RNA/DNA Biotechnology

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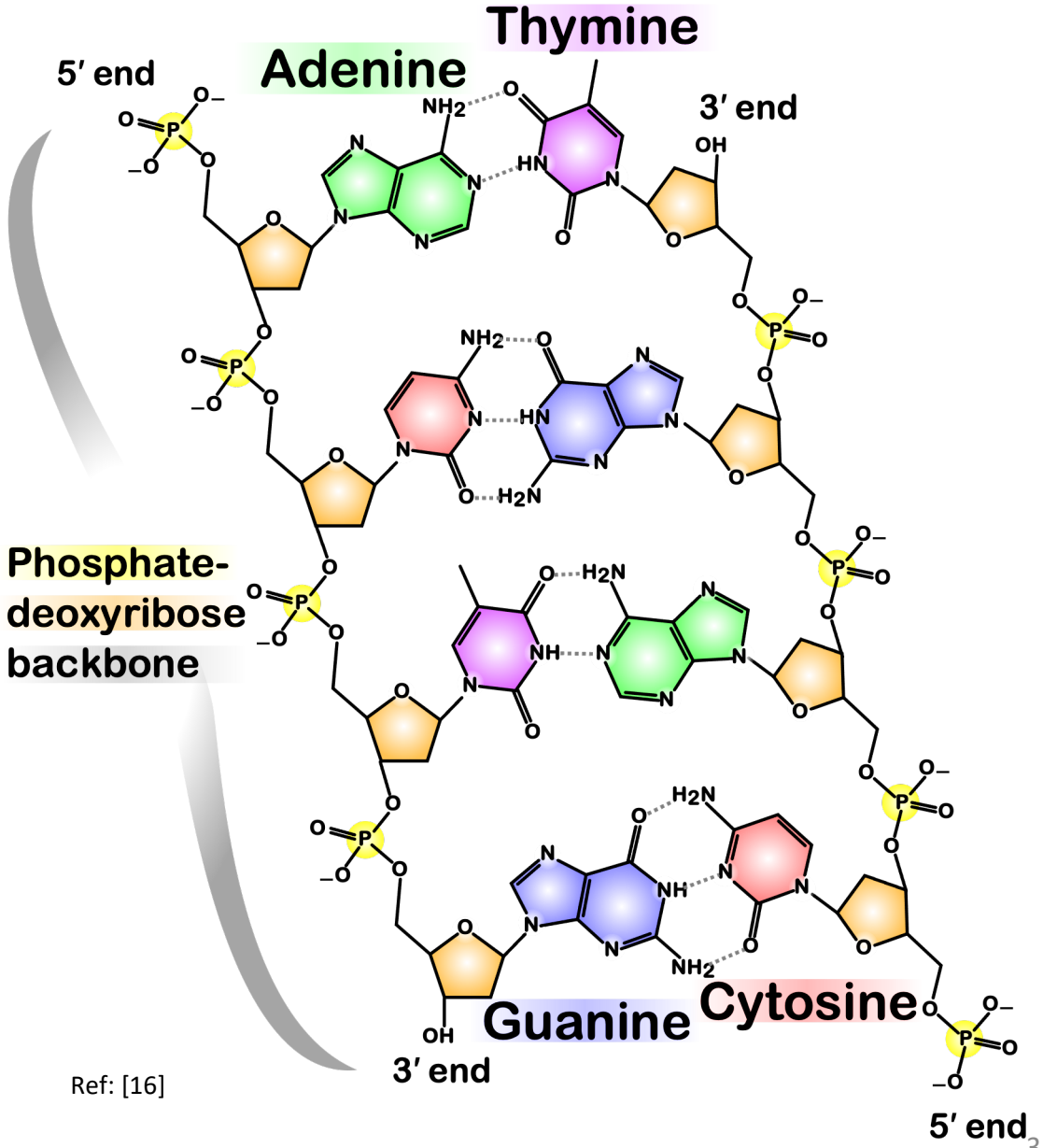
Content

- Basics of RNA, DNA
- Examples of biotechnology with DNA (nanostructure using DNA Origami)
- Aptamers via SELEX (evolutionary approach)
- DNAzymes

