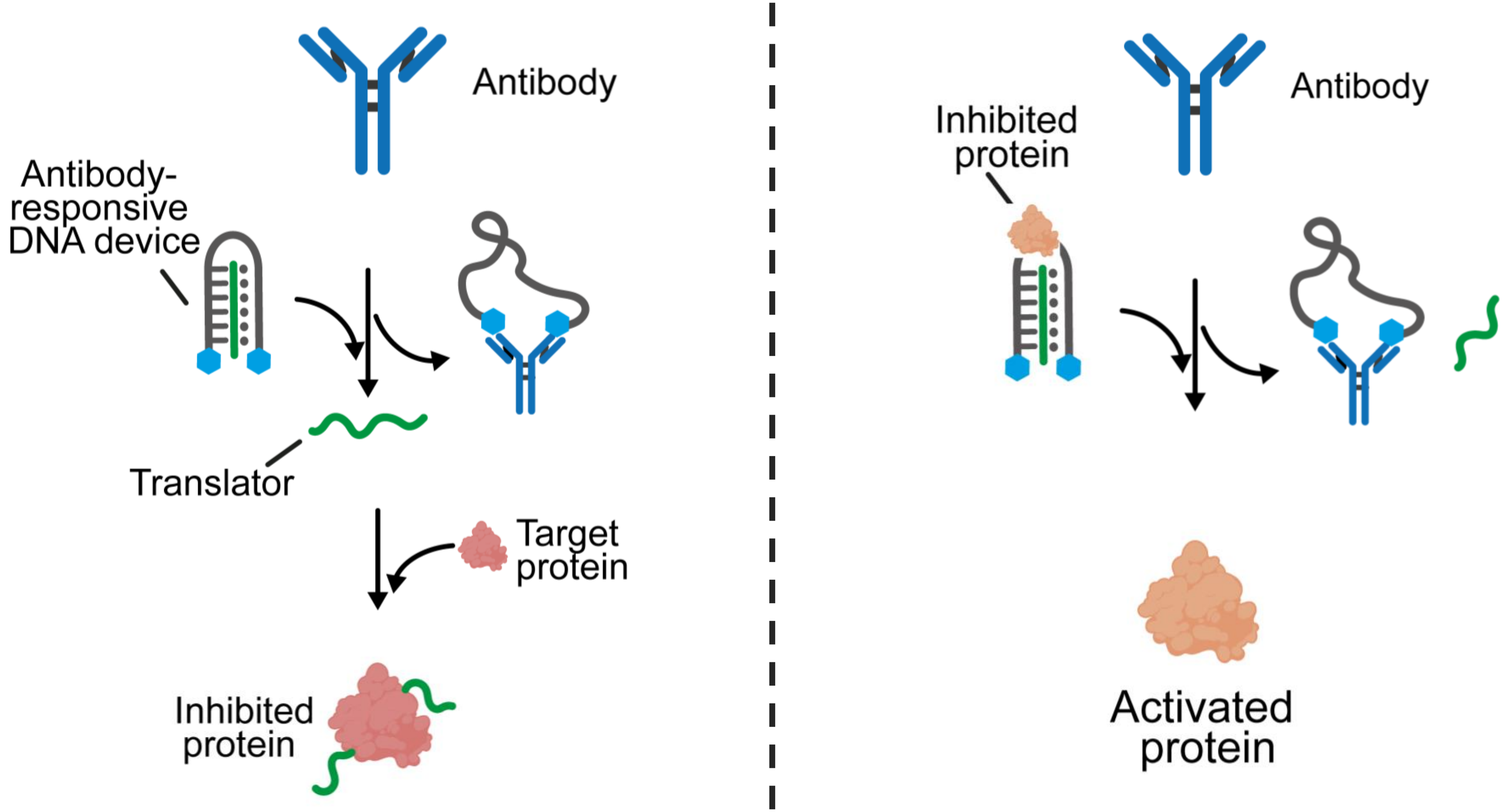
Protein-Protein Communication Mediated by an Antibody-Responsive DNA Nanodevices

Ranallo S. *et al.*, ***Angew. Chem. Int. Ed.*** 2022, 61, e202115680

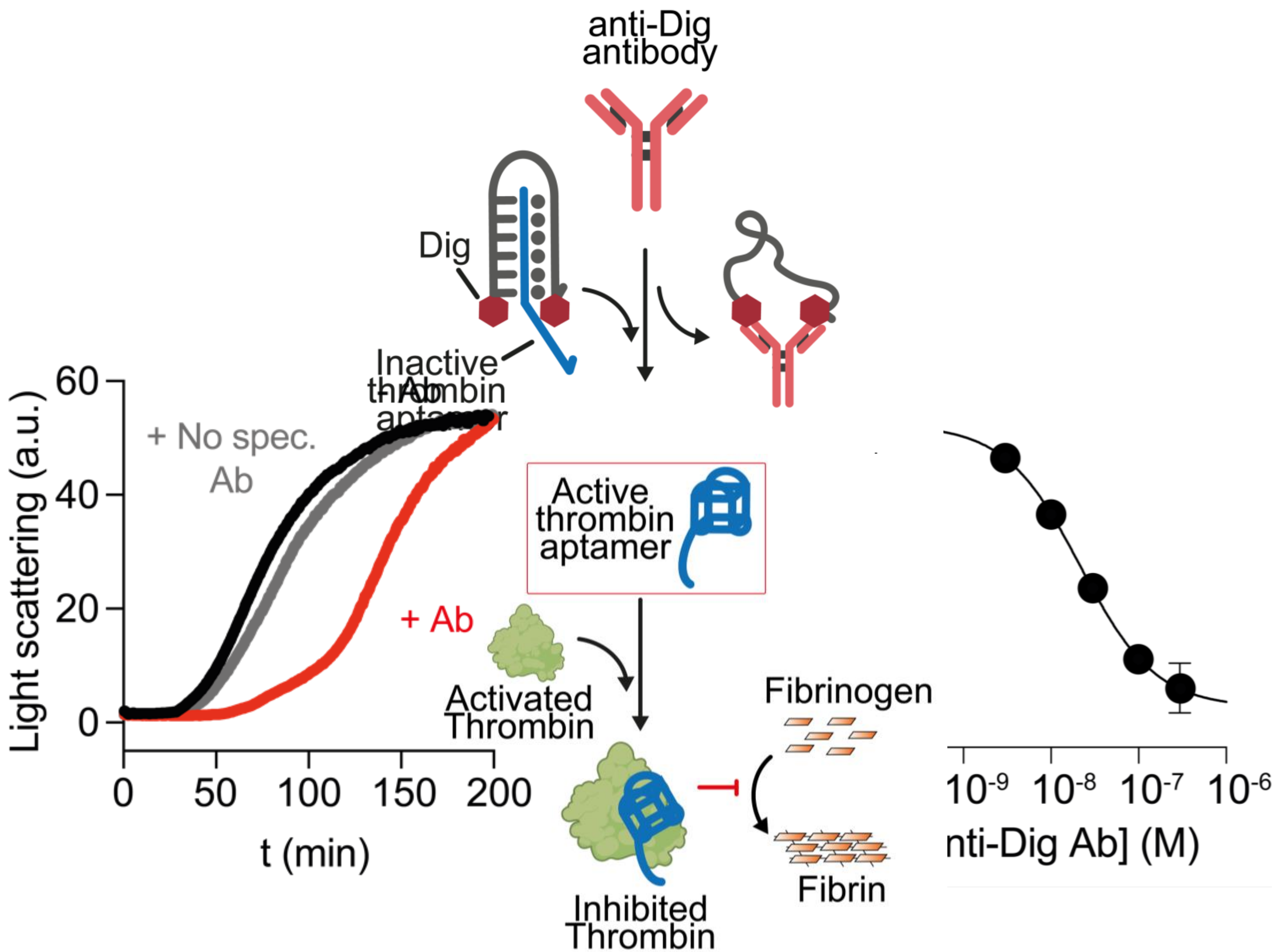
Working principle

Mechanism A: antibody-induced release of a protein inhibitor (translator)

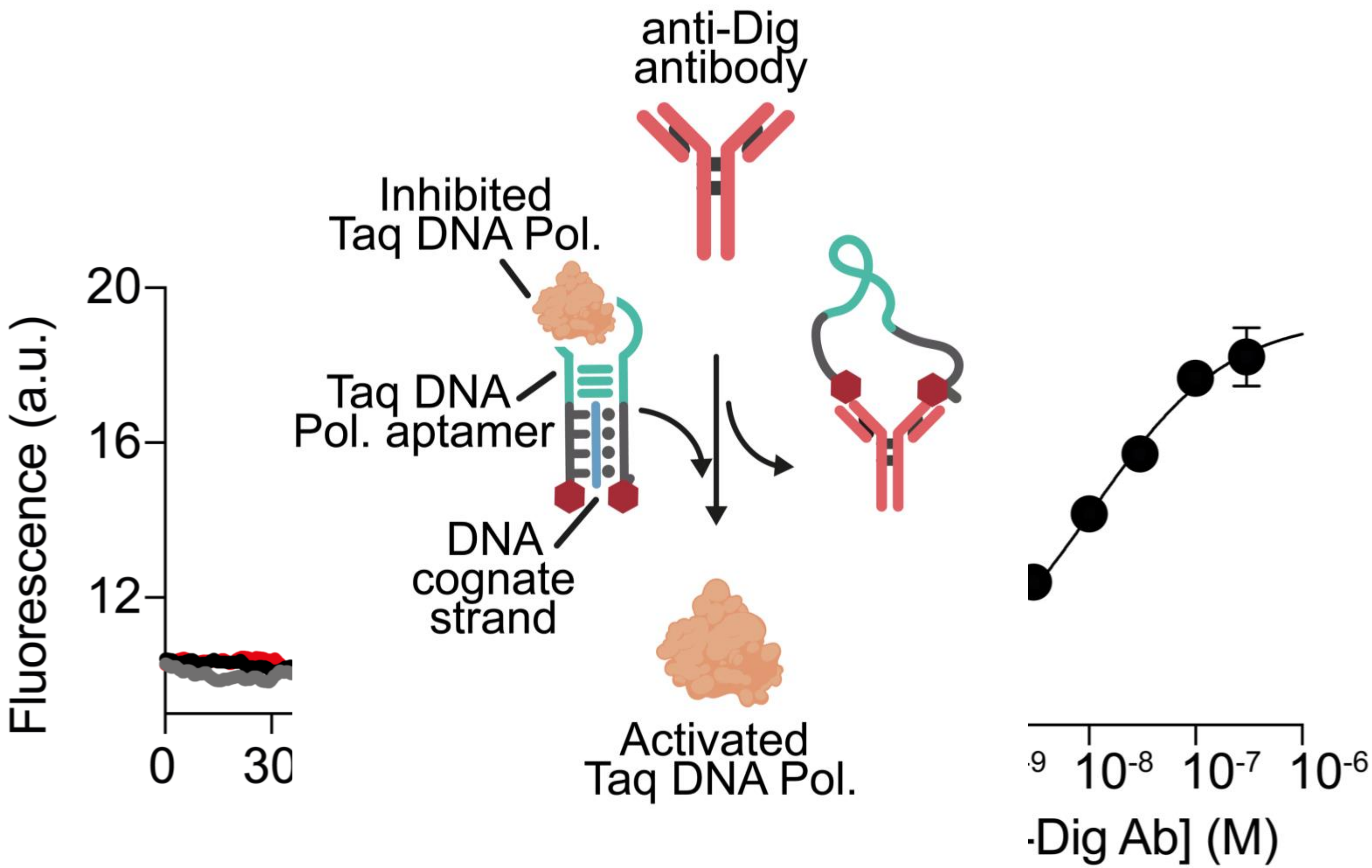
Mechanism B: antibody-induced activation of a target protein

