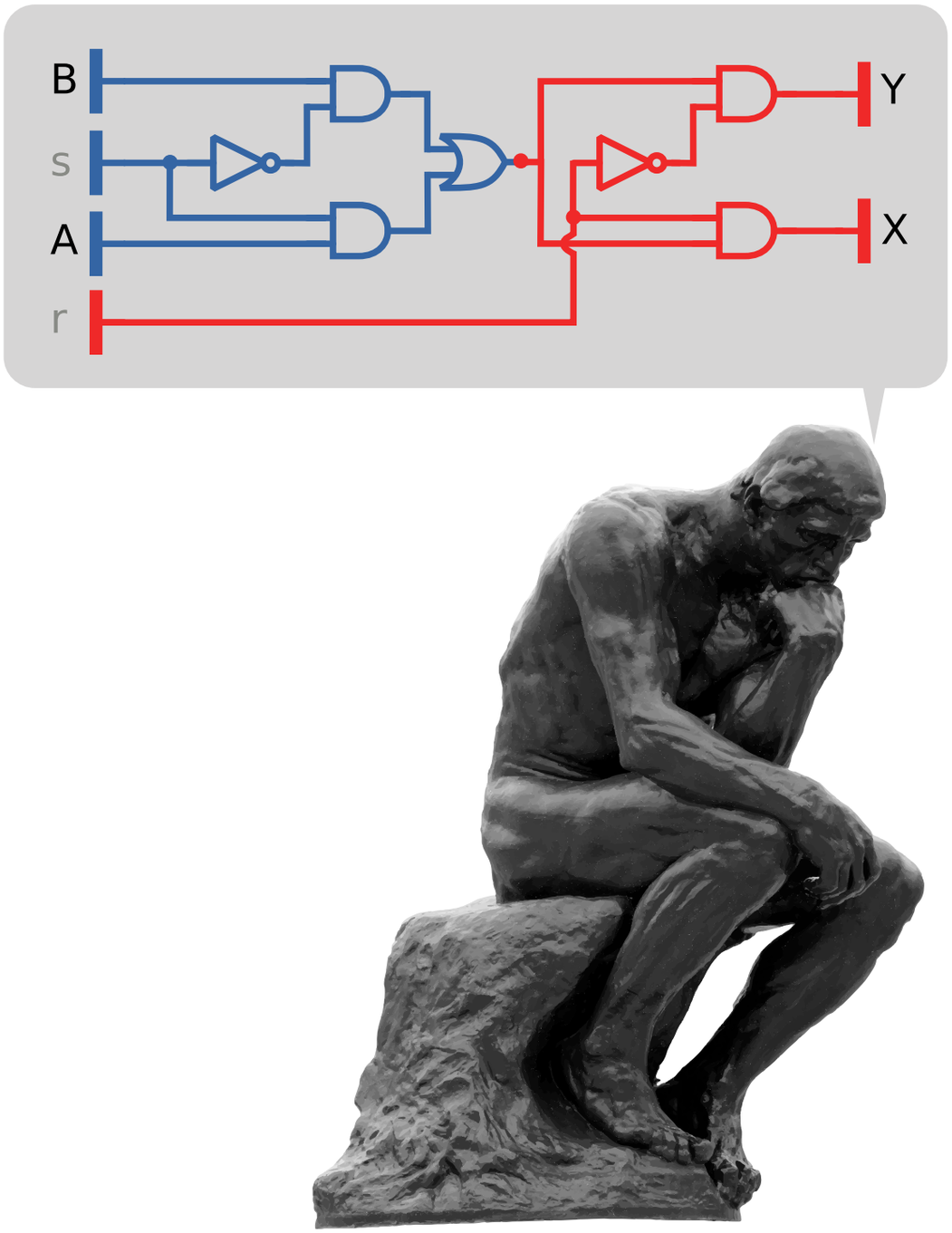
Engineering dynamics

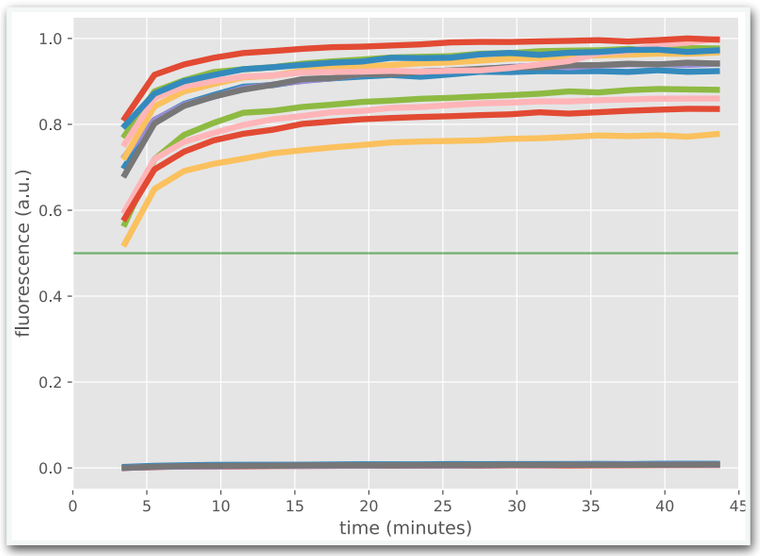
Theory & *Practice* of DNA strand displacement circuits

October 8, 2018 @ DNA 24

Chris Thachuk
Winfree Lab, California Institute of Technology

Today's tutorial in a nutshell





Molecular Circuits

Built upon DNA strand displacement cascades

Input	Computation	Output
5’–ACCACGATCACATTAC–3’	DNA strand displacement cascades	5’–GAGCTACATCAC–3’
5’–GCAACATACAT–3’		
5’–CCCATACATCACCAG–3’		5’–TAAATCATGATCAG–3’
5’–TACCACATGAGCAGCA–3’		

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5’–ACCACGATCACATTAC–3’

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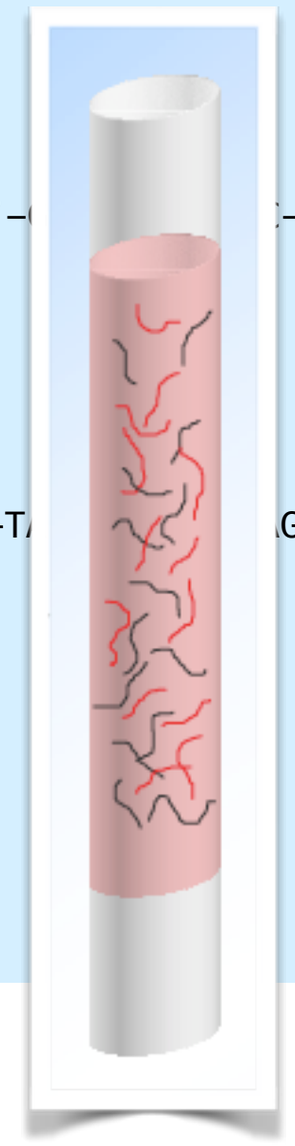
5’–TACCACATGAGCAGCA–3’