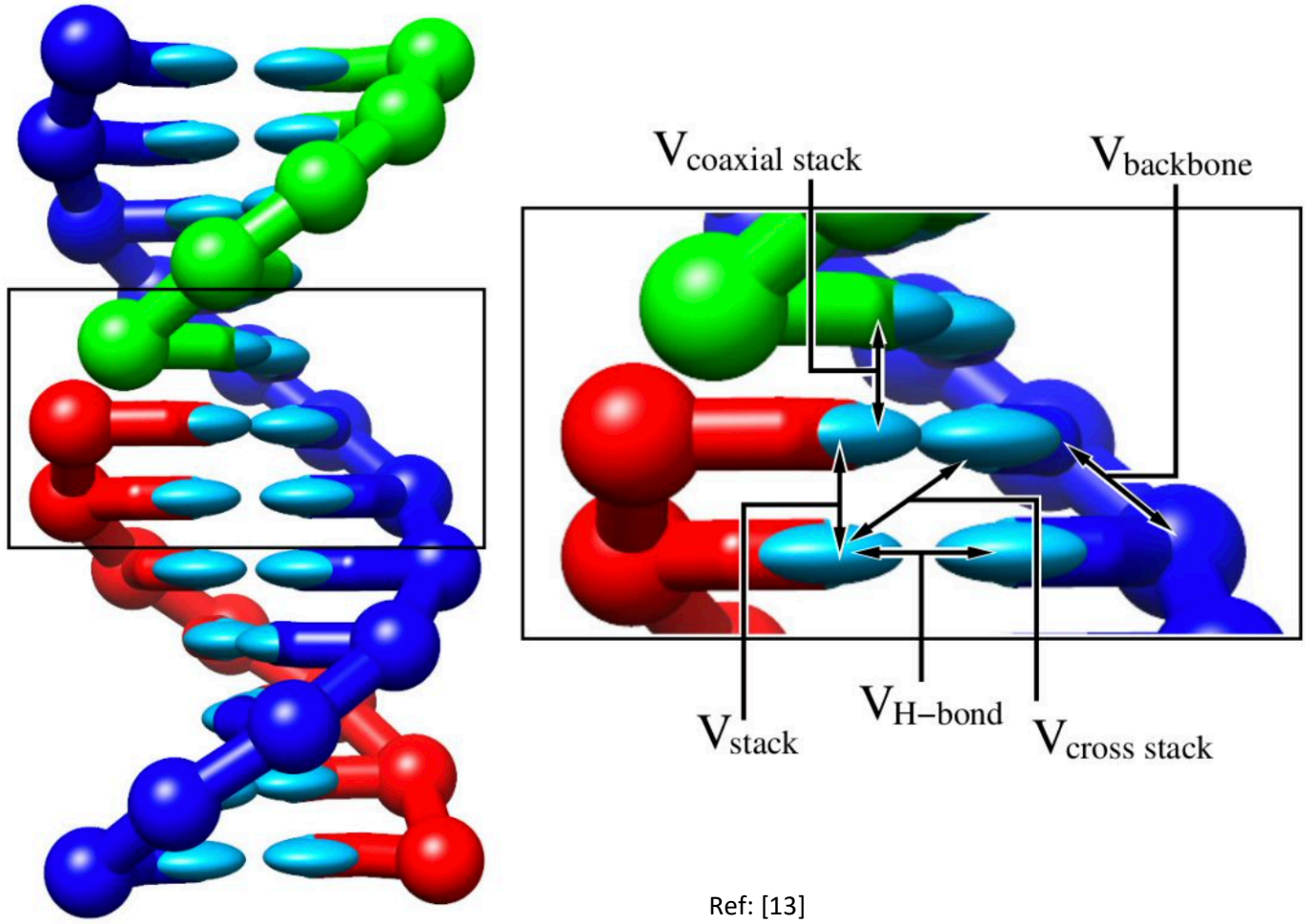
Quick recap of DNA

- Nucleotides aligned together
  - Alternating backbone:
    - Sugar (DNA: Deoxyribose, RNA: Ribose)
    - Phosphate
    - *Covalent Binding*
  - Nucleobase
    - **A**denine
    - **C**ytosine
    - **G**uanine
    - **T**hymine (DNA) / **U**racil (RNA)
    - *Watson-Crick base-pairing*
- DNA most commonly present as double helix (two strands), RNA as single strand



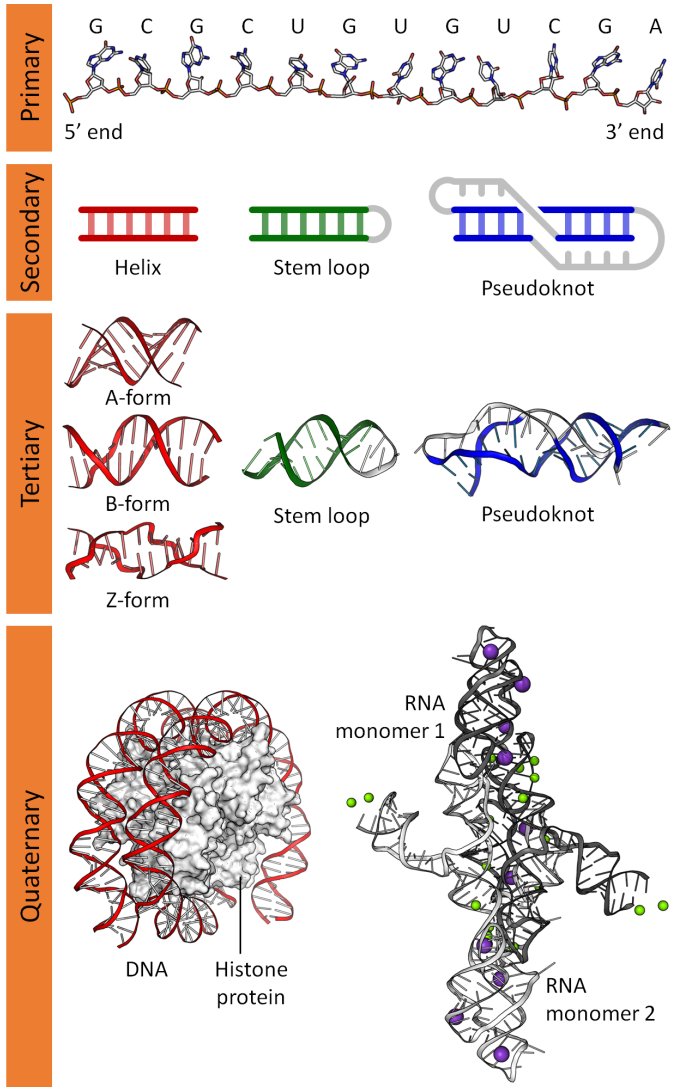
Ref: [16]

RNA/DNA Simulation (oxDNA)



Ref: [13]