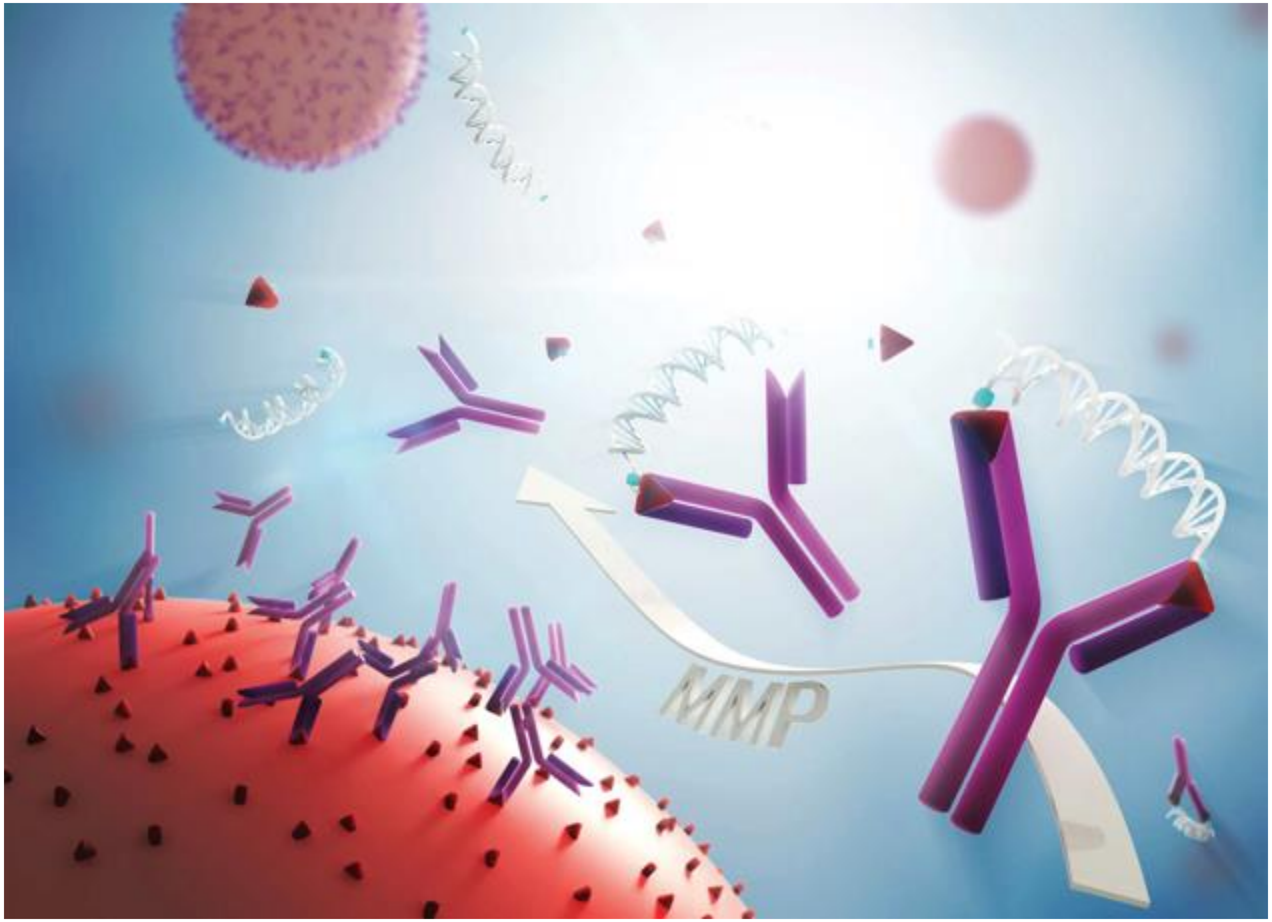


Antibody-protein communication to control the proteolytic activity of thrombin



Antibody-protein communication to control the activity of Taq DNA polymerase

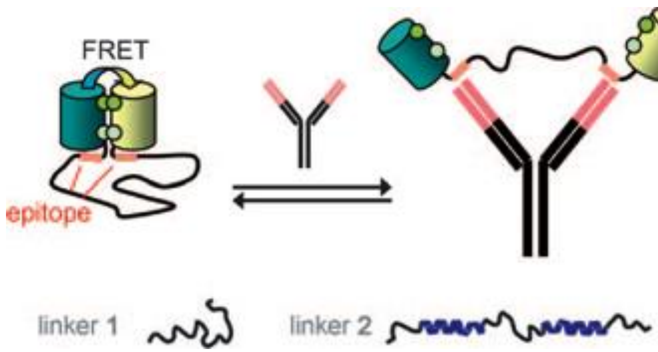




Antibody-Controlled Reactions, Logic Gates, and Circuits

Merkx M. *et al., **Org. Biomol. Chem.*** 2013, *11*, 7642–7649

Antibody detection by Using a FRET-Based Protein Conformational Switch

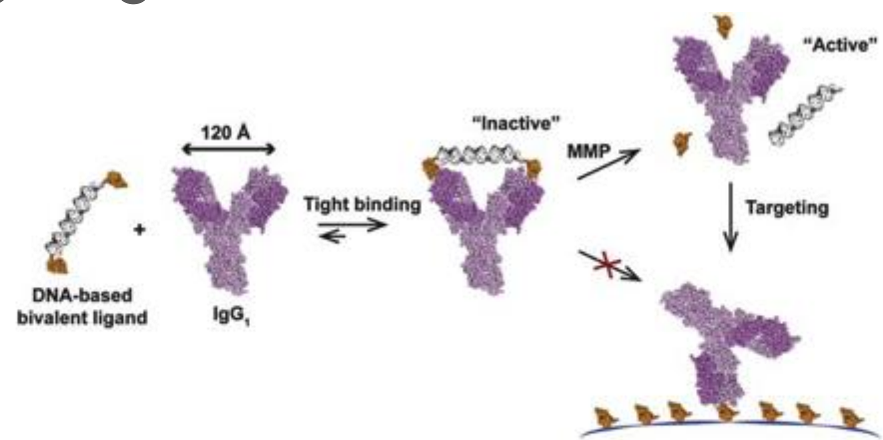


Merkx *et al.*, ***ChemBioChem*** 2010, *11*, 2264 – 2267

- ➢ Direct detection of antibodies in solution
- ➢ Translation of the antigen–antibody interaction into a change in Forster resonance energy transfer (FRET)
- ➢ Conformational switch approach

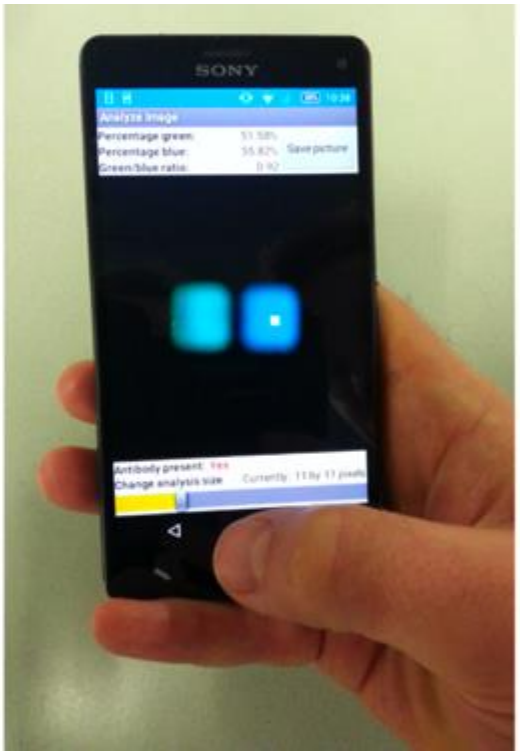
Reversible blocking of antibodies allows protease-activatable targeting

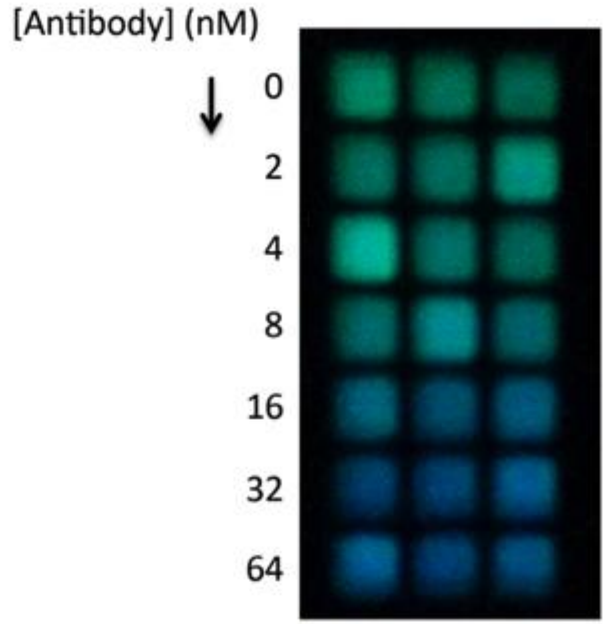
- ➢ Bivalent peptide–DNA conjugates
- ➢ Generic approach to introduce protease sensitivity into antibody-based targeting by taking advantage of the intrinsic ability of antibodies to engage in multivalent interactions



Merkx *et al.*, ***Chem. Sci.*** *2013, 4, 1442–1450.*

Detection of Antibodies in Blood Plasma Using Bioluminescent Sensor Proteins and a Smartphone (LUMAB sensor)