Computation

DNA strand displacement cascades

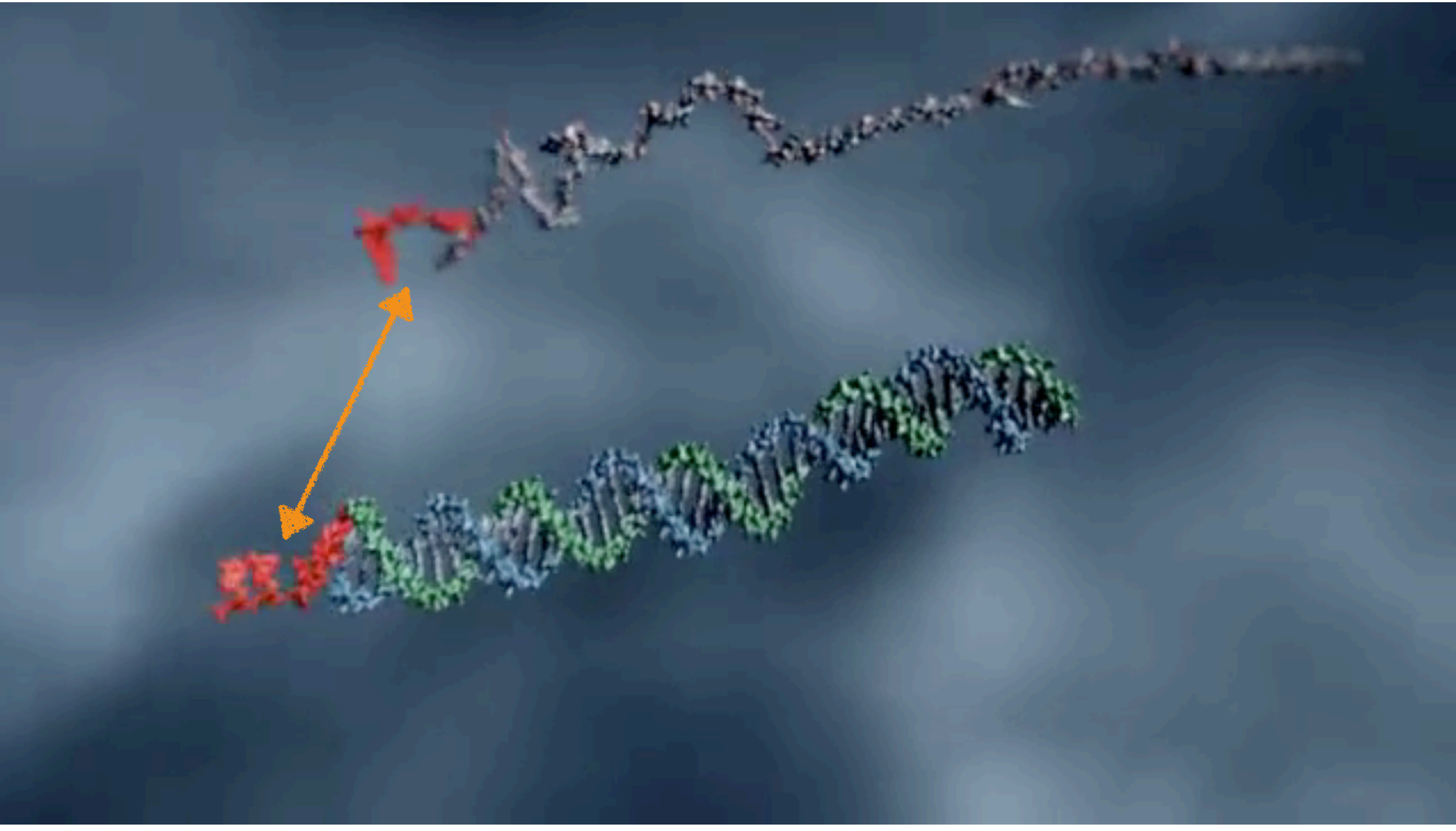
Output



Tutorial Outline

- **Review of strand displacement**
- Building and composing logic gates
- Tools for designing and verifying circuits
- Robustness of strand displacement

Review of DNA Strand Displacement (DSD)



B. Yurke, A. J. Turberfield, A. P. Mills Jr., F. C. Simmel, J. L. Neumann, Nature 406, 605 (2000).
A. J. Turberfield et al., Phys. Rev. Lett. 90, 118102

Video Courtesy of Biocomputation Group @ Microsoft Research

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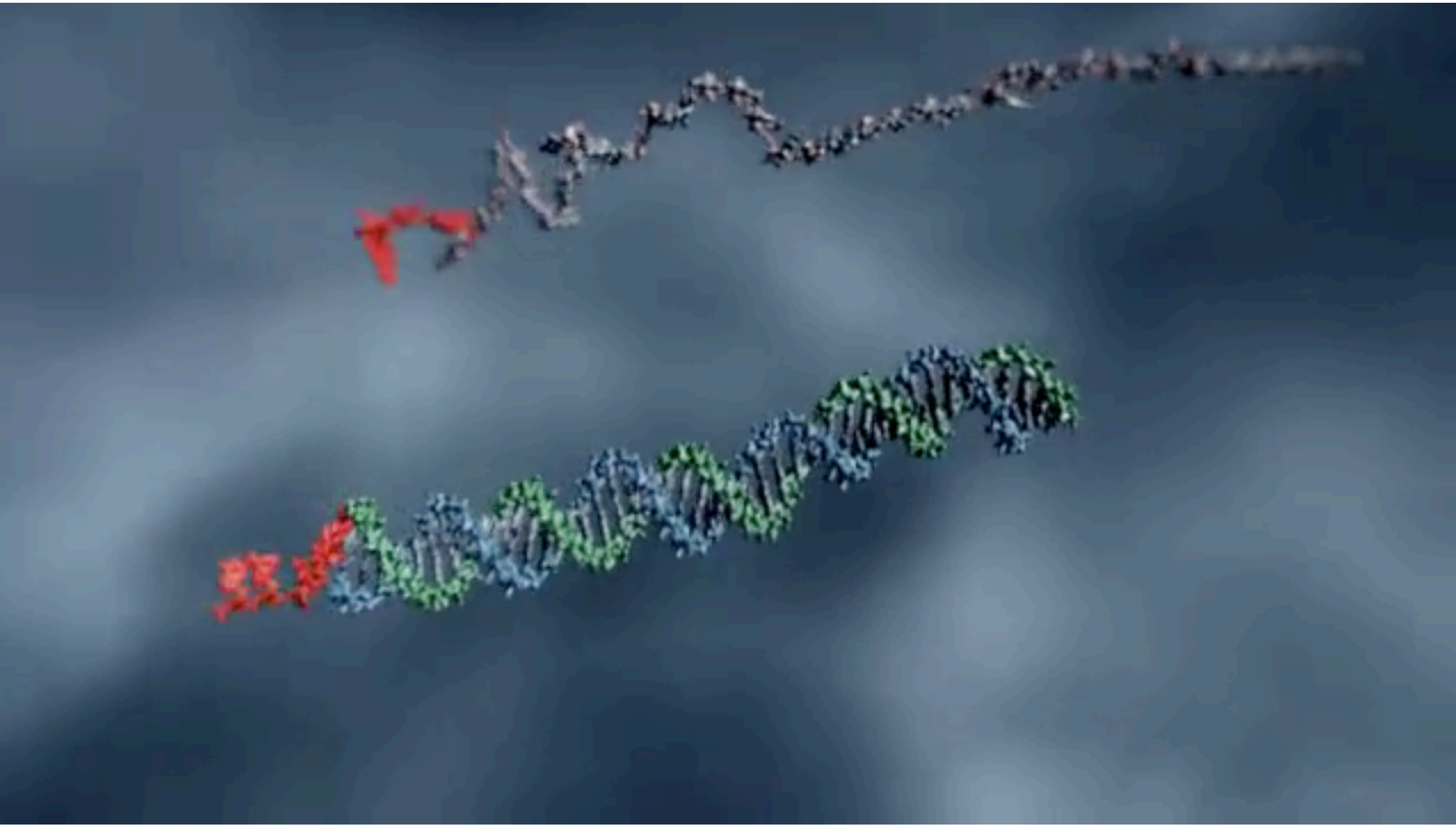