Nucleic Acid Structure



Linear sequence of nucleotides linked together via backbone

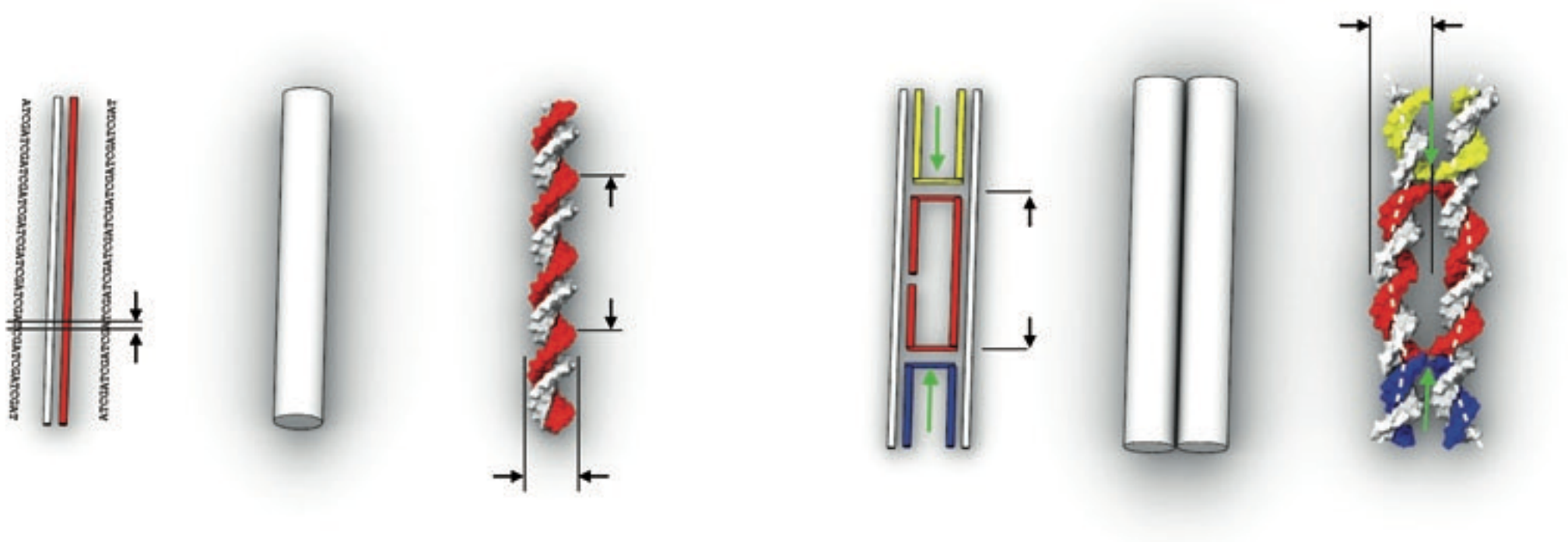
Set of interactions between bases

Location of atoms in 3D-space

Higher-level of organization of nucleic acids

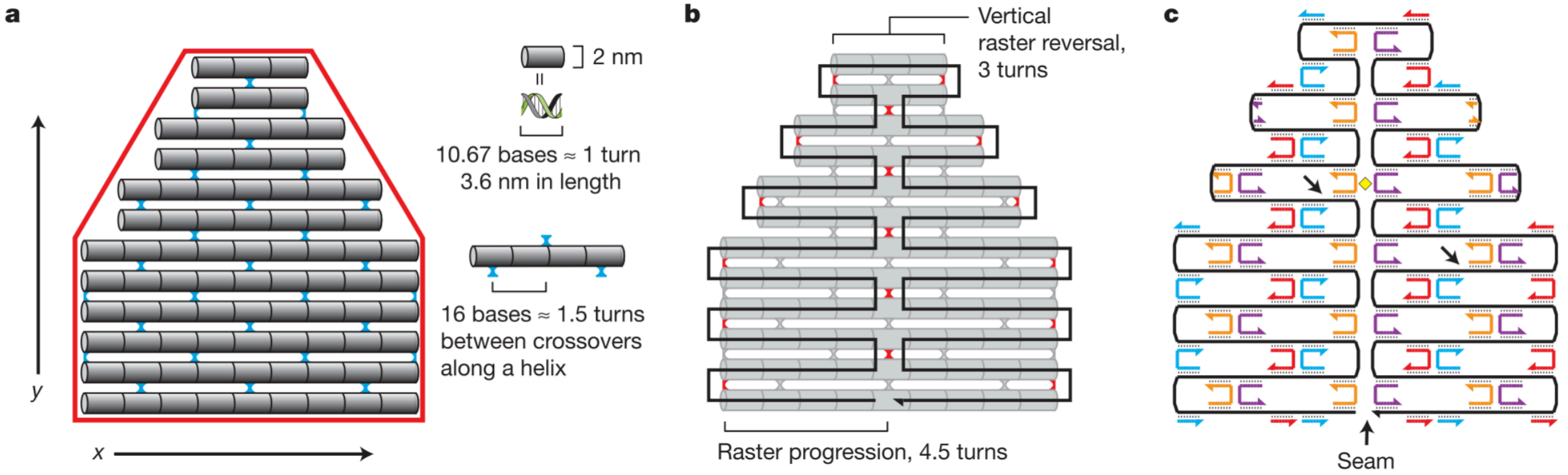
Ref: [17]

DNA Origami - Foundation



Ref: [5]

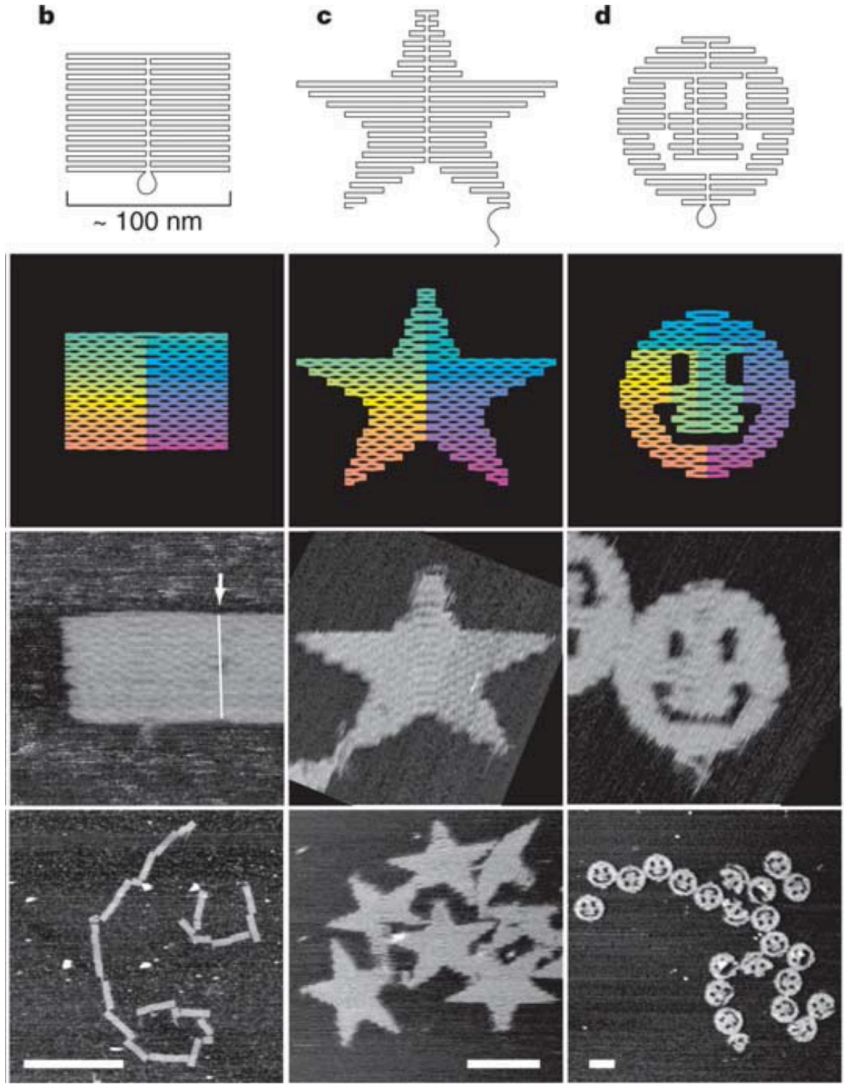
DNA origami



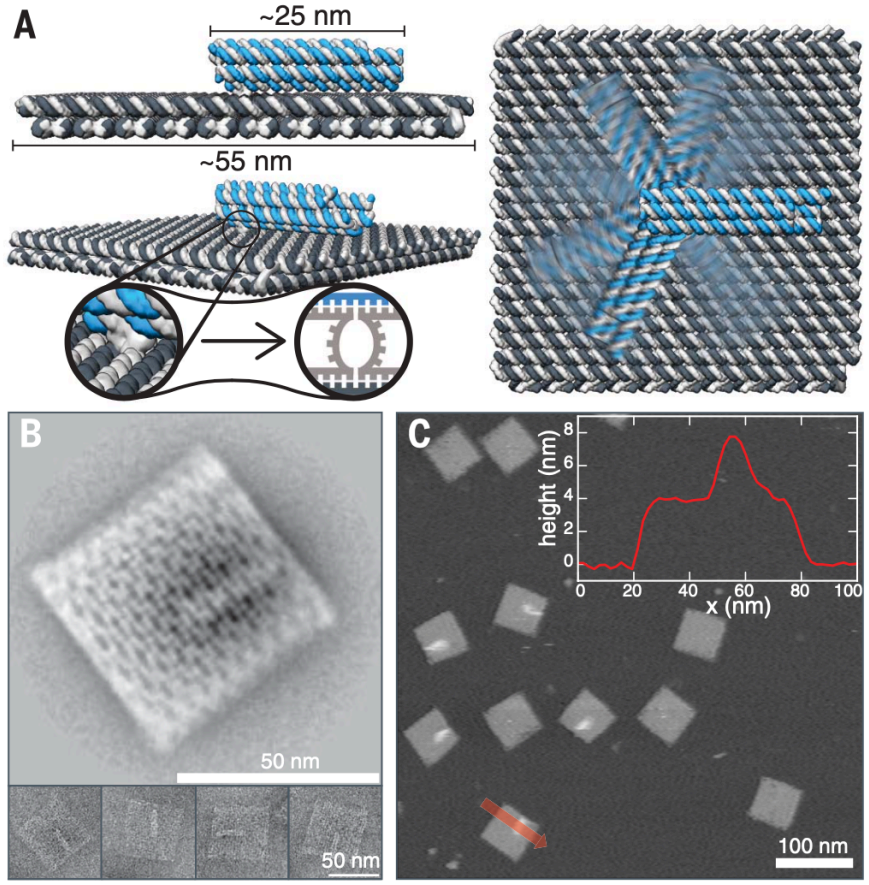
a) Design approximated shape
b) single stranded DNA scaffold (black) runs through every helix
c) design specific, short staples (also ssDNA) -> staples + scaffold = double helix in desired form