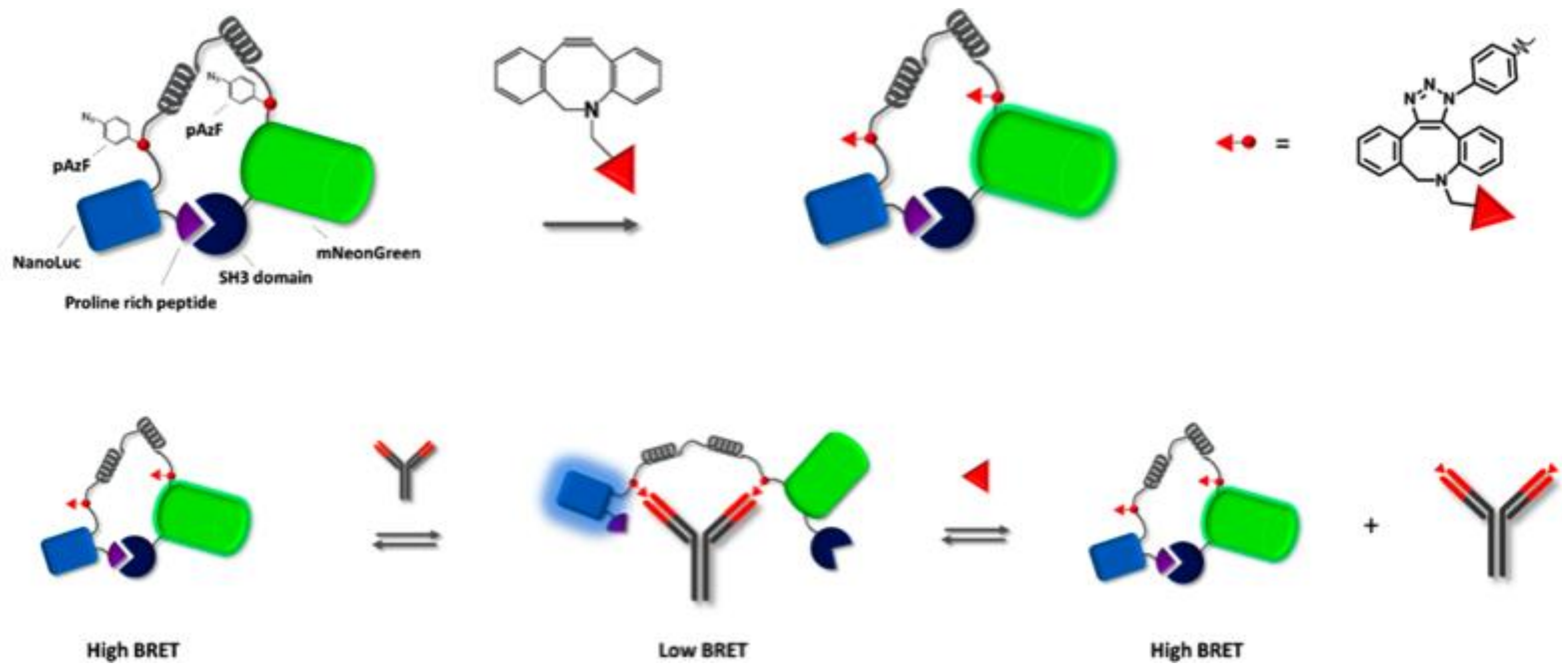
- Sens)
- Bindi iteraction

betwee

Change in color of the emitted light from green-blue to blue

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

Based on the same sensing principle…



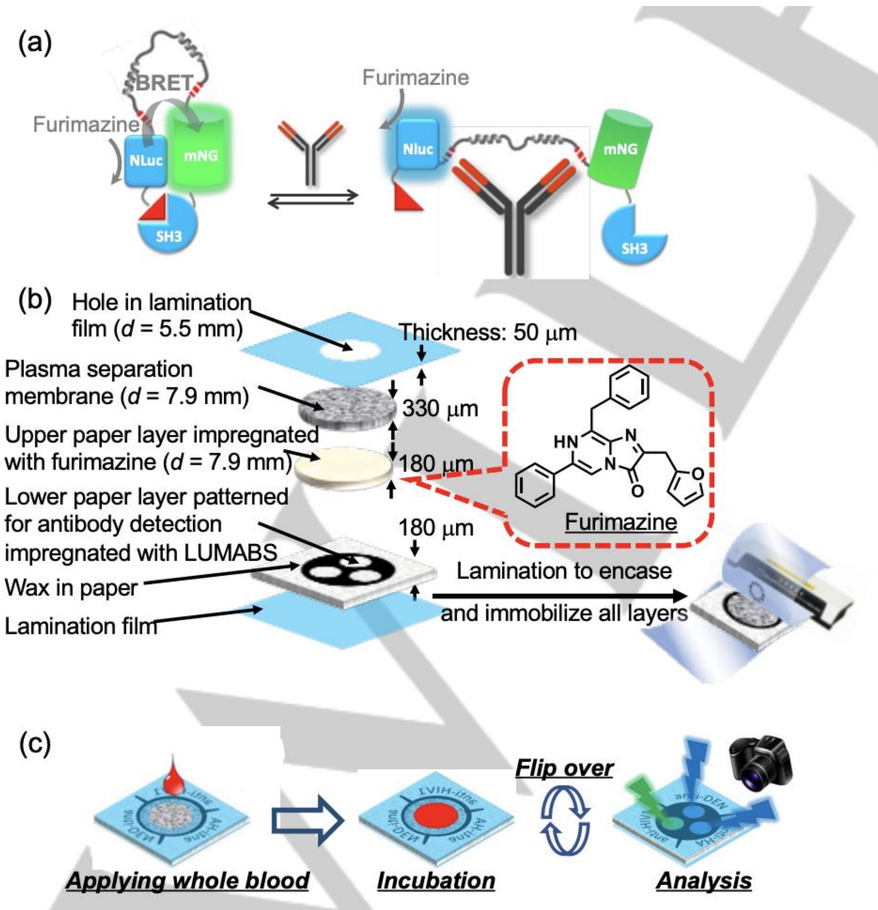
➢ Semisynthetic (LUMABS) sensors that recognize nonpeptide epitopes. The non- natural amino acid para-azidophenylalanine

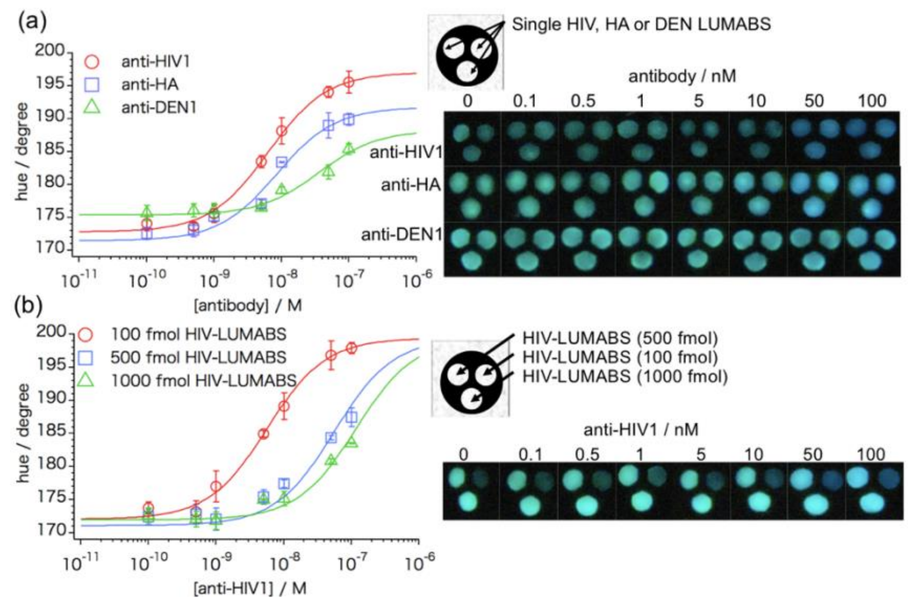
➢ Single-step immunoassays

Merkx *et al.*, ***ACS Sens.*** 2017, *2*, 1730−1736

Paper-based antibody detection using bioluminescent sensor protein

Simultaneous colorimetric detection and quantification of multiple antibodies in a single drop of whole blood (required volume 30 μl)

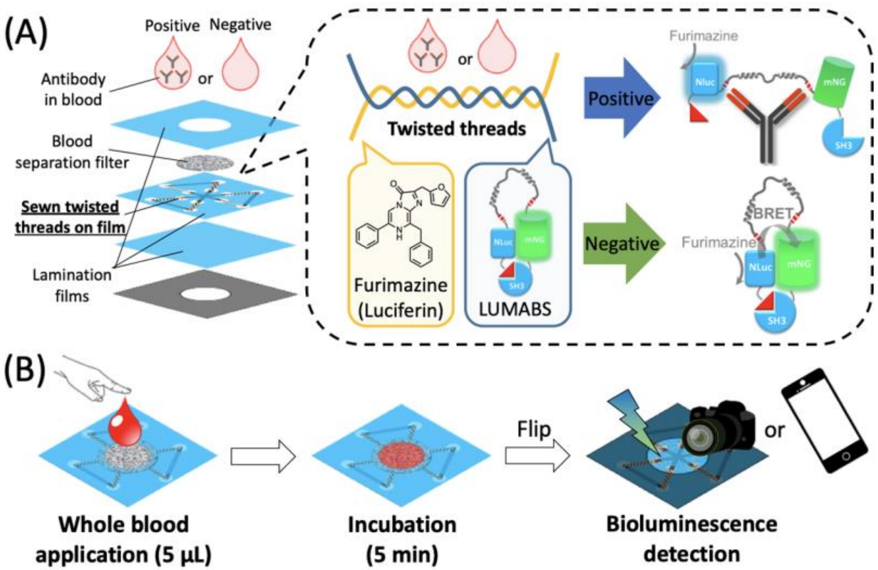


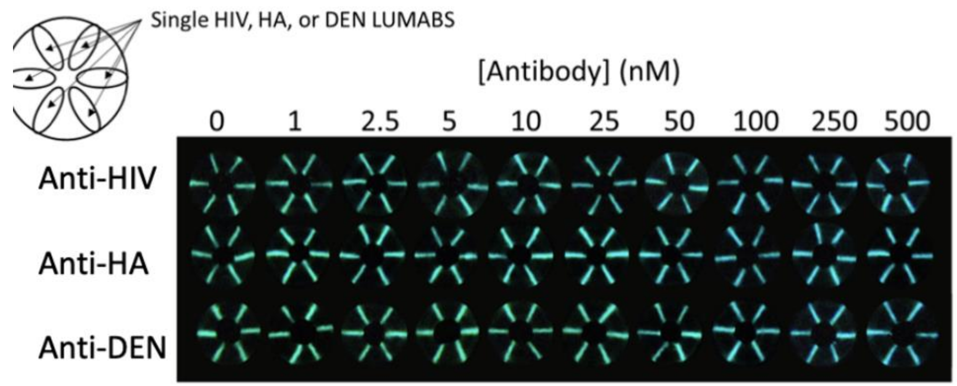


Merkx *et al.*, ***Angew. Chem. Int. Ed.*** 2018, *8*, 14473

Paper-based antibody detection using bioluminescent sensor protein

- ➢ Cotton threads as device substrates
- ➢ One drop of blood (5 μl volume)
- ➢Detection of the bioluminescence emission using a mobile phone with a 3Dprinted adapter
- ➢First report of a thread-based device for biomarker detection in a single drop of whole blood utilizing a bioluminescent sensor protein.





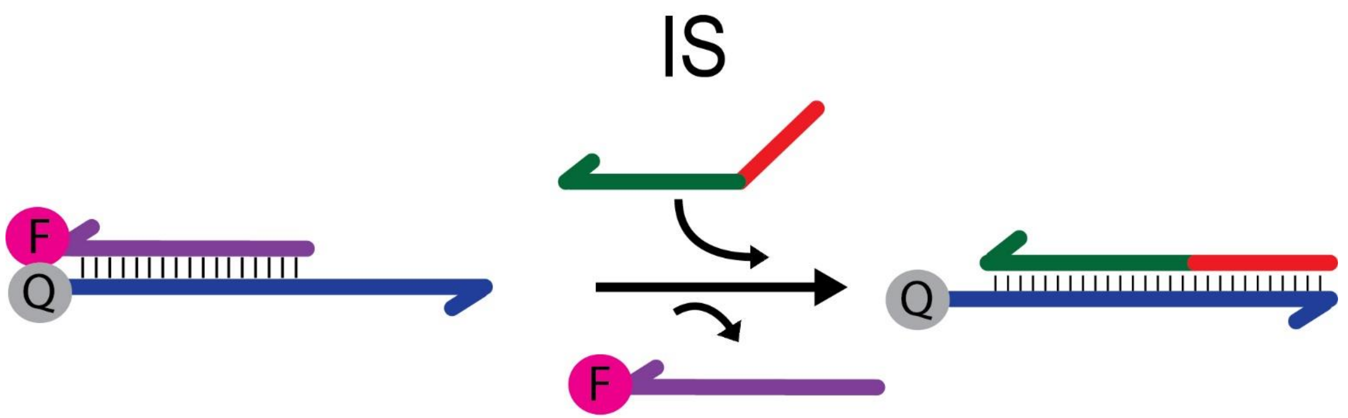
Merkx *et al.*, ***ACS Sensors*** 2020, *5*, 1786

Orthogonal regulation of DNA nanostructure self-assembly and disassembly using antibodies

Ranallo et al., **Nat Commun.** 2019, *10*, 5509.