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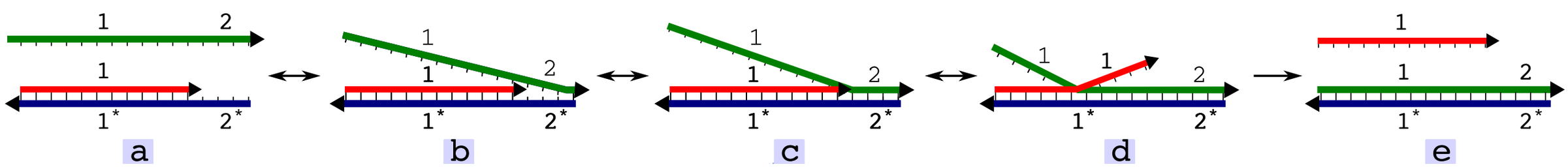
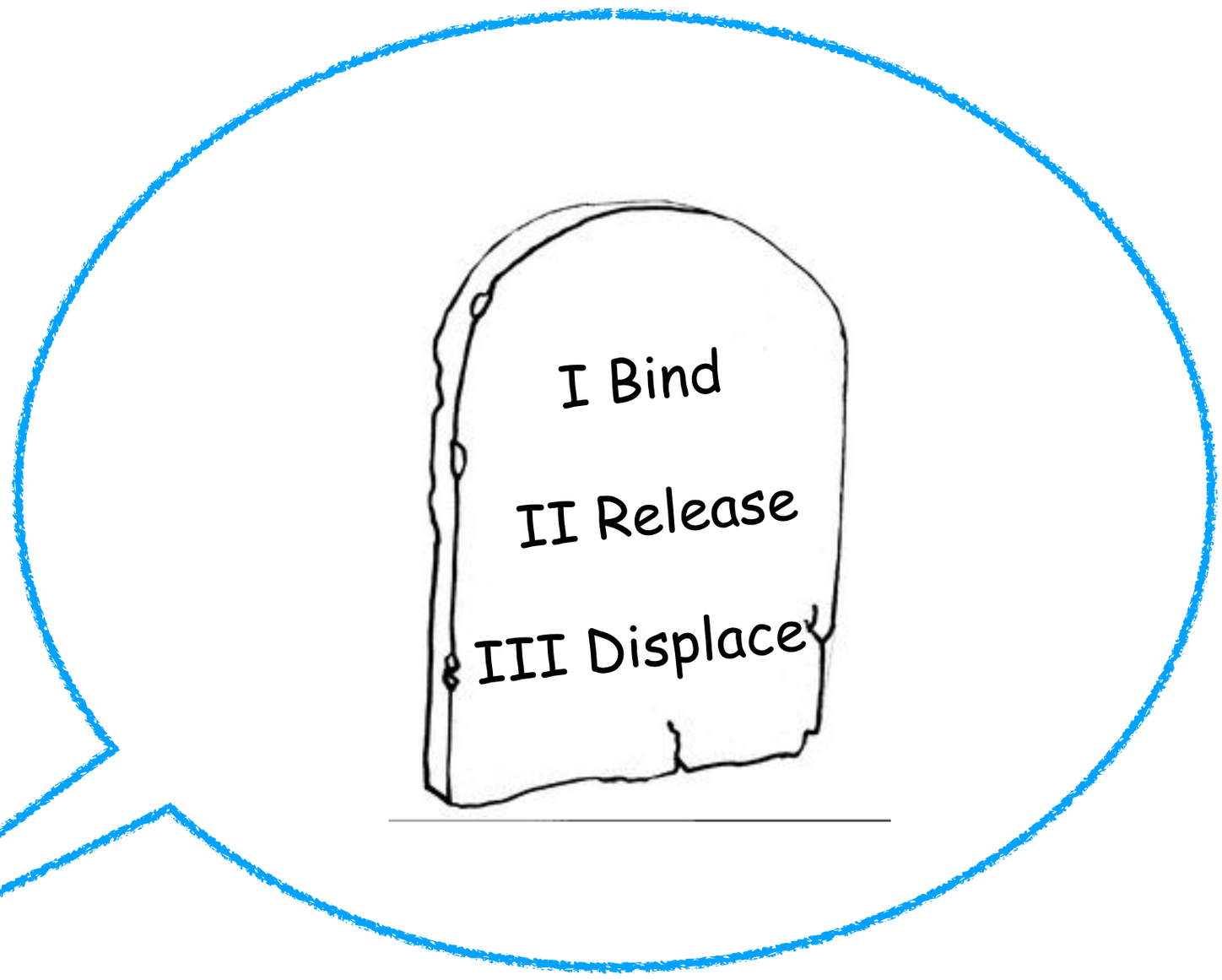


Adapted from Zhang & Seelig 2011

Strand Displacement Cascades

=

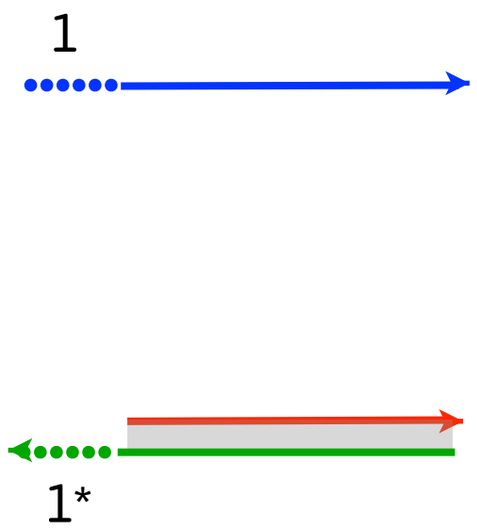
Three Rules



Domain level rules for DSD

Rule 1: ***Bind***

Example



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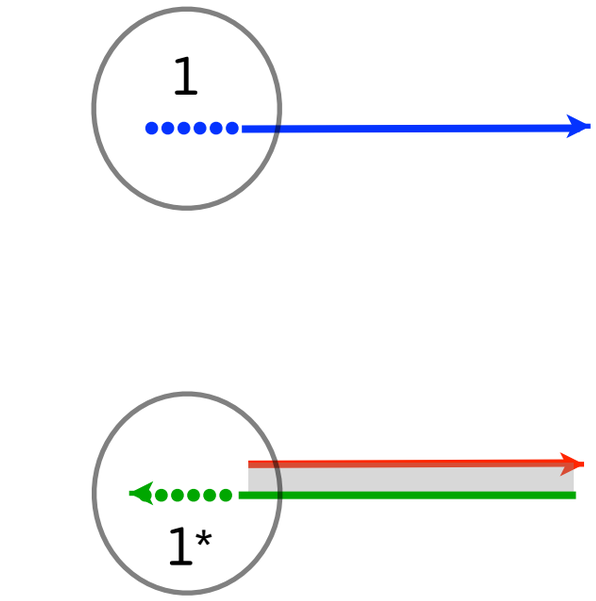

single-stranded
complementary
domains

Domain level rules for DSD

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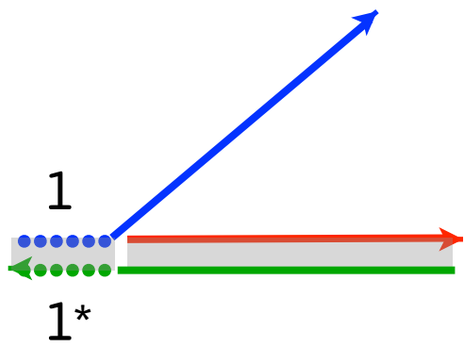
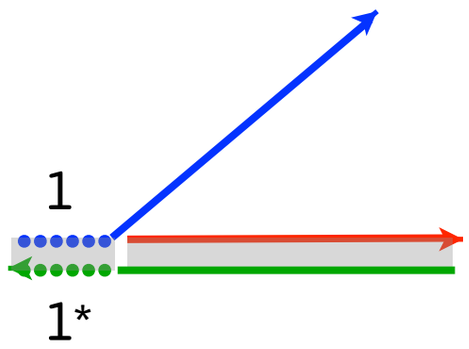


Domain level rules for DSD

Rule 1: ***Bind***

Two single-stranded complementary domains can bind

Example



Domain level rules for DSD

Rule 2: ***Release***

Example

Domain level rules for DSD

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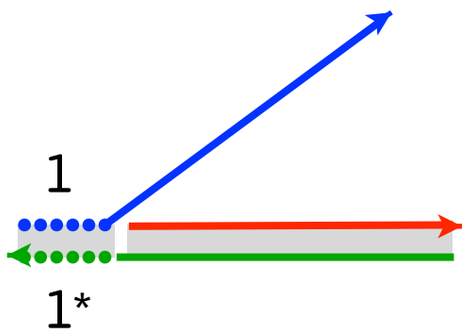
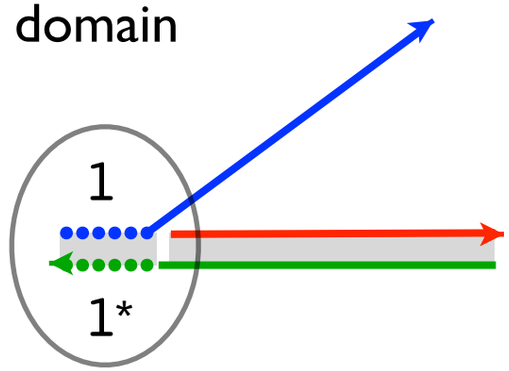


Domain level rules for DSD

Rule 2: ***Release***

Example

blue strand bound by only
a short domain



Domain level rules for DSD

Rule 2: ***Release***

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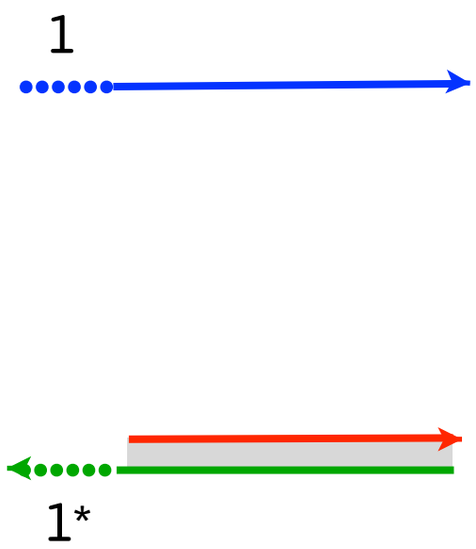
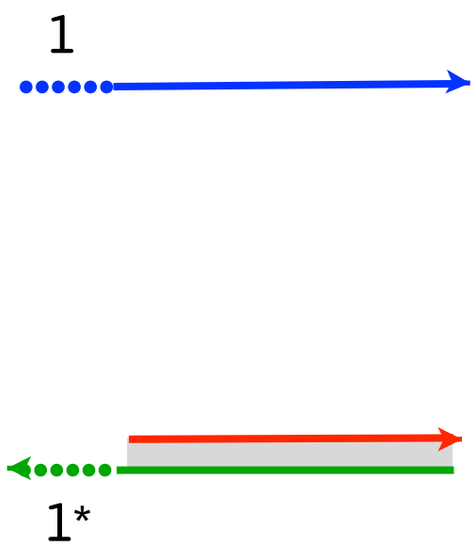