Ref: [2]

DNA Origami - Examples

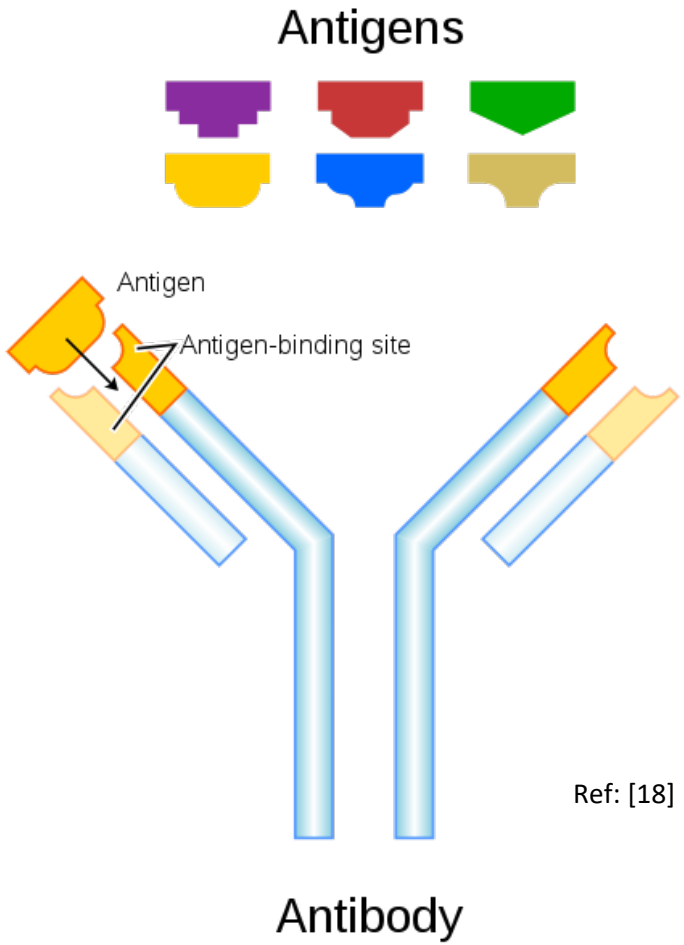


Ref: [2]



Ref: [9]

Motivation: Custom Antibody?



Ref: [18]

- Y-shaped protein that neutralizes pathogens
- Binds to unique molecule of pathogen (the antigen)
- difficult to produce in labs

What are Aptamers

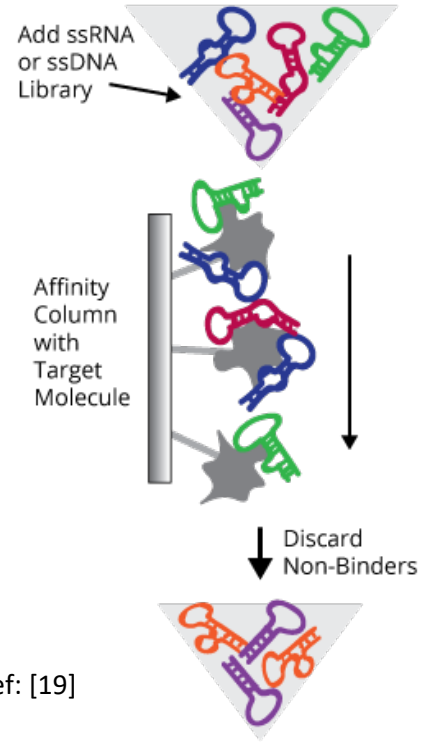
Latin *aptus* – fit; Greek *meros* – part

- short single-stranded DNA/RNA molecules
- bind to specific target
- bind via tertiary structure

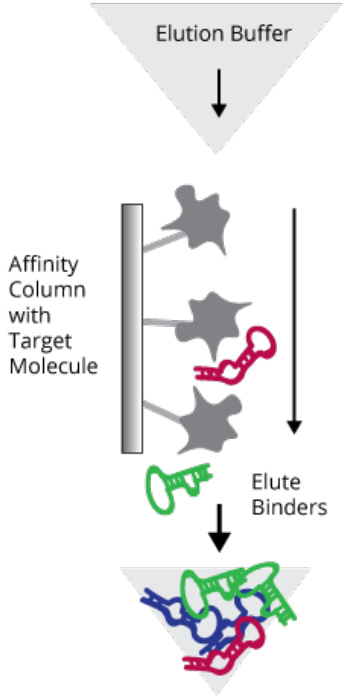
Evolutionary Approach: SELEX

SELEX: Systematic Evolution of Ligands by Exponential enrichment

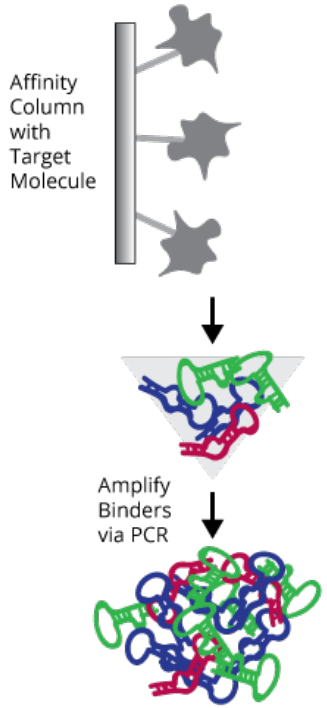
Step 1
Mix library with target, discard non-binders



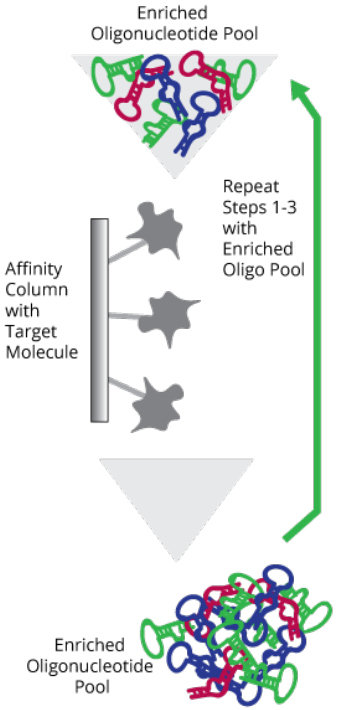
Step 2
Isolate binders



Step 3
Amplify binders via PCR



Step 4
Repeat from Step1



Ref: [19]

