Engineering dynamics

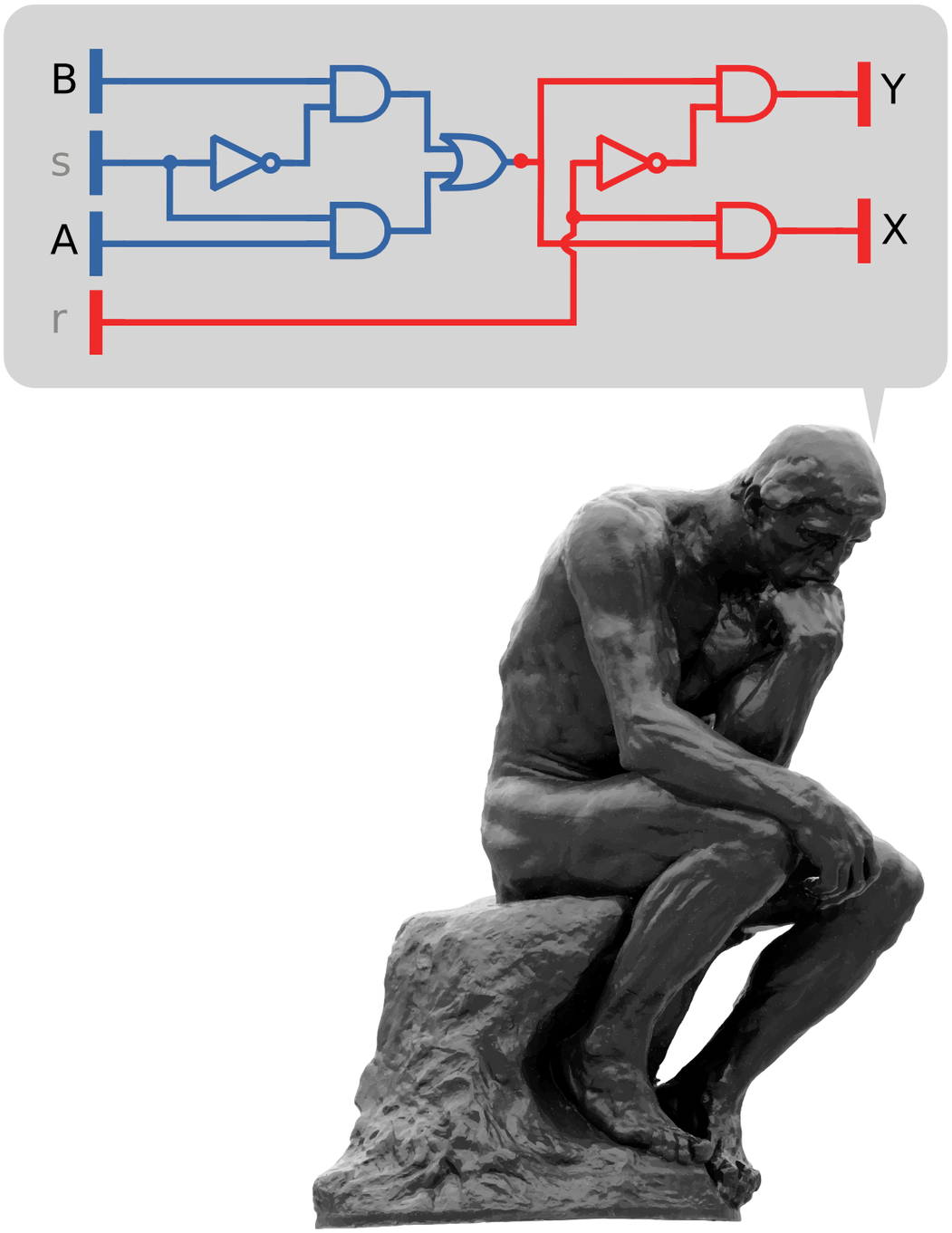
Theory & *Practice* of DNA strand displacement circuits