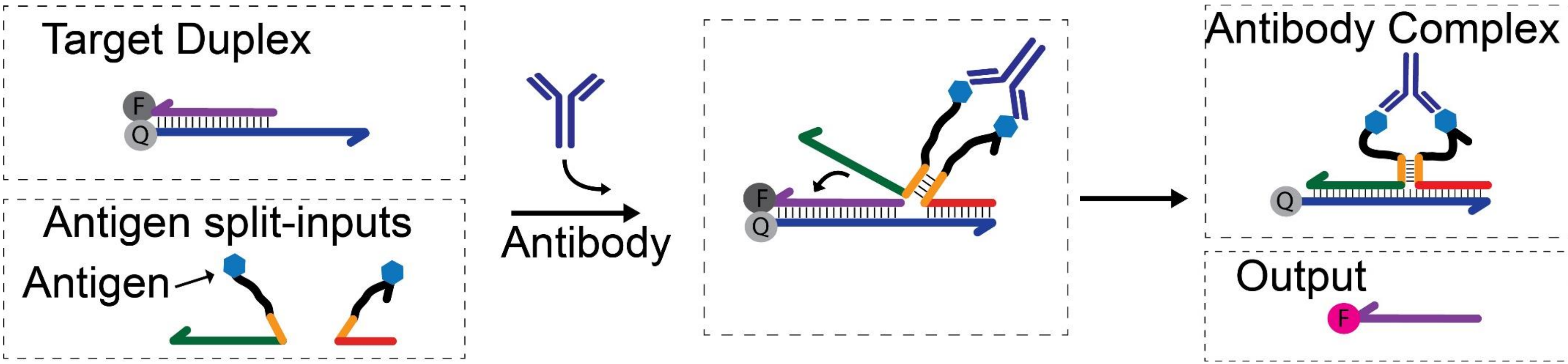
Idea

Toehold mediated strand displacement reaction

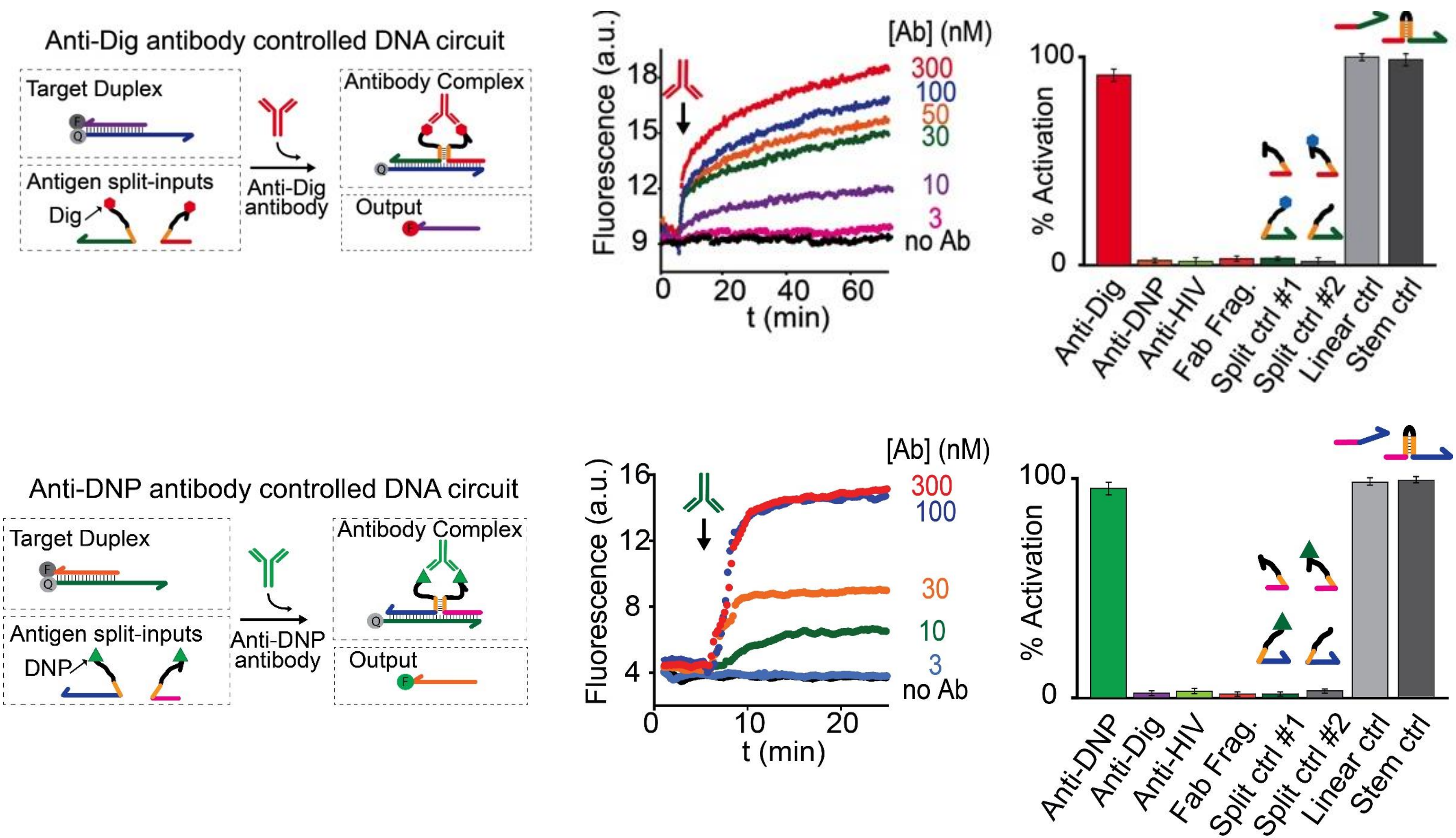


Working principle

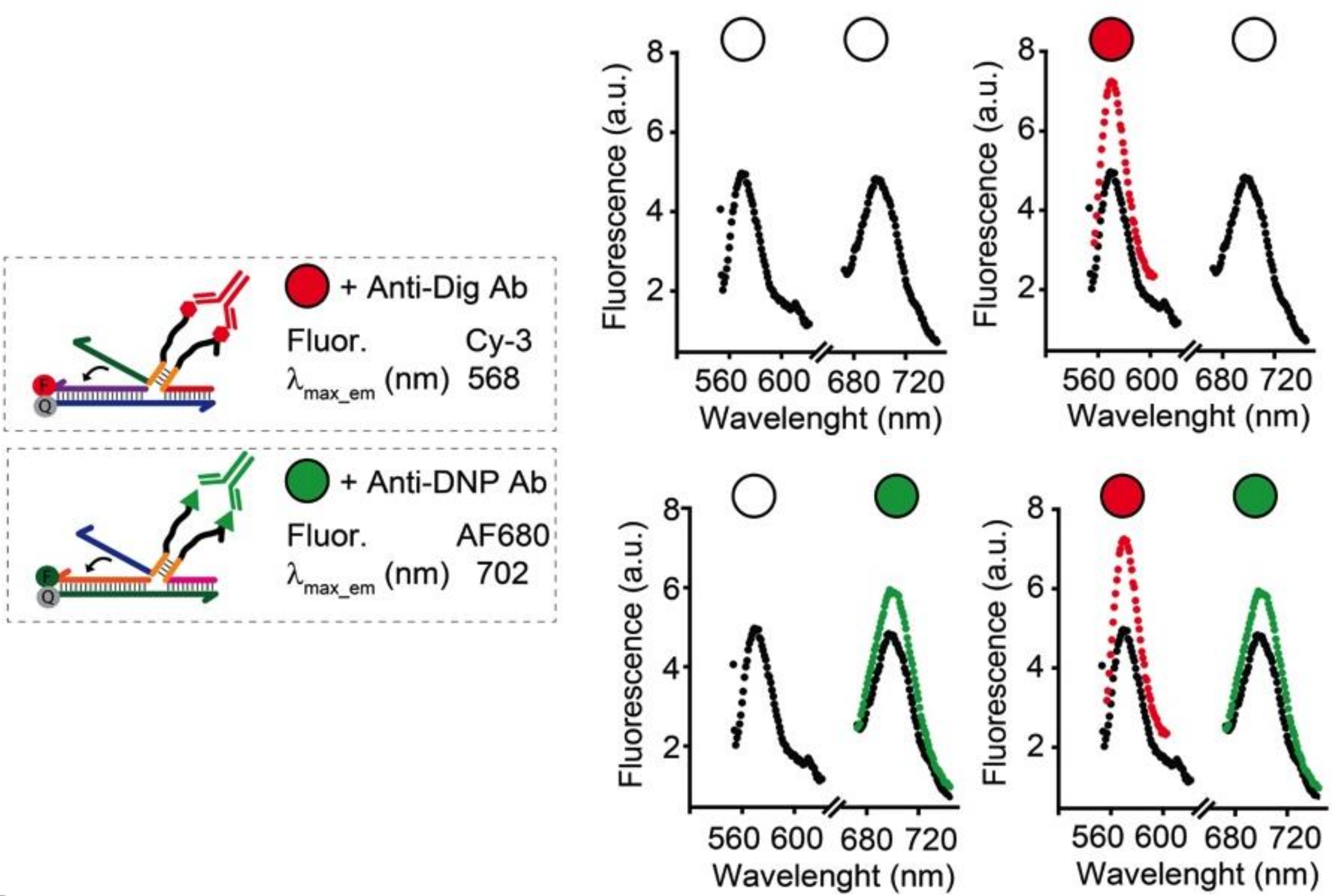
Antibody-controlled DNA circuit



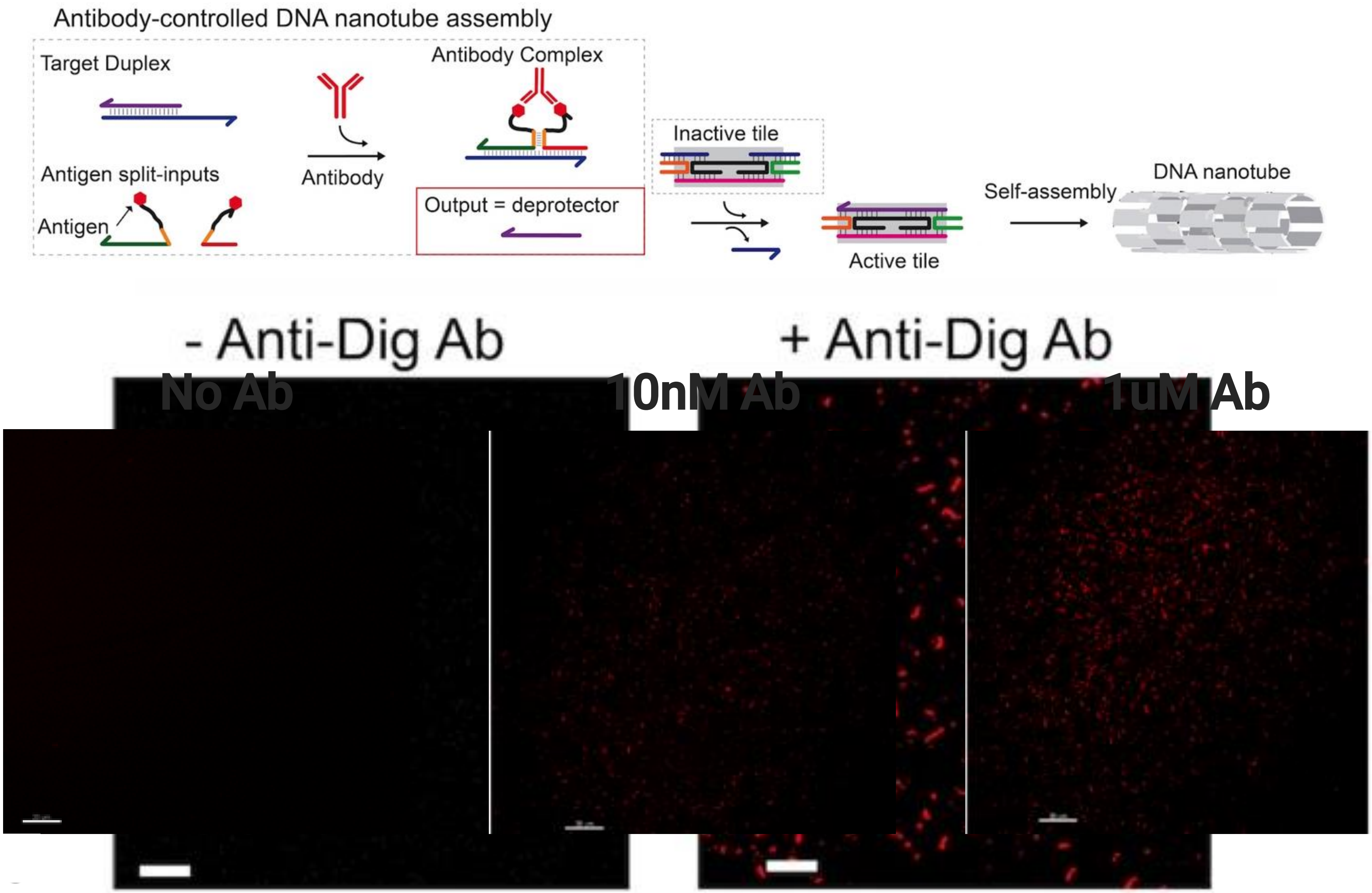