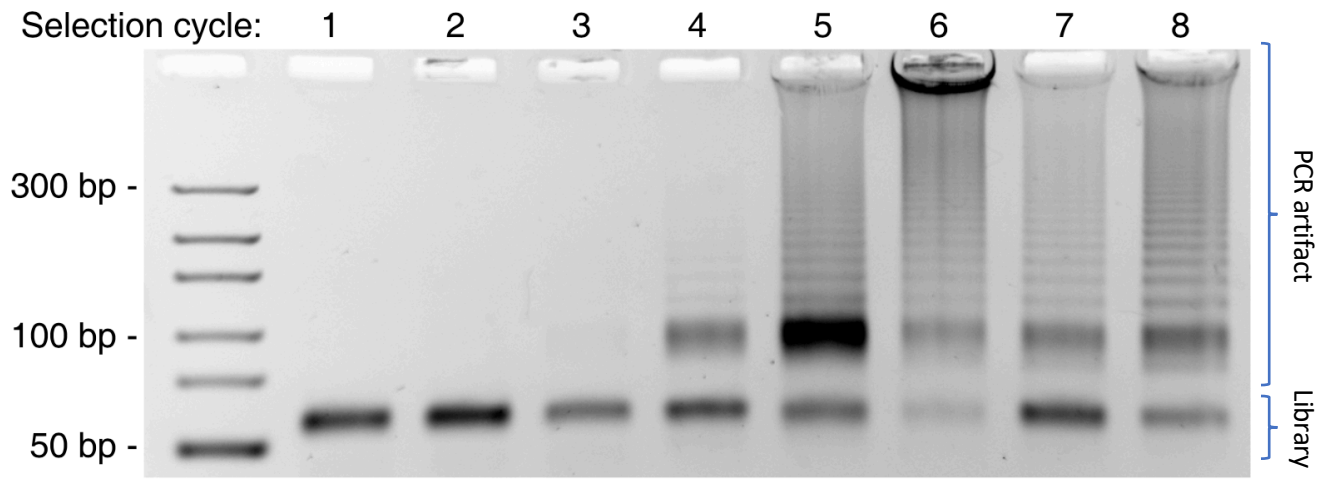
Aptamers vs Antibodies

Aptamers

- are more flexible for targets
- can be highly selective
- development very fast (2-3 months) and easy
- can be very small
  - enhanced access to tissues
  - are non-immunogenic
- more stable
- are easy to store long-term

Limitations of SELEX

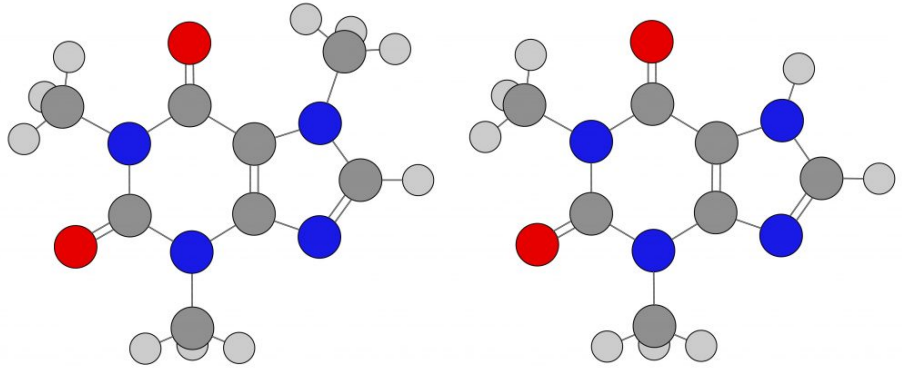
- PCR bias
- small targets loose interaction site
- May bind to similar targets
- stability in body fluids
- low success rate of selection procedure
- only few SELEX-labs



Ref: [8]

Improvement of SELEX

- Negative SELEX: discarding aptamers binding to a similar (different) target
- Enhanced alphabet
- Mutation during SELEX

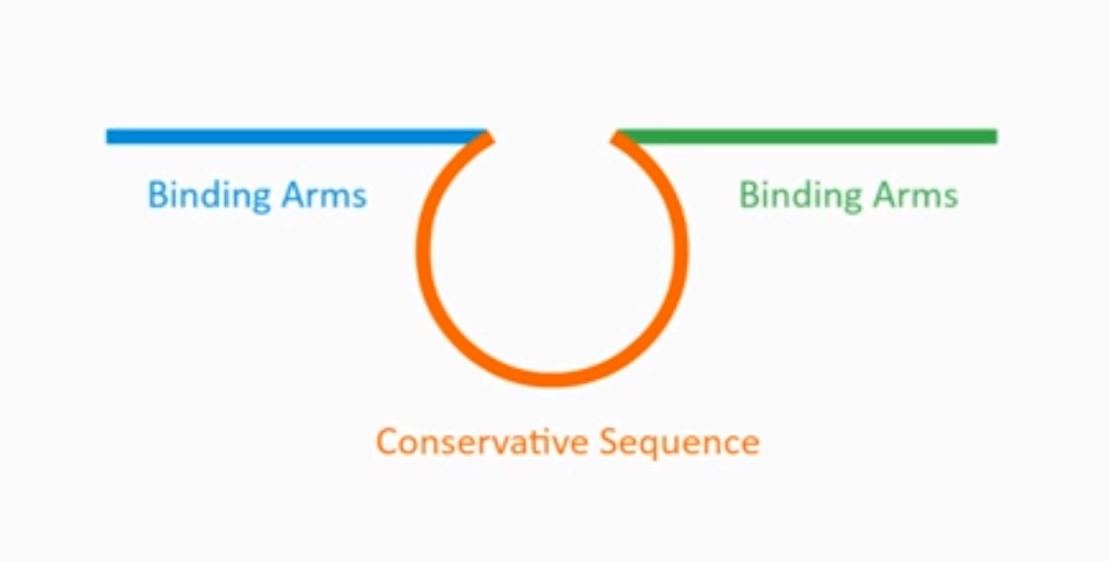


Caffeine Theophylline

Ref: [20]

DNAzymes

- Not all enzymes are proteins (1980s) -> Ribozymes
  -> **RNA World Hypothesis**
- Motivated to create Enzymes out of DNA
- Mechanism
  1. DNAzyme binds to target mRNA
  2. Catalytic domain becomes active
  3. DNAzyme-RNA complex dissociates

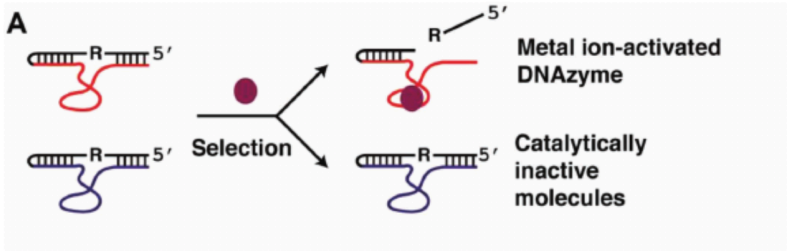


Ref: [6]

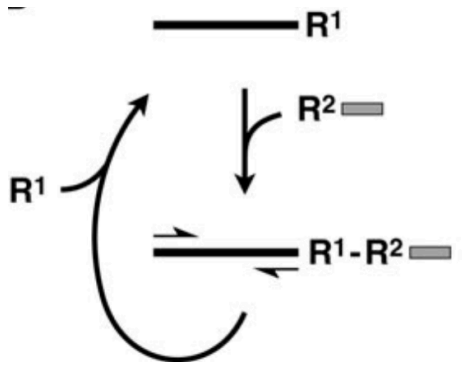
Example Selection for RNA-Cleaving DNAzyme