Domain level rules for DSD

Rule 2: ***Release***

Any strand bound by only a short domain can **release**

Example



Domain level rules for DSD

Rule 3: ***Displace***

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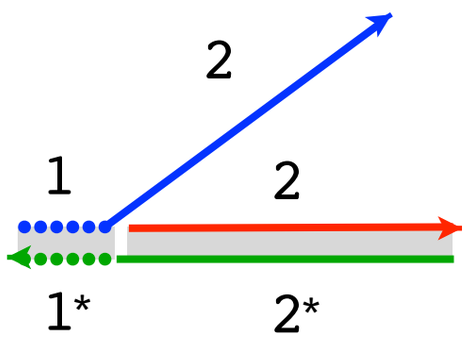

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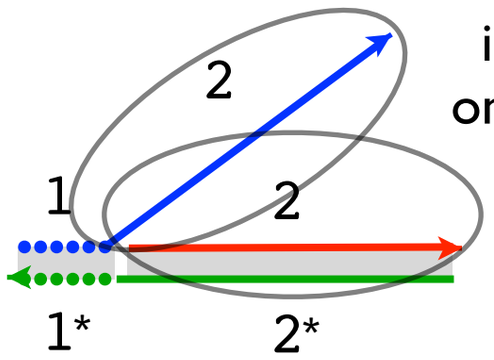

identical domains,
one bound, one free

Domain level rules for DSD

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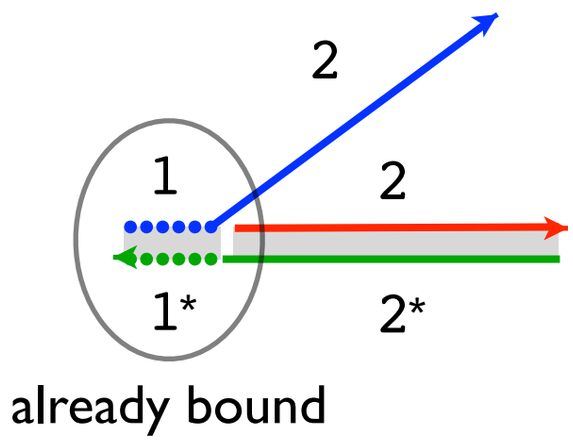

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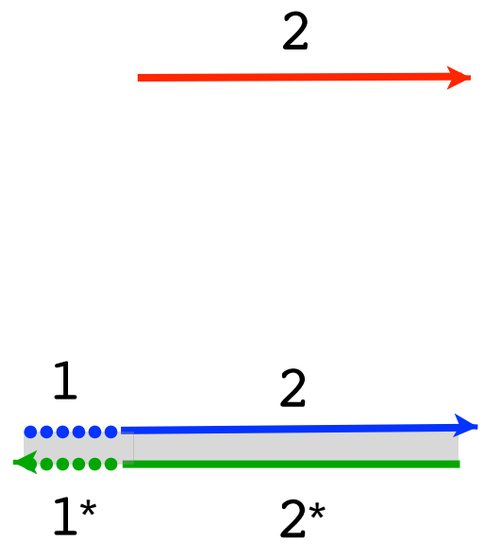
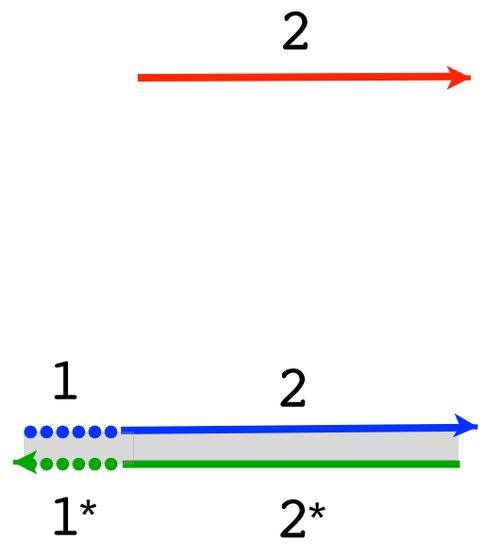


Domain level rules for DSD

Rule 3: ***Displace***

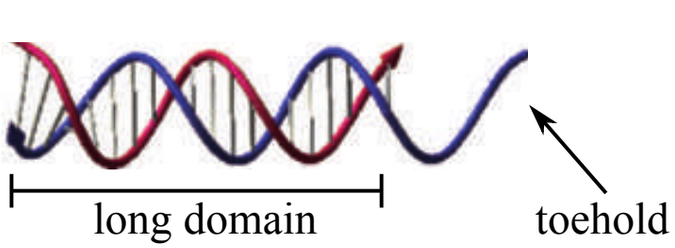
A domain can **displace** an identical domain of another strand,
if neighboring domains are already bound

Example



Why do we use toeholds?

Adapted from Zhang & Seelig 2011



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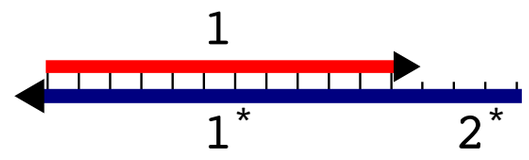

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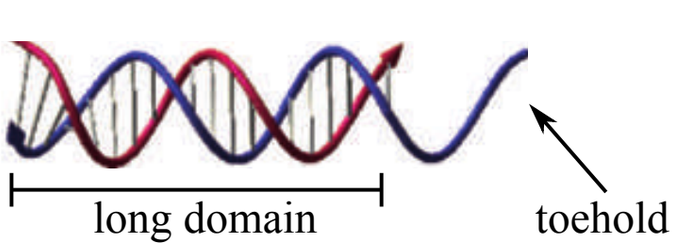

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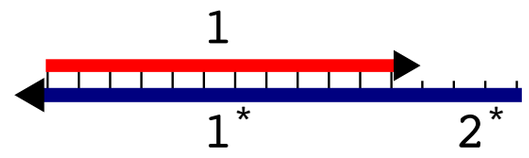
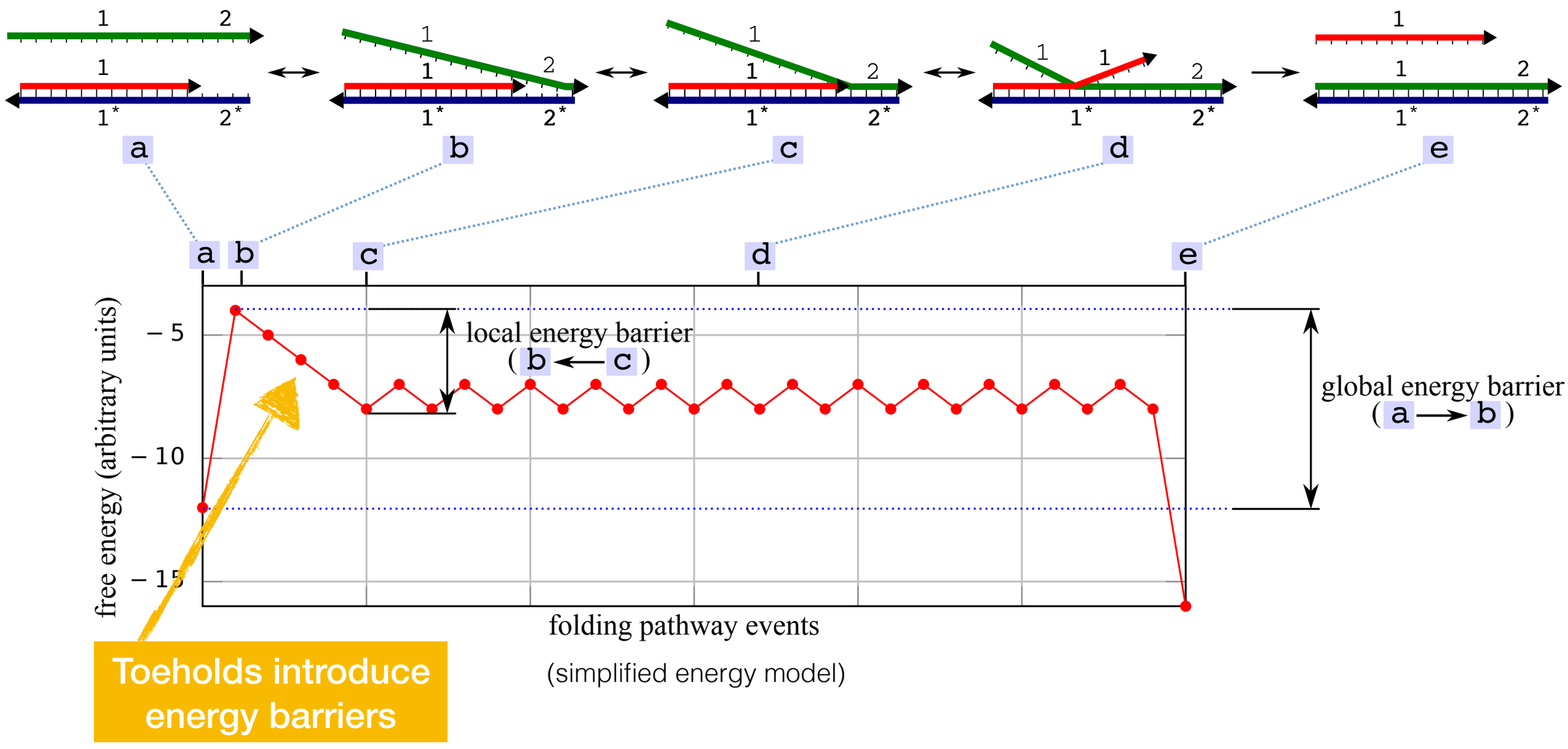