Designing antibody-controlled DNA circuits



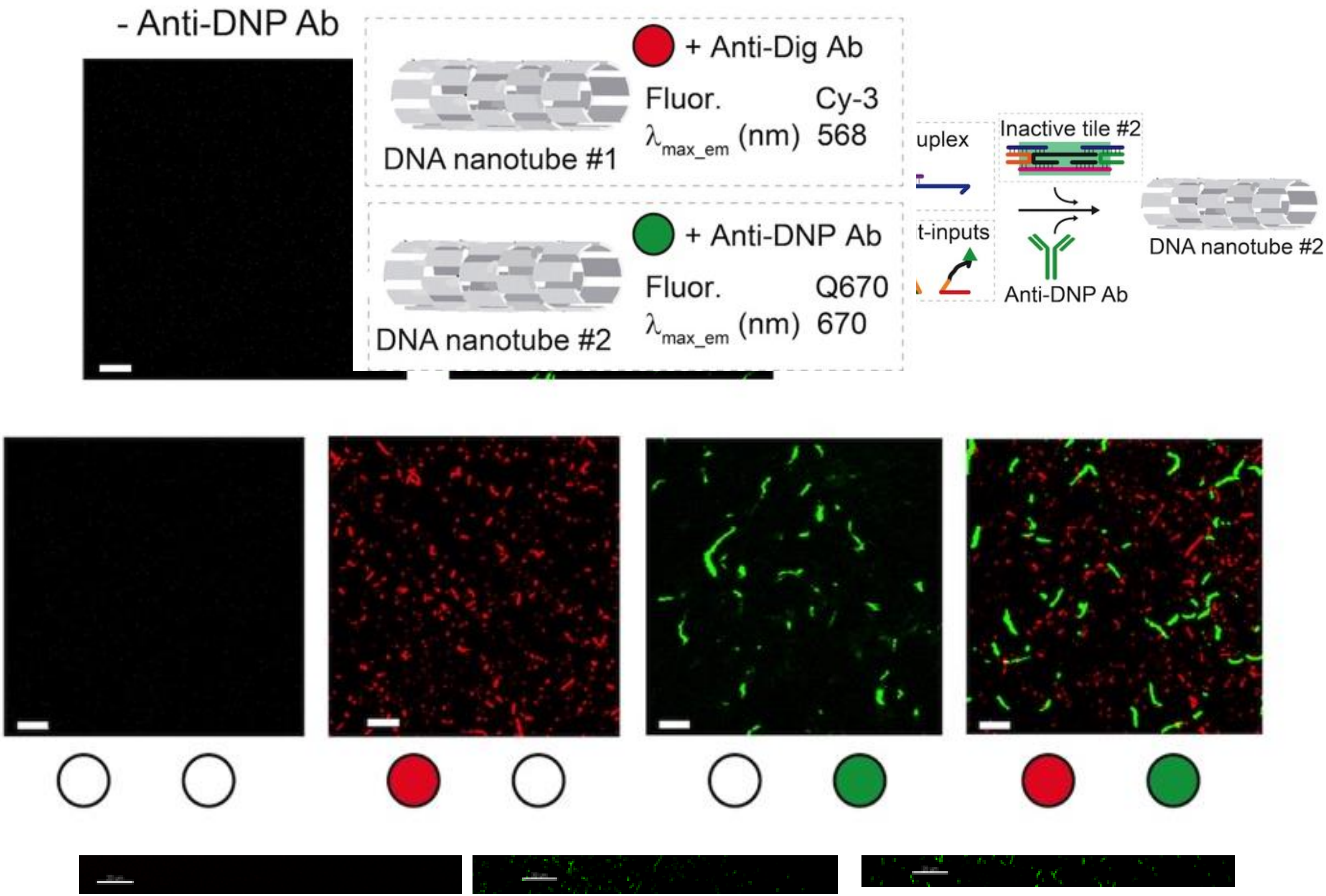
Orthogonal control of two antibody-controlled circuits



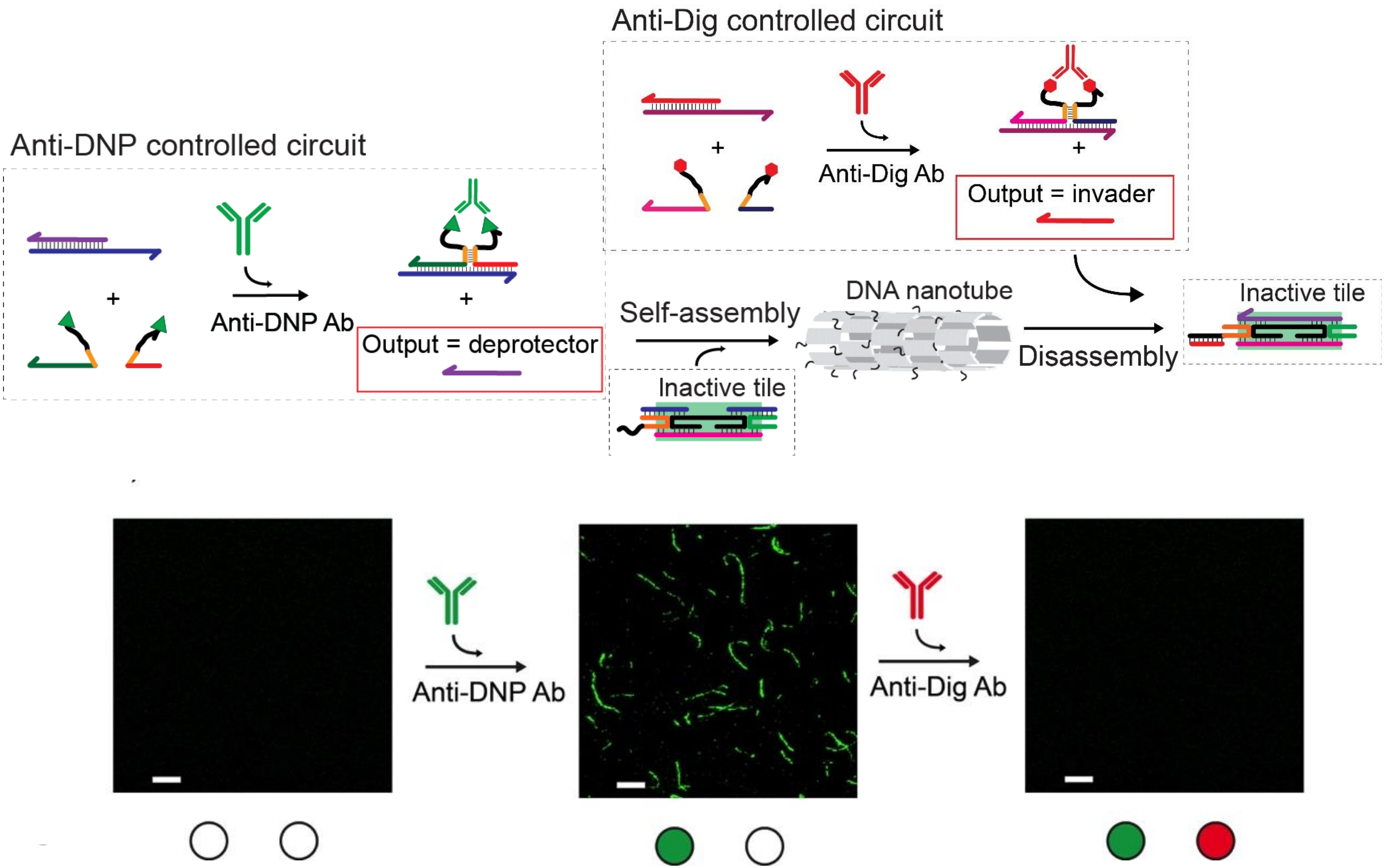
Antibody-controlled DNA nanostructures assembly