Enzyme catalizing bond formation between R1 and R2:

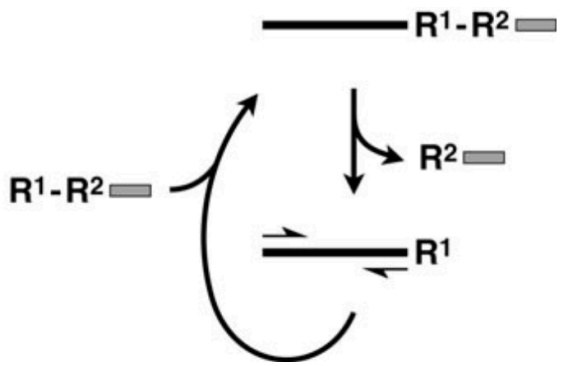
- R1 is attached to RNA
- R2 (with chemical tag) is added
- -> Tag attached to RNA
- Isolation of RNA (by Tag) and amplification (Tag is not amplified)



Bond-Forming DNAzyme

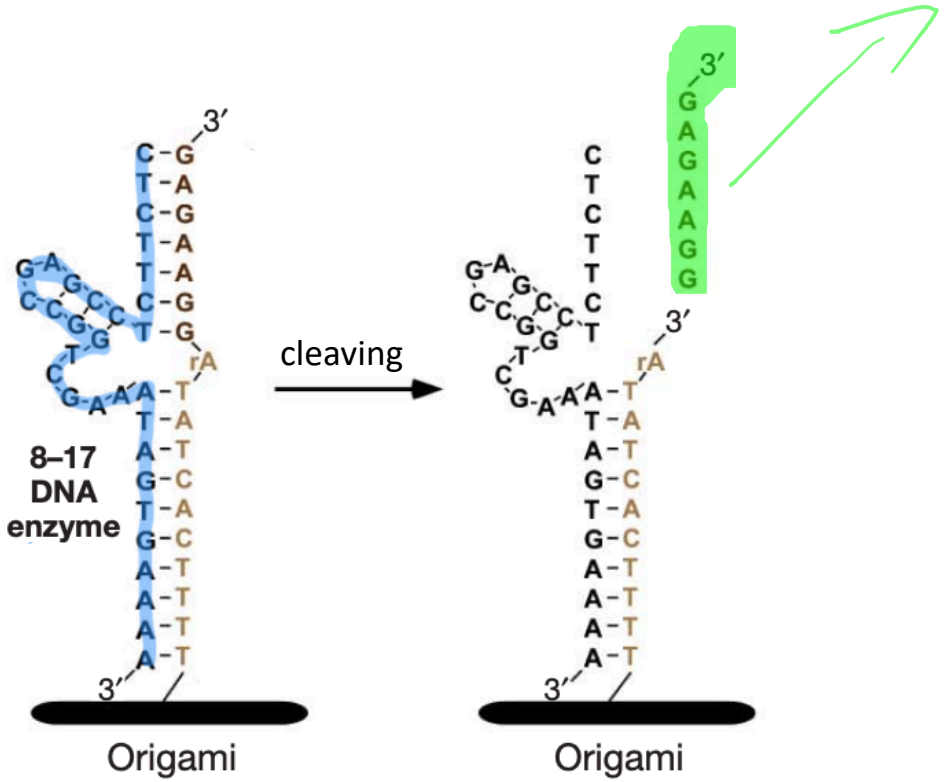


Bond-Breaking DNAzyme



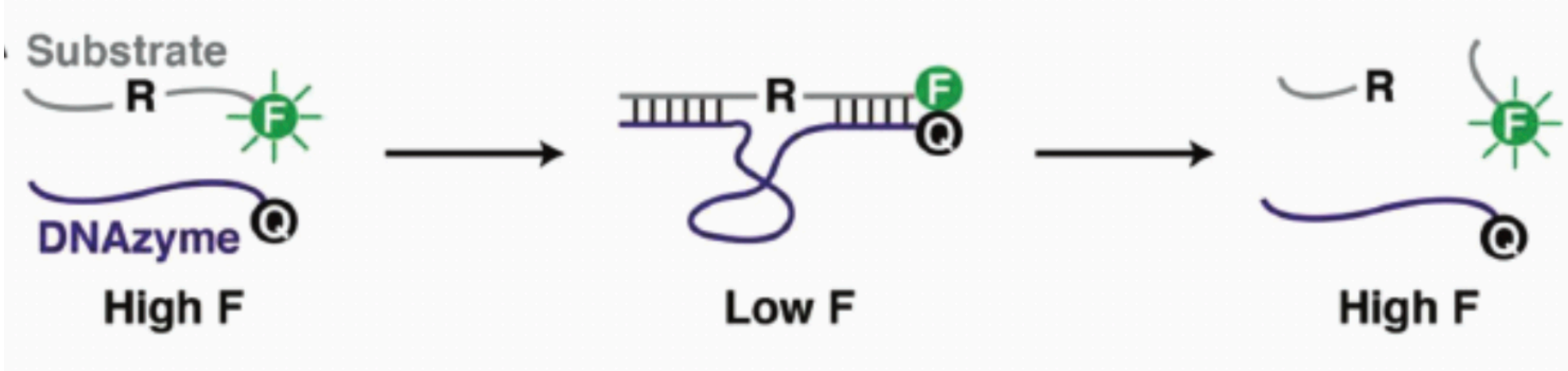
Ref: [3]
Ref: [10]

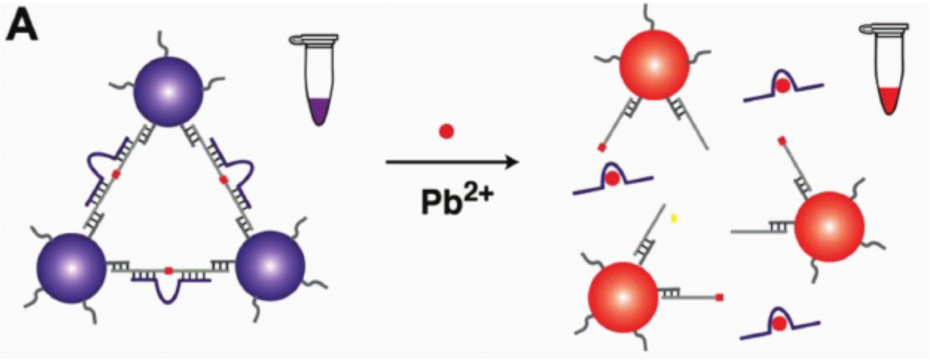
RNA-Cleaving DNAzyme



Ref: [4]