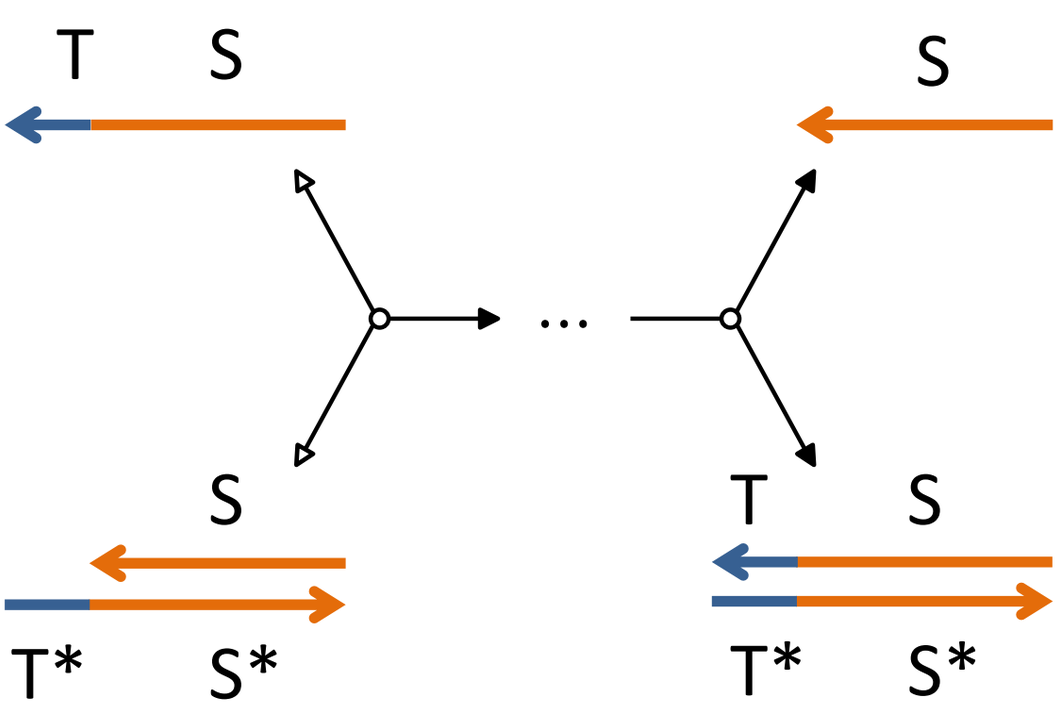


Toeholds introduce energy barriers

Toehold-mediated DNA strand displacement

T: toehold domain (typically 3-7 nucleotides)
S: branch migration domain (typically 15-20 nucleotides)

$$A + B \overset{k}{\rightarrow} C + D$$



The rate of strand displacement grows exponentially with toehold length for short toeholds.

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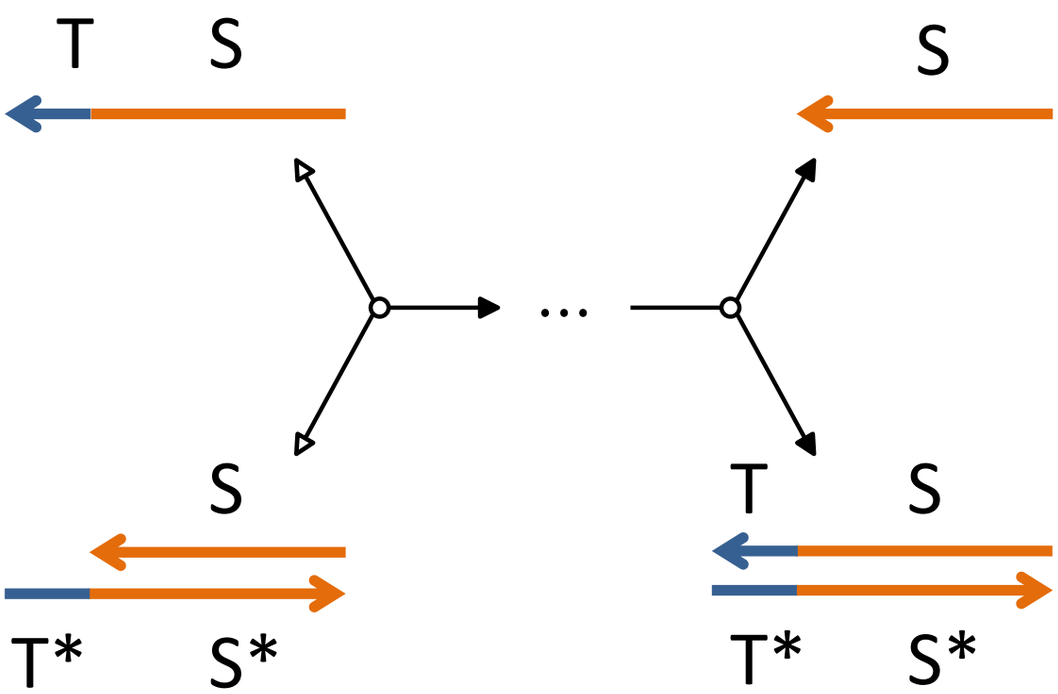

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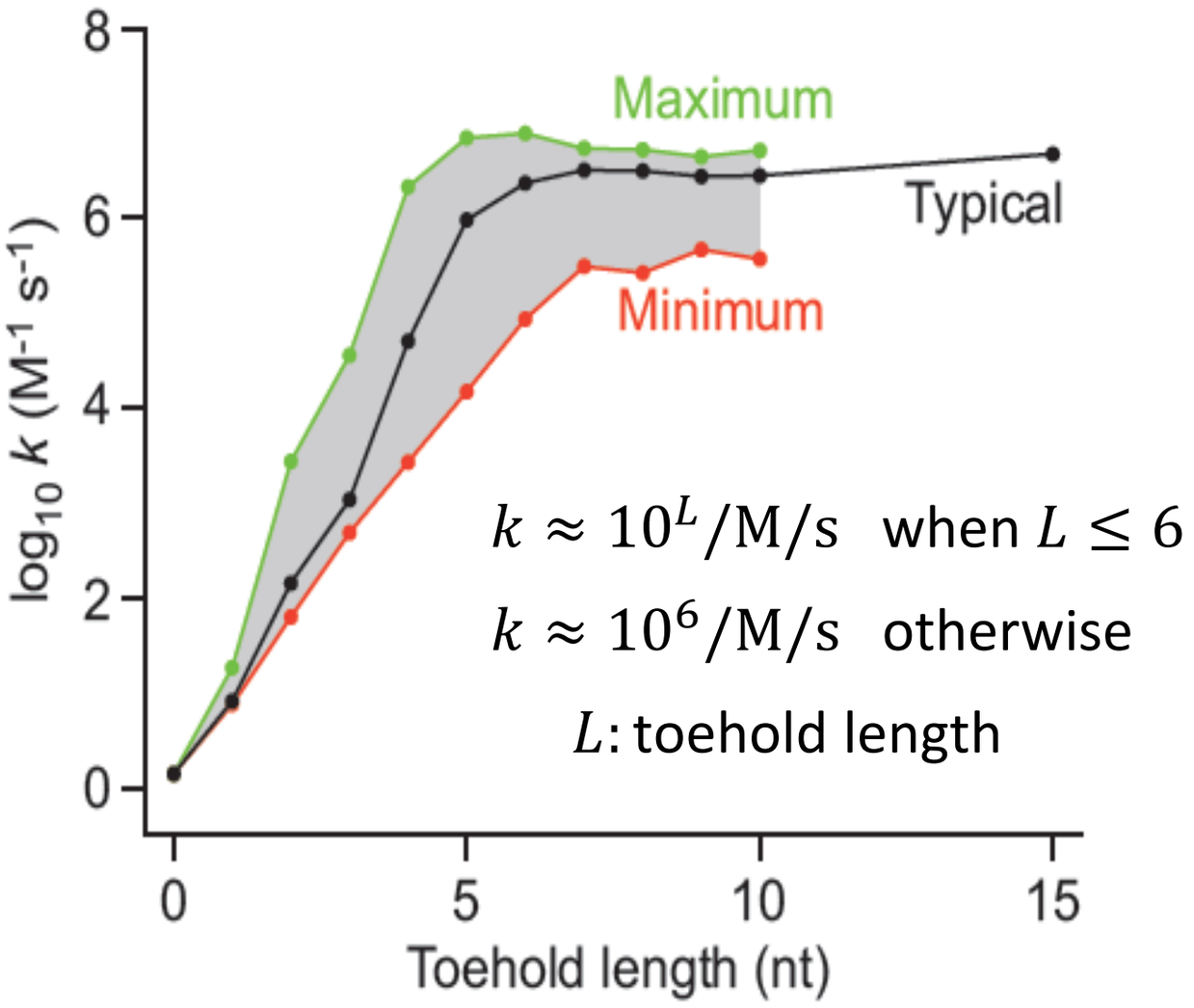