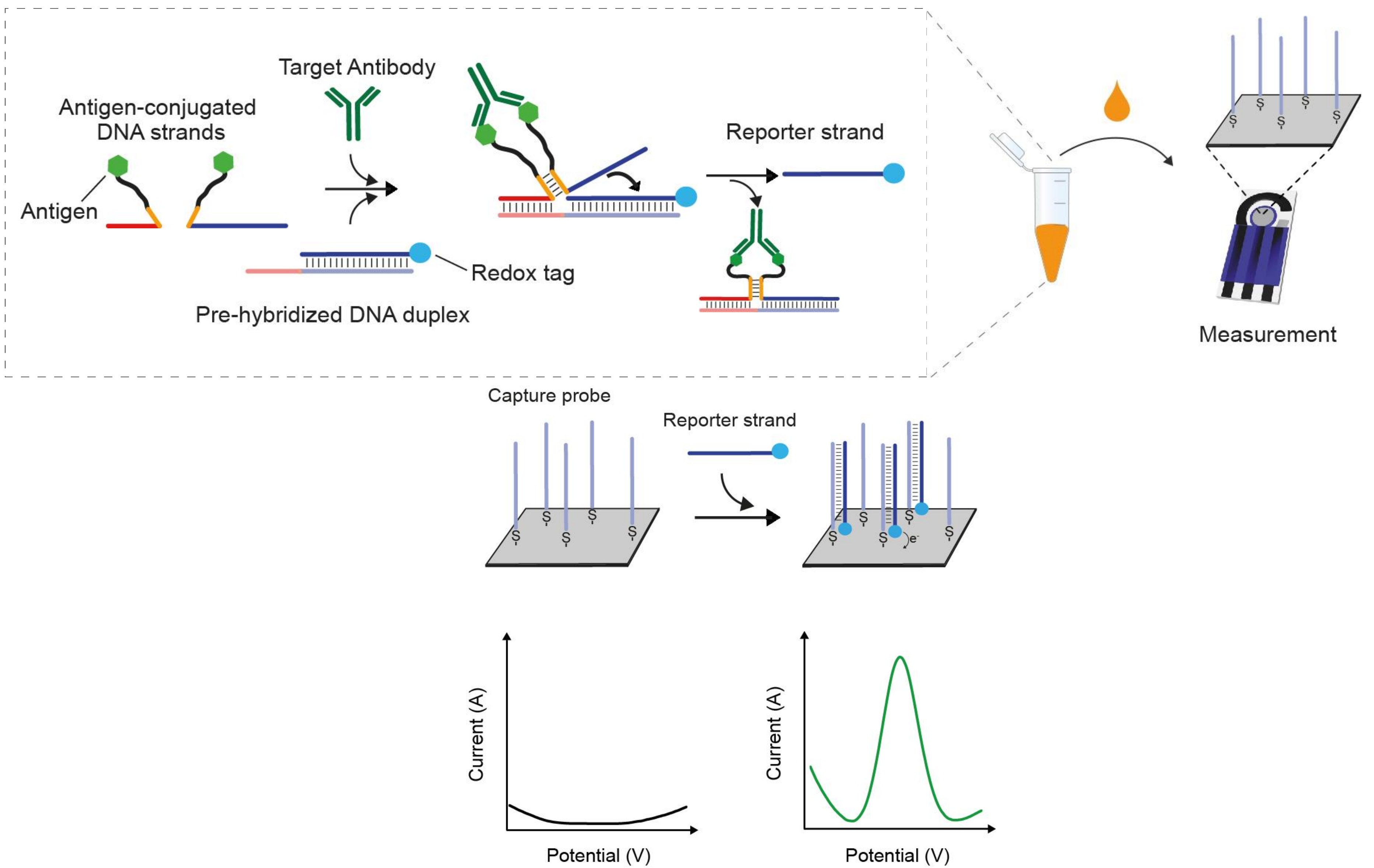
Antibody-controlled DNA nanostructures assembly