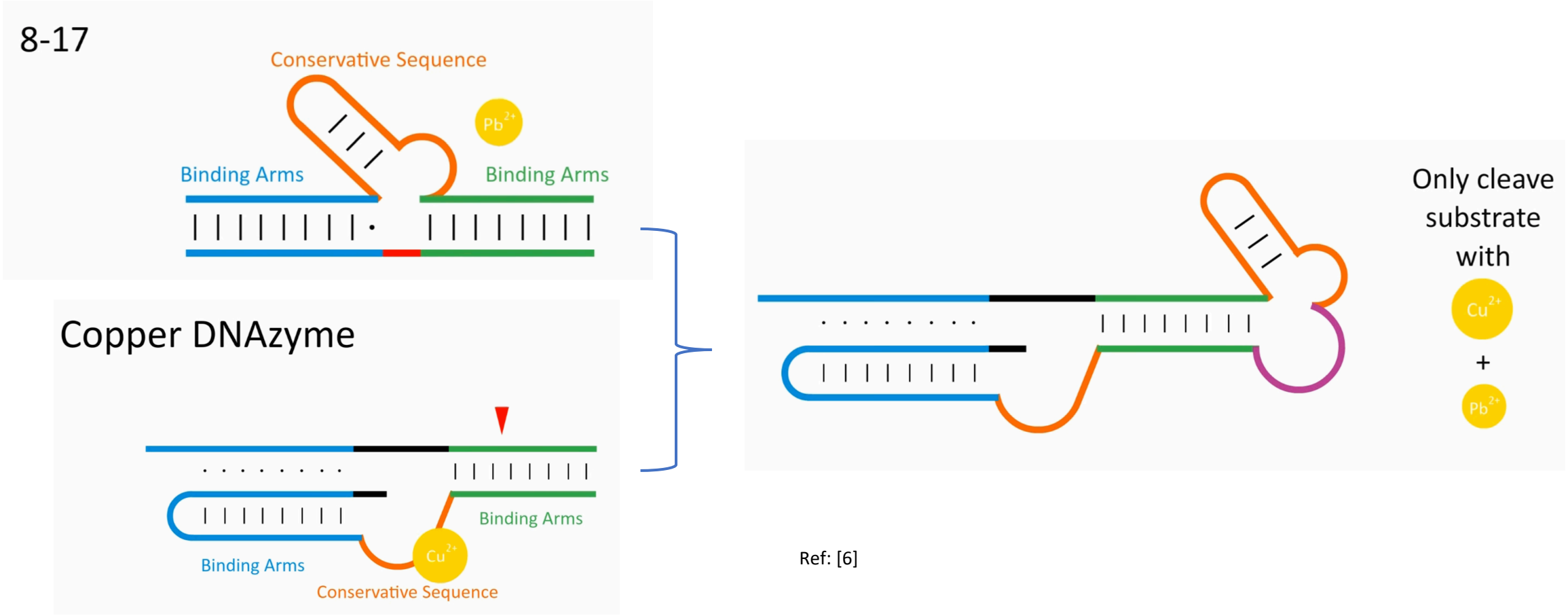
RNA-Cleaving DNAzyme as Biosensor





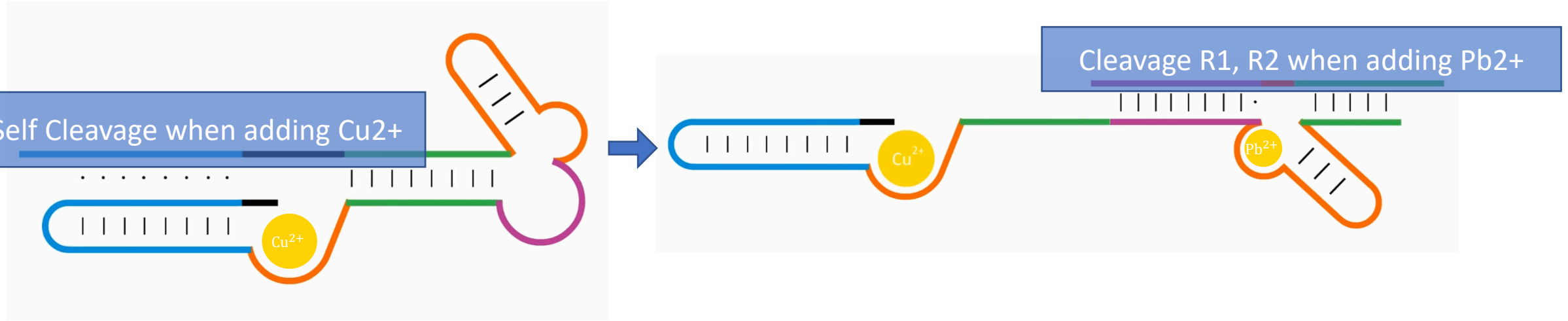
Ref: [10]

Logic AND-Gate with combined DNAzyme

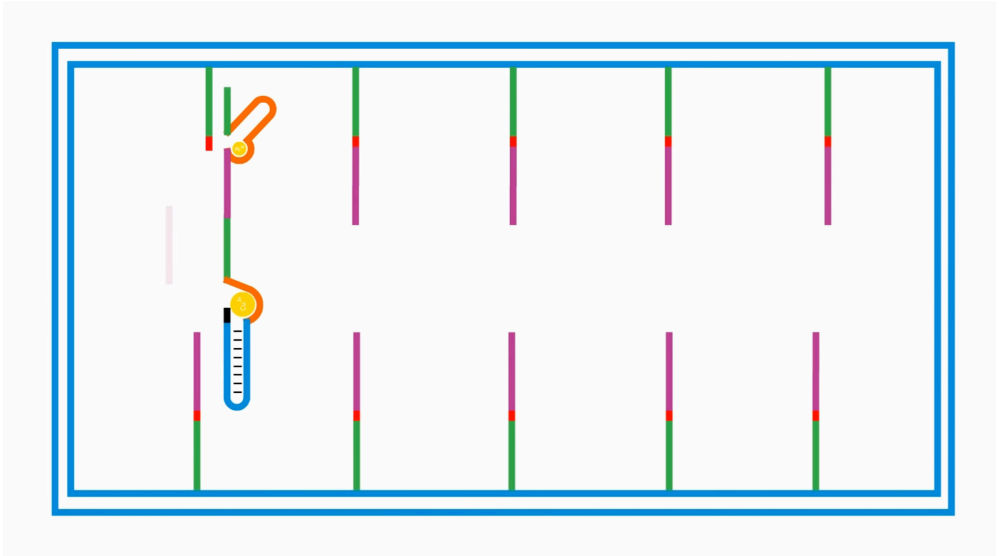


Ref: [6]