Zhang and Seelig, *Nature Chemistry* 2011

Kinetics of toehold-mediated strand displacement

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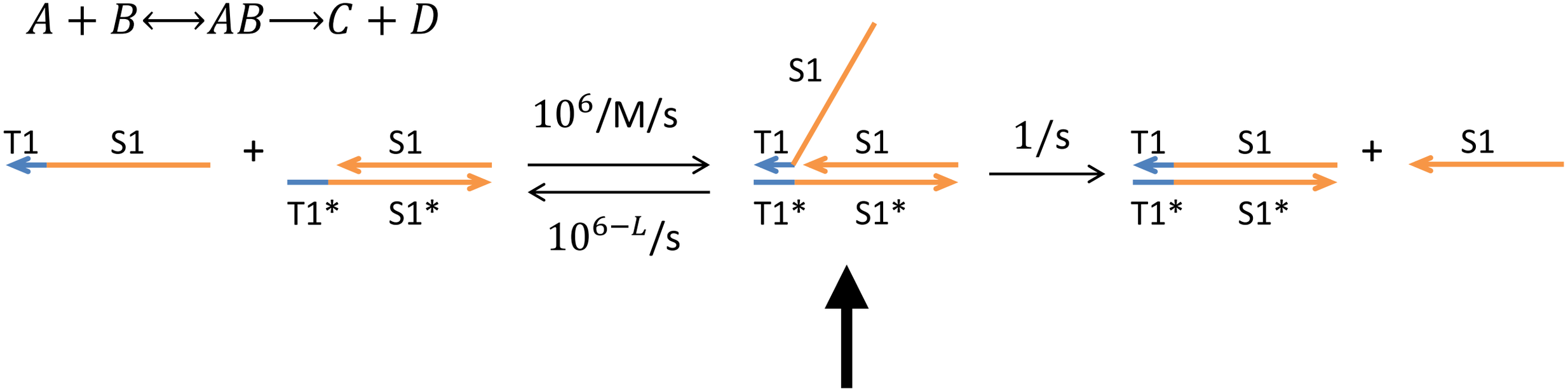

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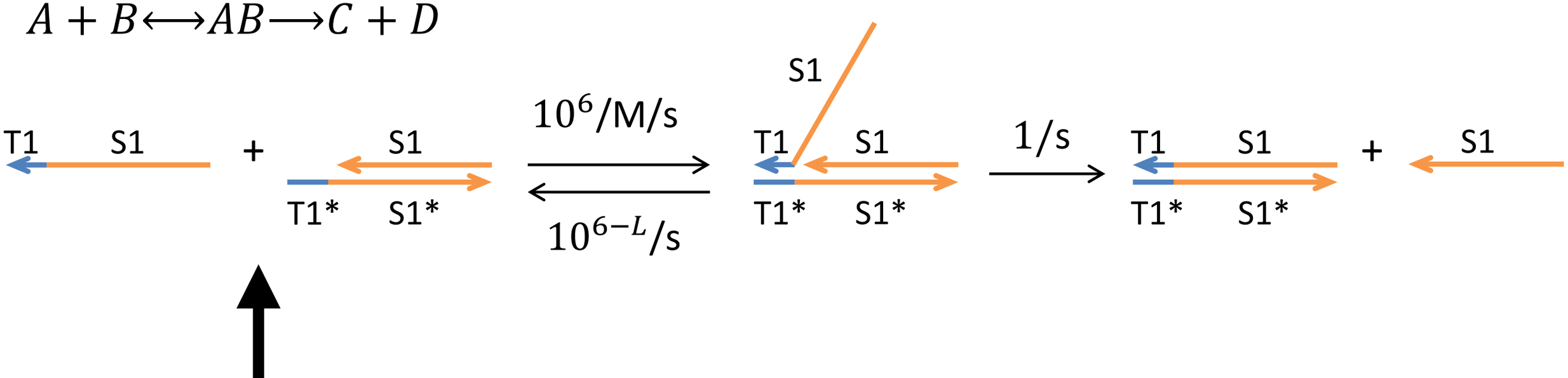

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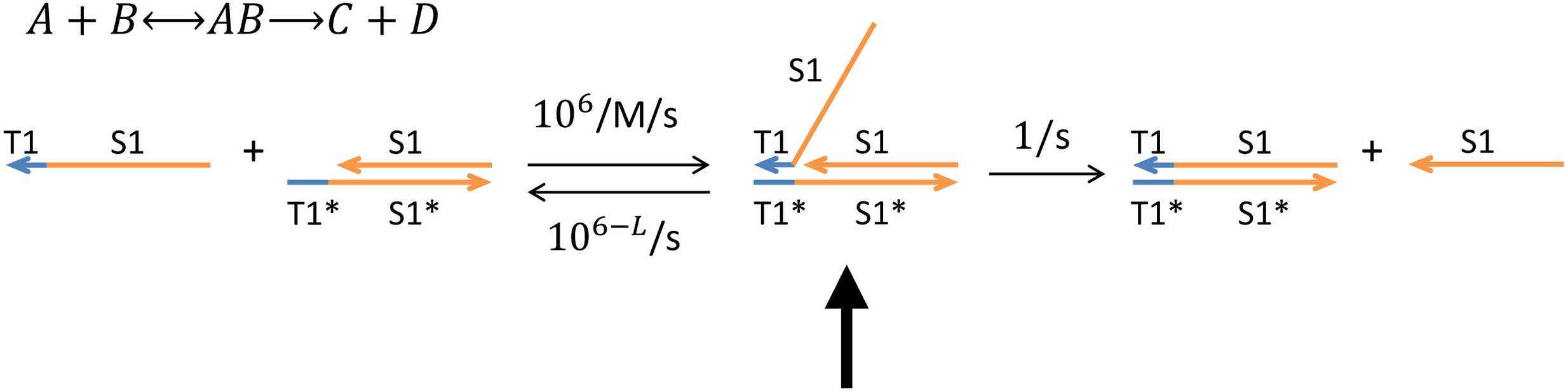

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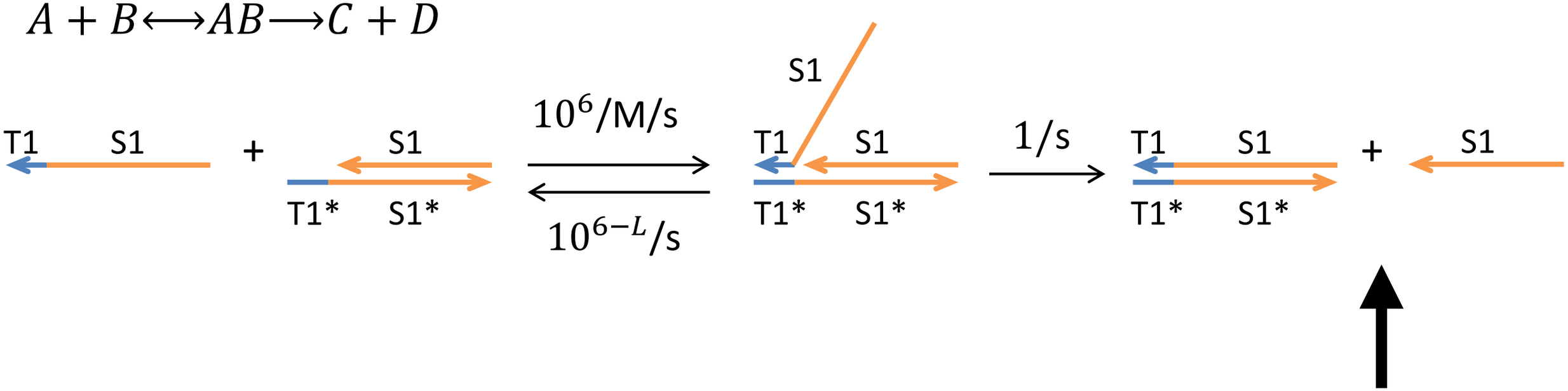