Orthogonal assembly and disassembly of antibody-controlled DNA nanostructures

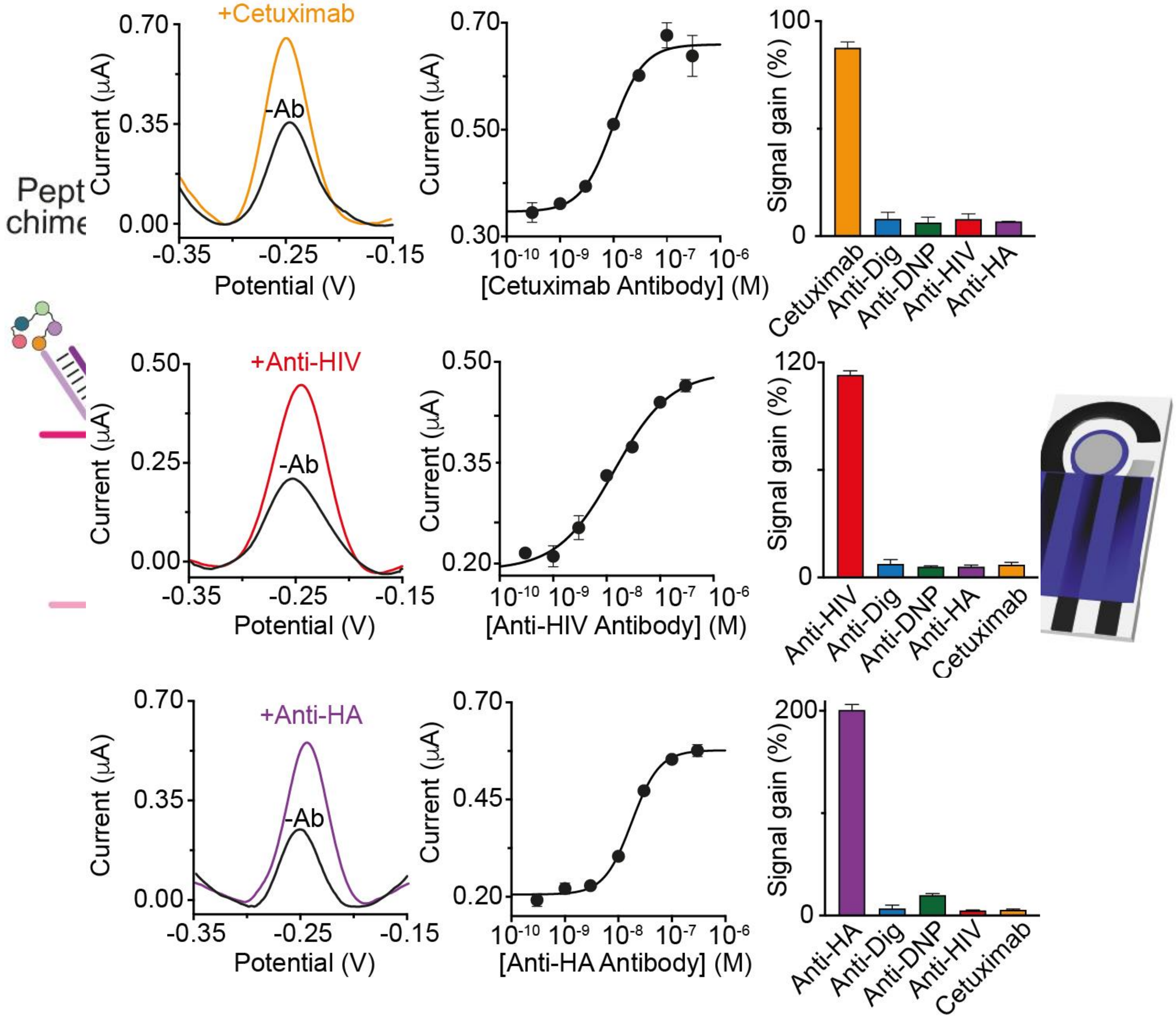


Electrochemical DNA-based Platform for antibody detection

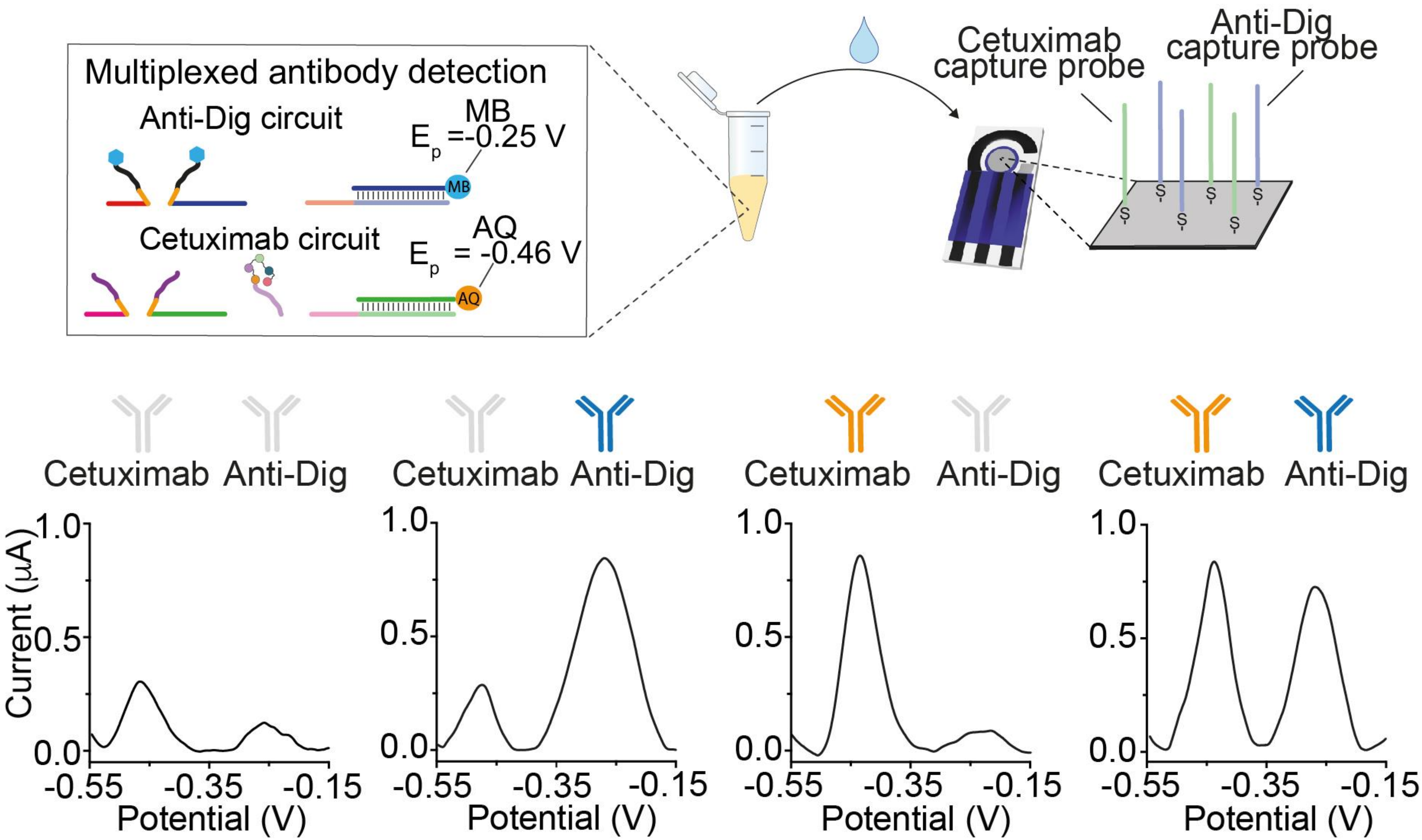


Bracaglia S., Ranallo S. *et al.*, **ACS Sens.** 2021, 6, 2442–2448

Electrochemical platform: detection of clinically-relevant antibodies

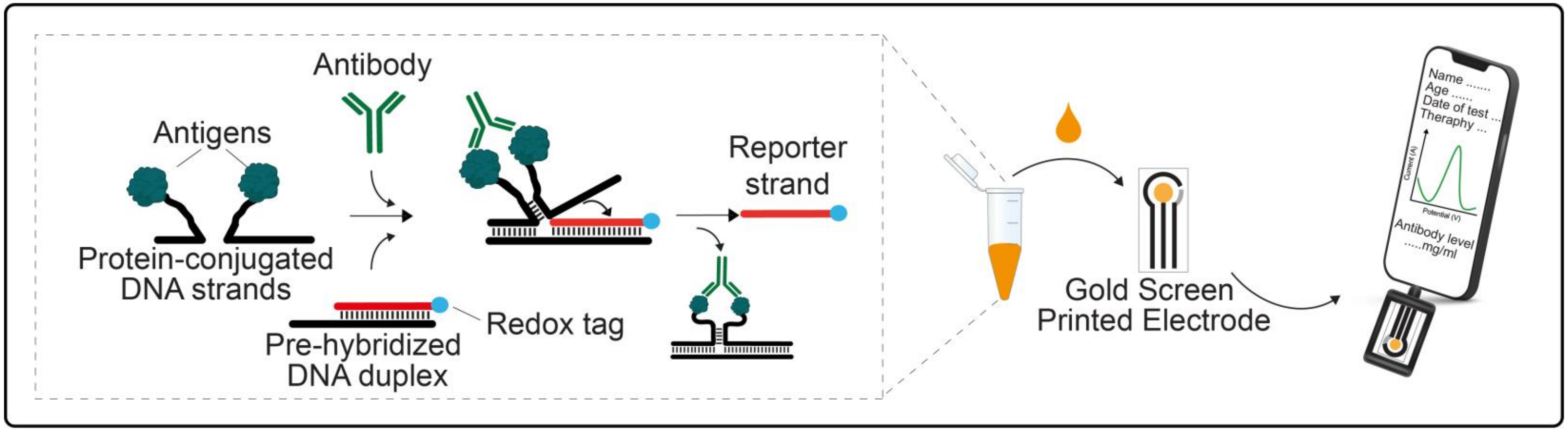


Orthogonal platform: multiplexed antibodies detection

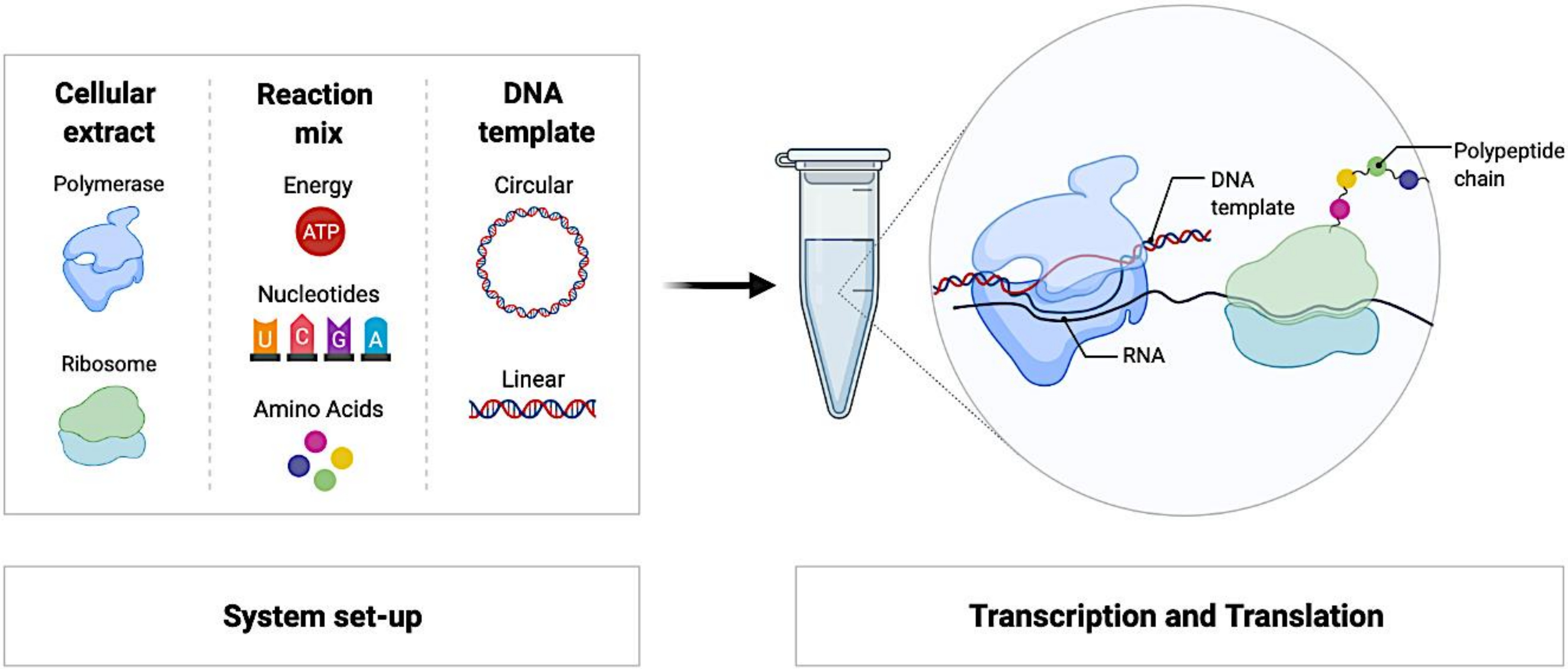


Electrochemical DNA-based platform for antibody detection

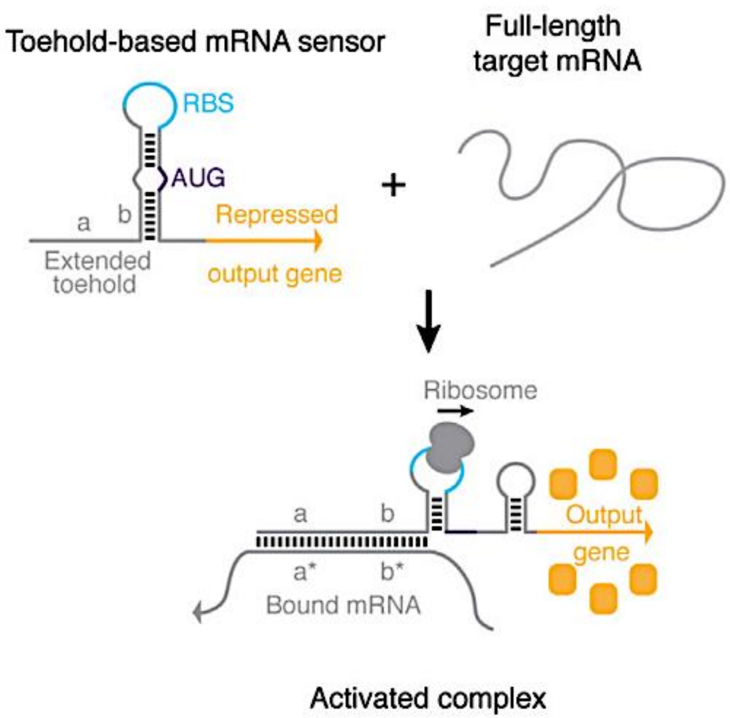
Sensitive | Low volume | Versatile | Low cost | Home test



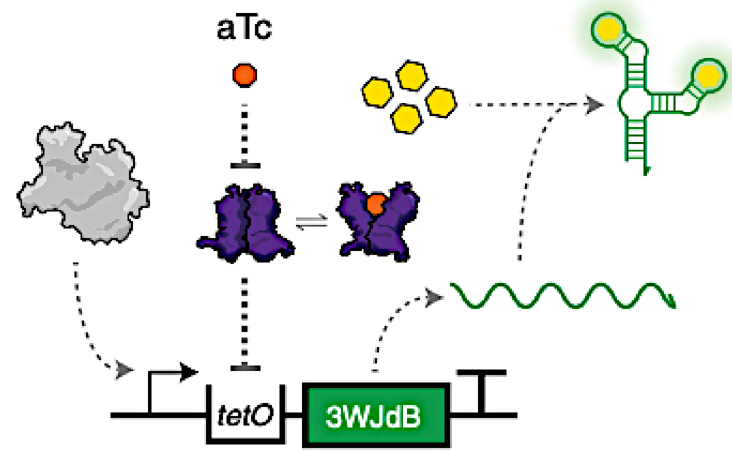
Cell-free Transcription/Translation system