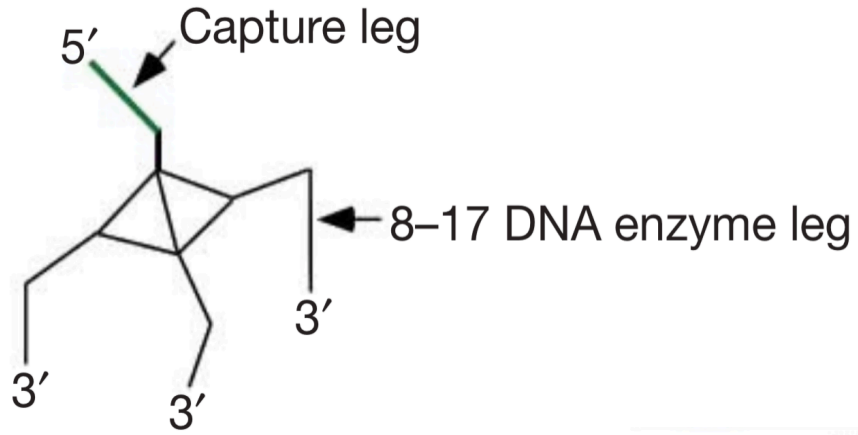
Logic AND-Gate with combined DNAzyme

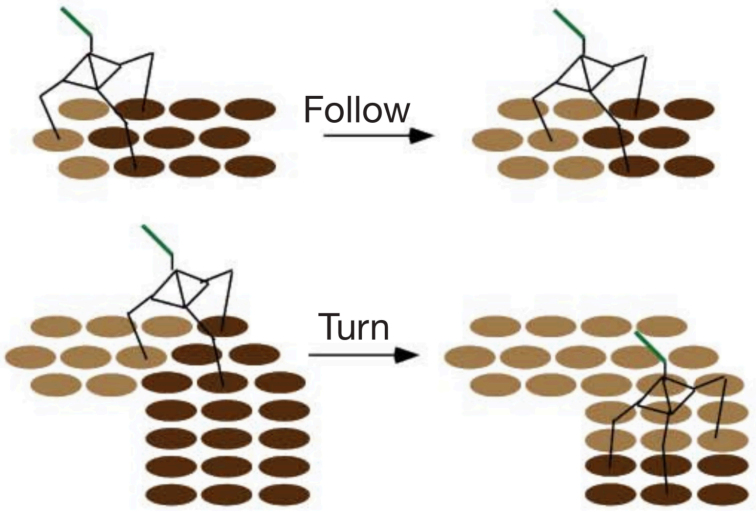


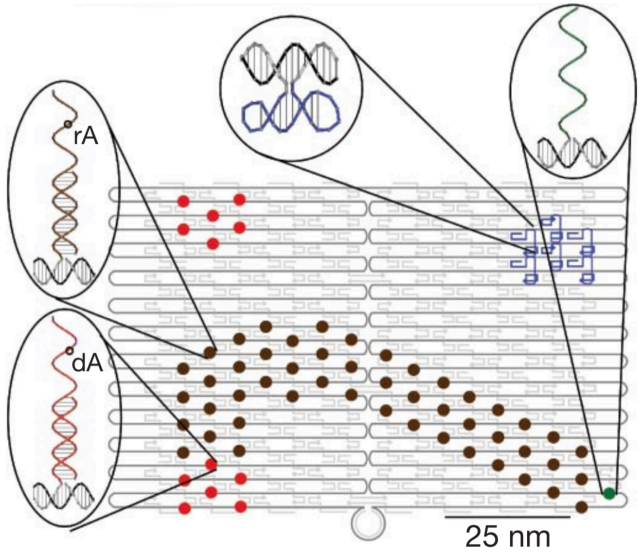
Ref: [6]

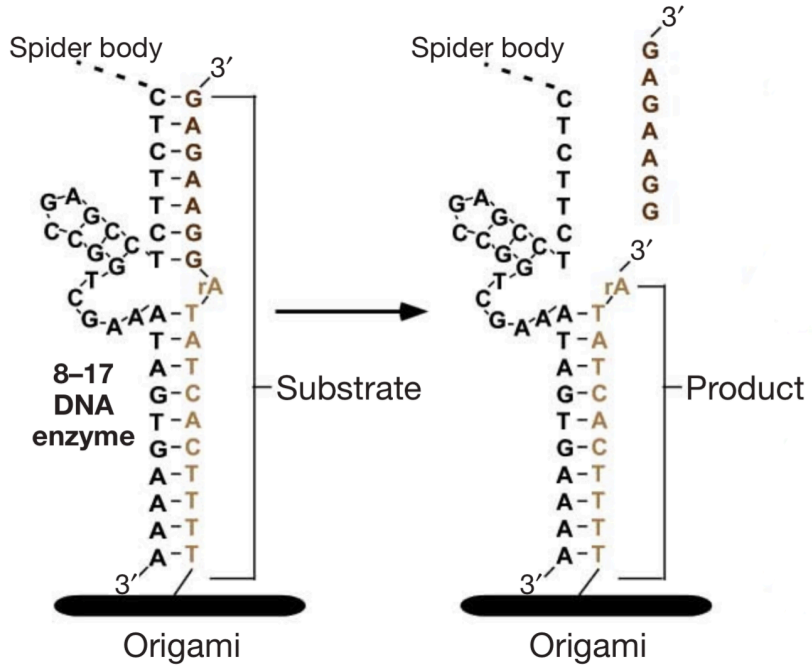


DNA-Spider with DNAzyme legs









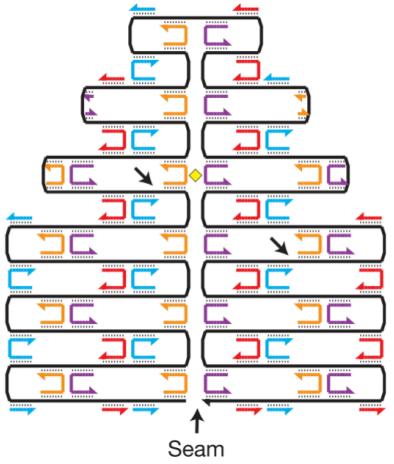
- DNAzyme leg binds to substrate
- Performs cleaving action
  -> cleaves substrate
  -> binding is now weaker
- DNAzyme is more likely to bind to neighboring, uncleaved substrate

Ref: [4]

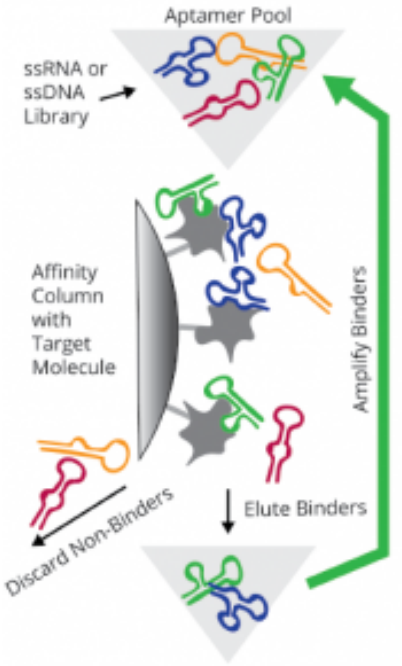
Summary

- DNA not only information storage, but also functional structure (DNA-Origami, Aptamer, DNAzyme)
- Aptamers are nanostructures, created using evolutionary mechanism (SELEX), that bind to specific targets
- SELEX is very improvable (chemistry/ expanding genetic alphabet) -> superior properties, enlarged target space
- Enzymes made of DNA/RNA
  -> RNA World Hypothesis
- Biosensors, Logic and Spiders made of DNAzymes

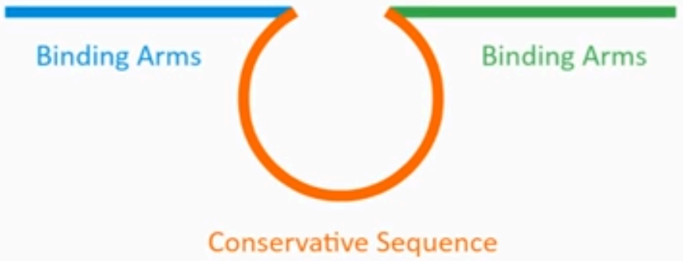
DNA-Origami



Aptamers via SELEX



DNAzyme



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