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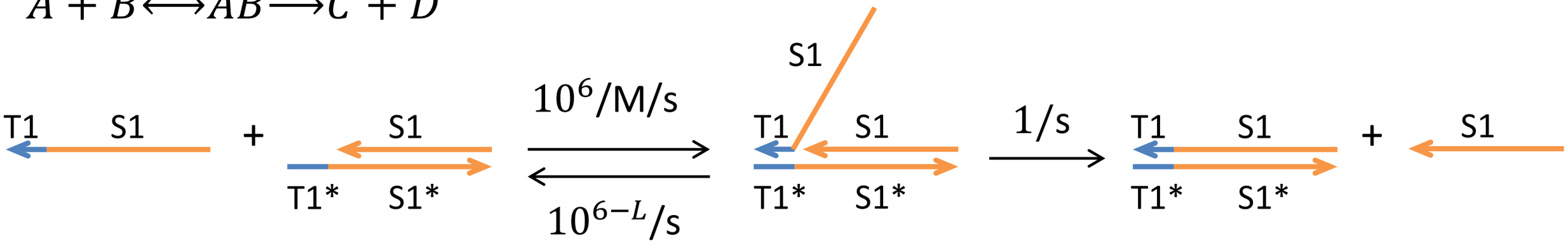

simplify: $A + B \xrightarrow{k_{eff}} C + D$ $\quad k_{eff} = ?$

collision rate: $10^6[A][B]$

collision success probability: $\dfrac{1/\text{s}}{1/\text{s} + 10^{6-L}/\text{s}}$

net rate of success: $\underbrace{10^6 \cdot \dfrac{1}{1 + 10^{6-L}}}_{k_{eff}}[A][B]$

$k_{eff} \approx 10^L$/M/s when $L \leq 6$

otherwise $k_{eff} \approx 10^6$/M/s

L: toehold length |T1|

Zhang et al, *JACS* 2009

Srinivas et al, *NAR* 2013

This approximation is valid for low concentrations of A and B (e.g. [A]=[B]=100nM) such that the unimolecular reaction is sufficiently faster than the bimolecular reaction.

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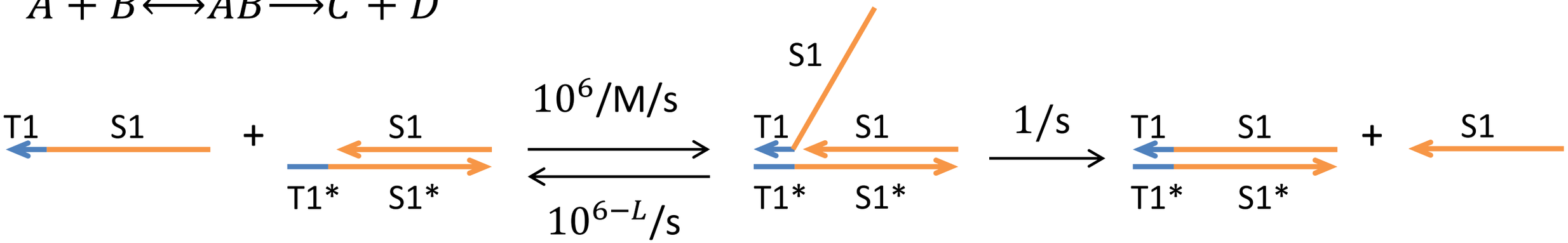

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Strand displacement in the lab

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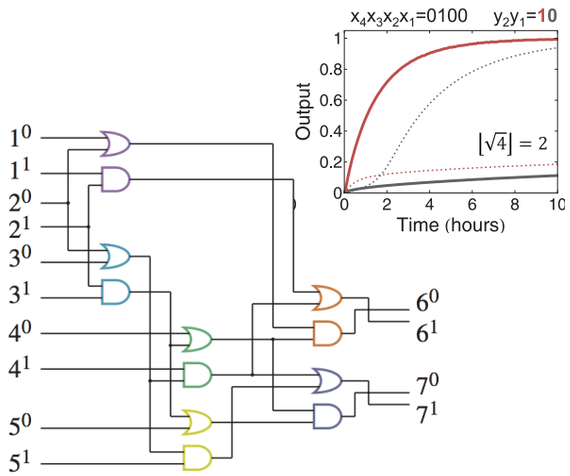
molecular
logic circuits



- Large autonomous biochemical networks built from scratch

  Qian, Winfree, *Science* 2011

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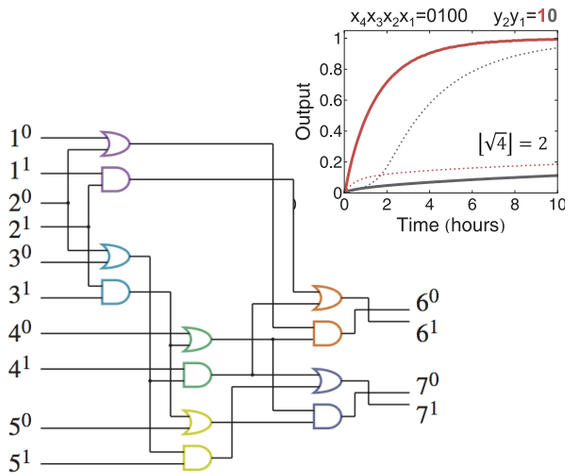

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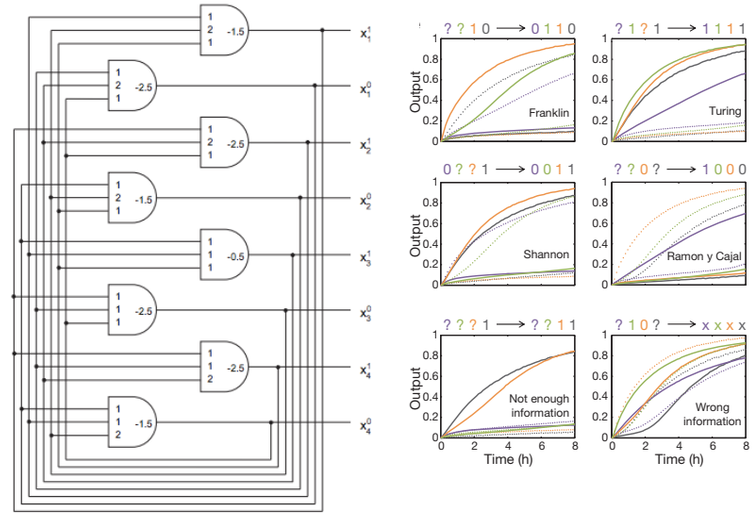
strand displacement cascades

molecular artificial neural networks

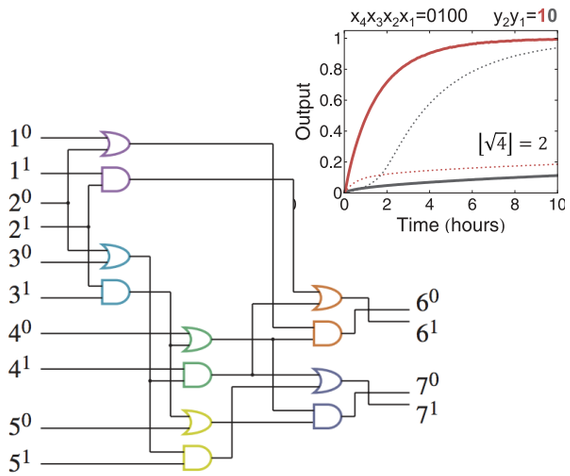


- Biochemical system doing inference

Qian, Winfree, Bruck *Nature* 2011

Strand displacement in the lab

molecular logic circuits



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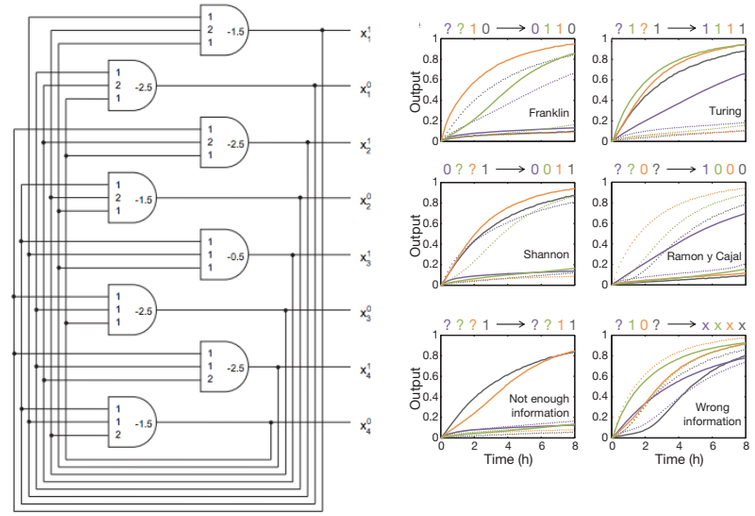