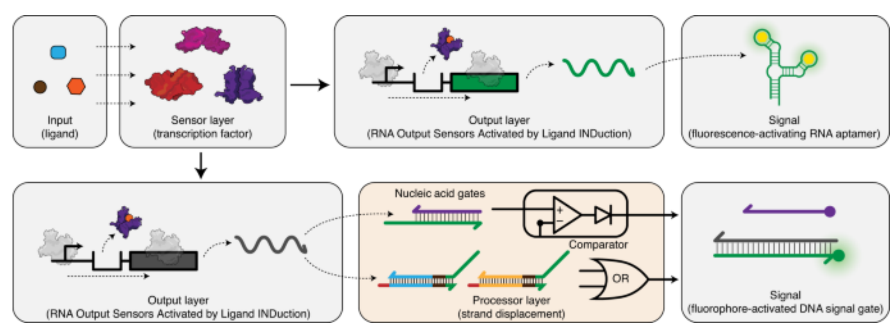
Cell-free biosensors



Pardee, K. *et al.* ***Cell***. 2014, 159, 940-954

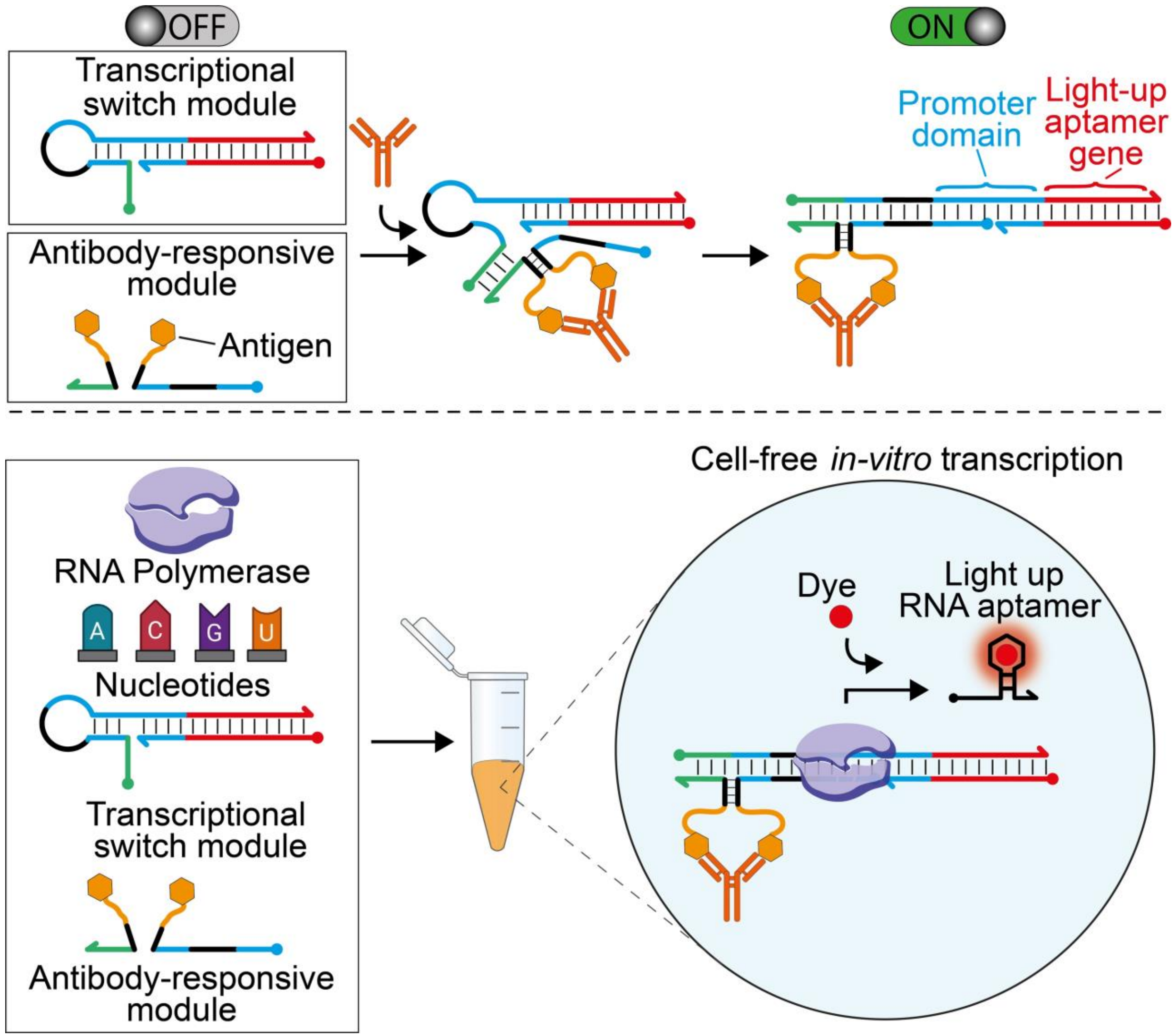


Jung, J. K. *et al.*, **Nat. Biotechnol.** 2020, *30*, 1451-1459



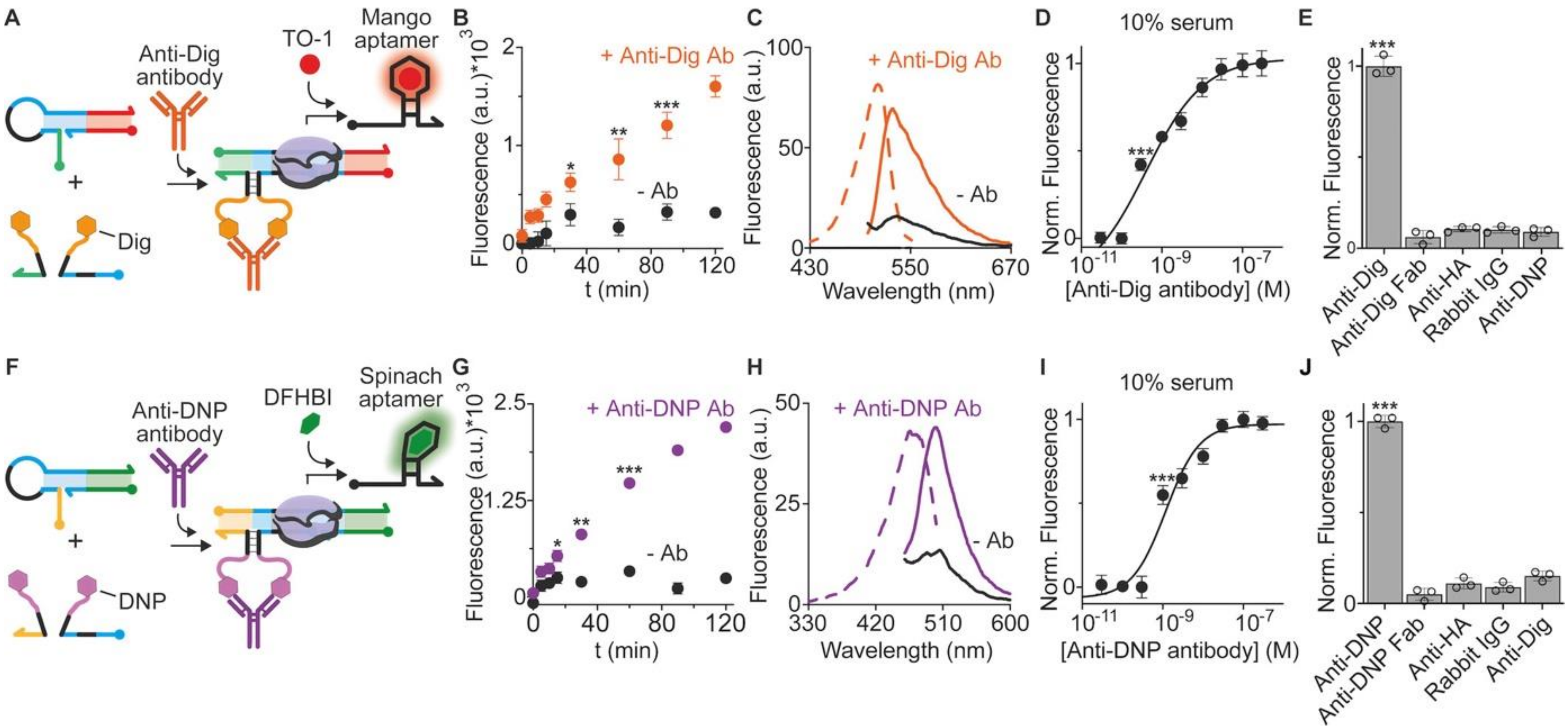
Jung, J. K. *et al.*, **Nat Chem Biol 2022,**
DOI:10.1038/s41589-021-00962-9

Cell-free biosensors: Transcriptional switch

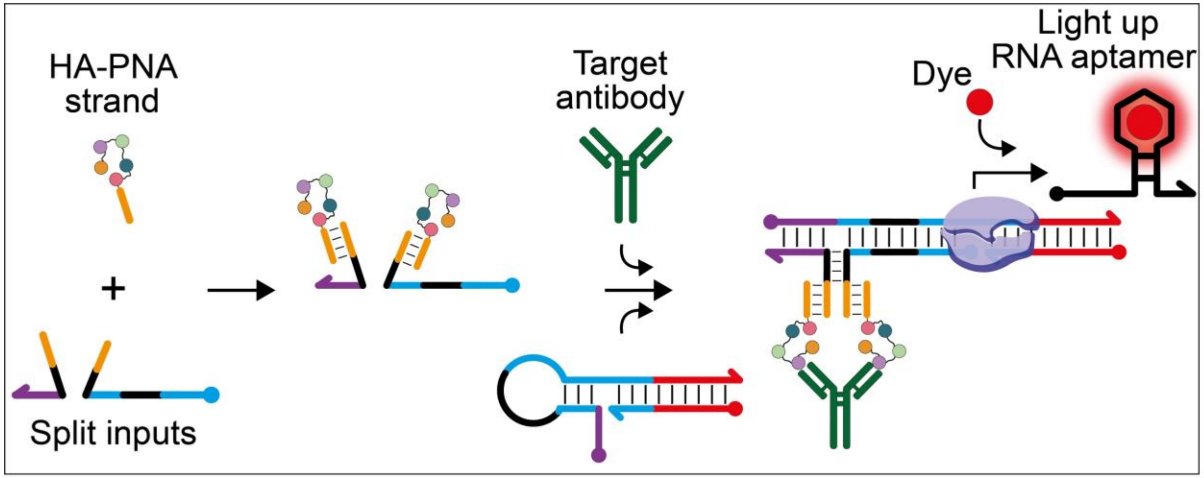


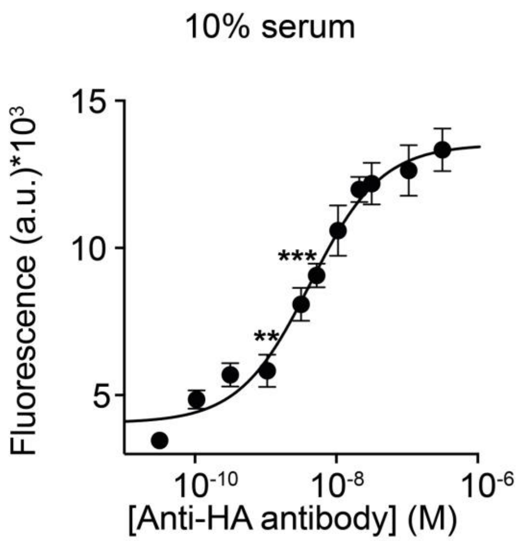
Patiño Diaz, A. Bracaglia, S., Ranallo S., Patiño Padial T., *et al.* **J. Am. Chem. Soc.** *2022, 144, 13, 5820−5826*

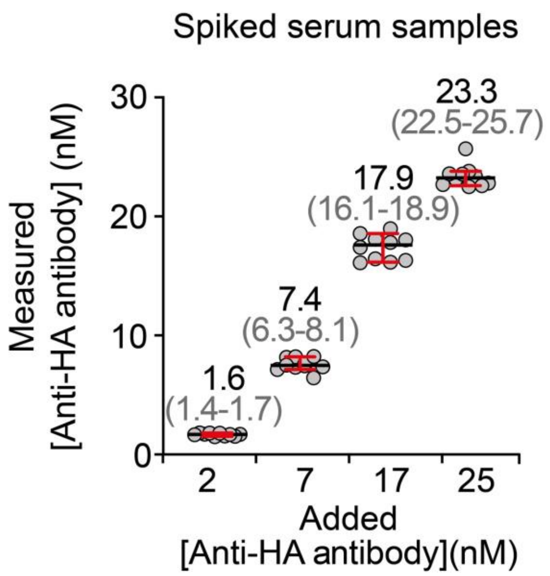
Cell-free biosensors: Transcriptional switch



Cell-free biosensors: Transcriptional switch







Conclusions

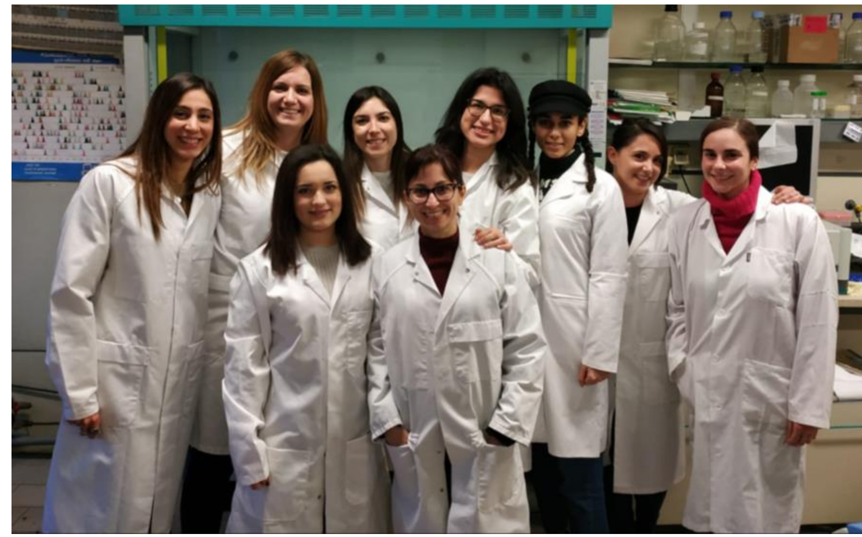
- ✓ Antibody-powered DNA-based nanomachines for diagnostic and drug-delivery applications (optical and electrochemical biosensors).

- ✓ Antibodies as new molecular tools to control the assembly of DNA-based nanostructures and for non-natural communication with proteins

- ✓ Combines the advantages of synthetic biology with those of electrochemical platforms particularly suitable for point-of-care (POC) applications.

Acknowledgements

"DNA-NANO-AB"



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Thanks for your attention!