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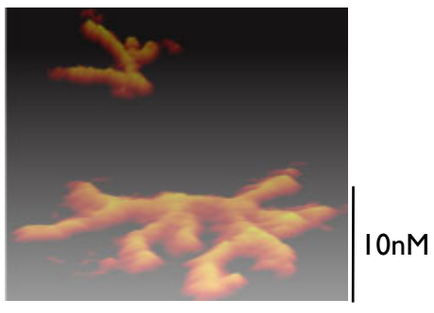
strand displacement cascades

controlling assembly of nanoscale structures

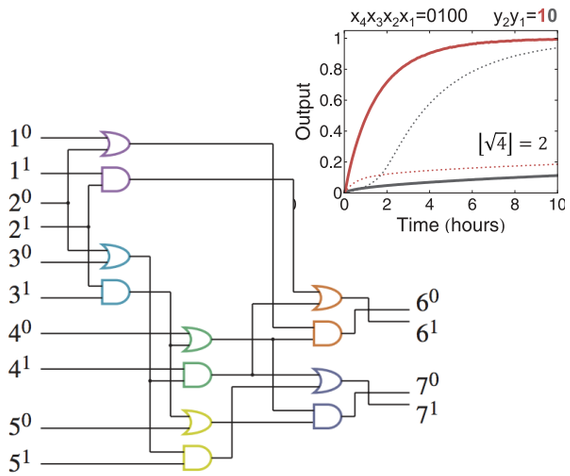


- Prescribed nanoscale structures seen under atomic force microscope

Yin, Choi, Calvert, Yurke, Pierce *Nature* 2008

Strand displacement in the lab

molecular logic circuits



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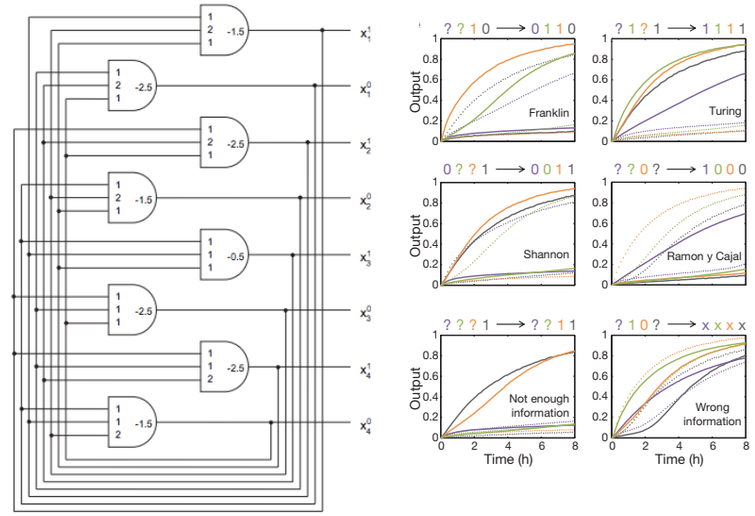

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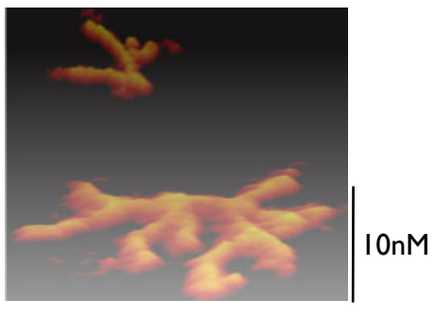

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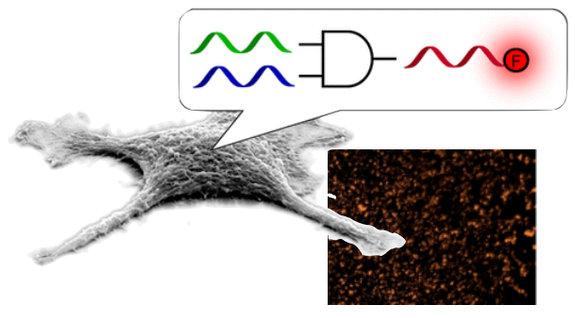
strand displacement in vivo



- Logic on biological signals

Hemphill, Deiters *J Am Chem Soc* 2013

Strand displacement in the lab

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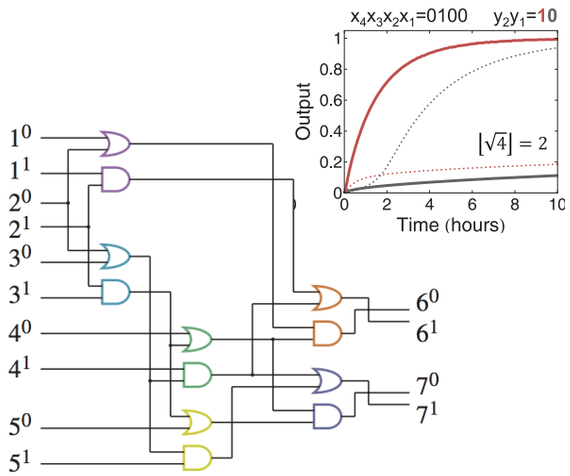

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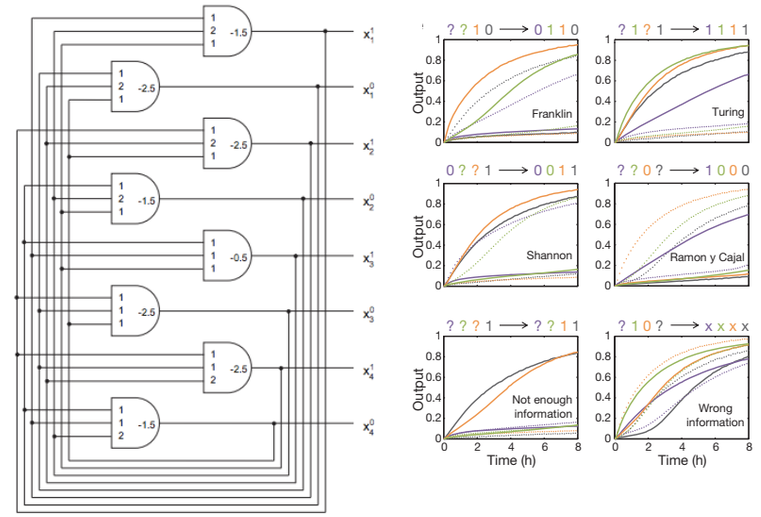
Qian, Winfree, *Science* 2011

molecular artificial neural networks

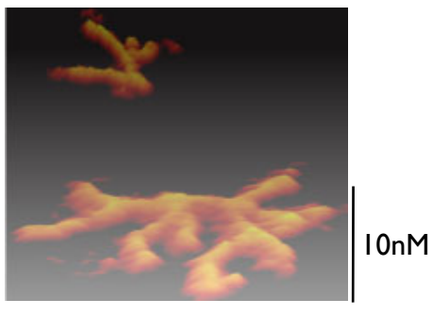


- Biochemical system doing inference

Qian, Winfree, Bruck *Nature* 2011

strand displacement cascades

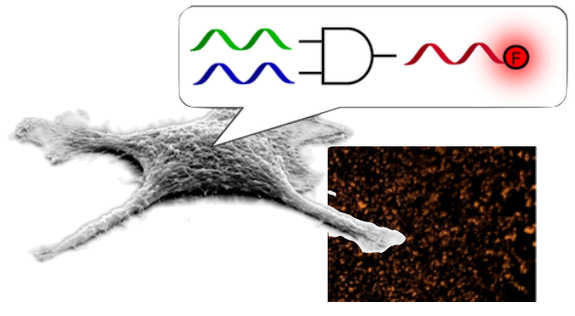
controlling assembly of nanoscale structures



- Prescribed nanoscale structures seen under atomic force microscope

Yin, Choi, Calvert, Yurke, Pierce *Nature* 2008

strand displacement in vivo



- Logic on biological signals

Hemphill, Deiters *J Am Chem Soc* 2013