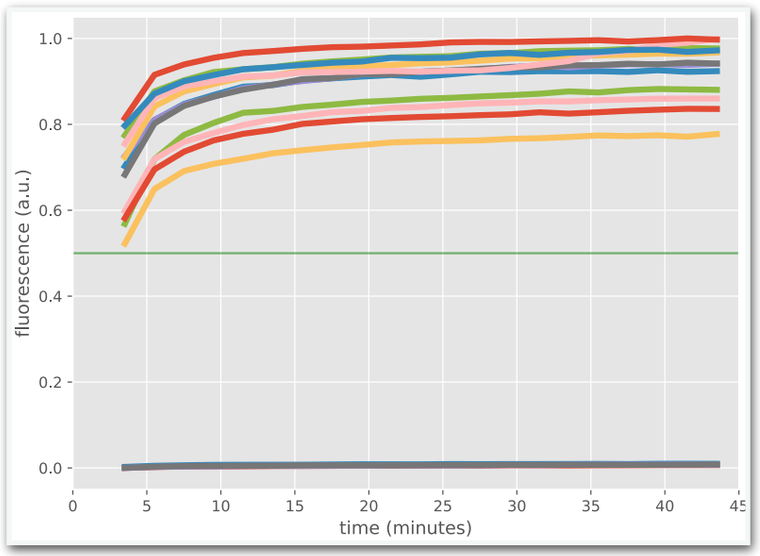
October 8, 2018 @ DNA 24

Chris Thachuk
Winfree Lab, California Institute of Technology

Molecular Programming Coat of Arms

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’
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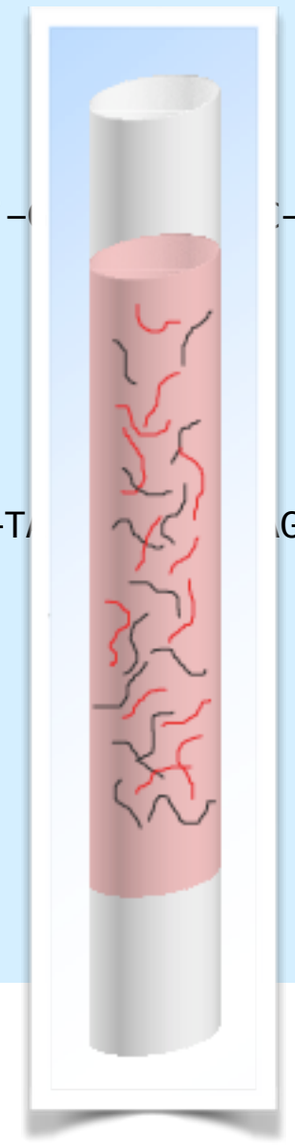
Computation

DNA strand displacement cascades

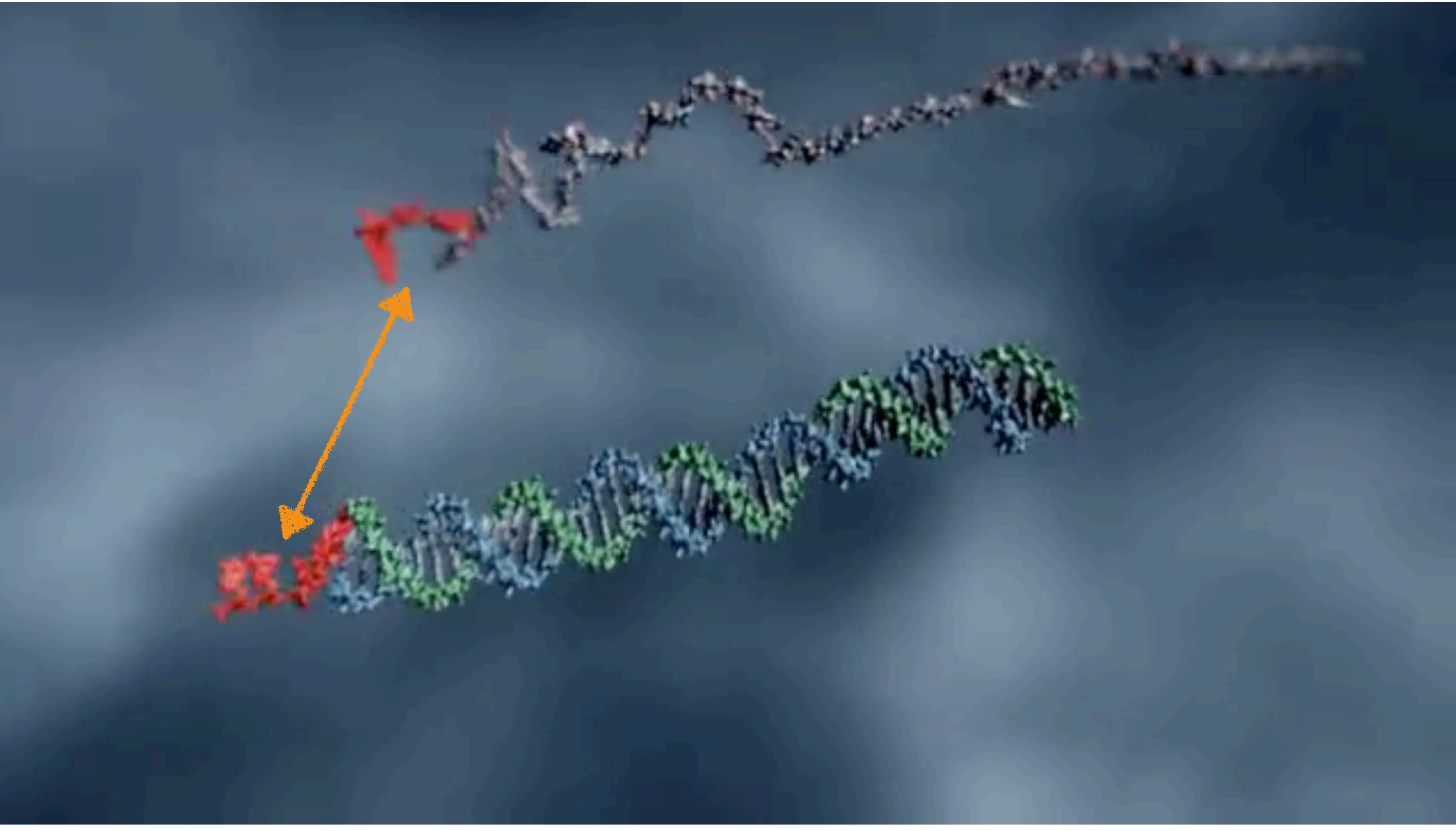
Output



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).
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Video Courtesy of Biocomputation Group @ Microsoft Research

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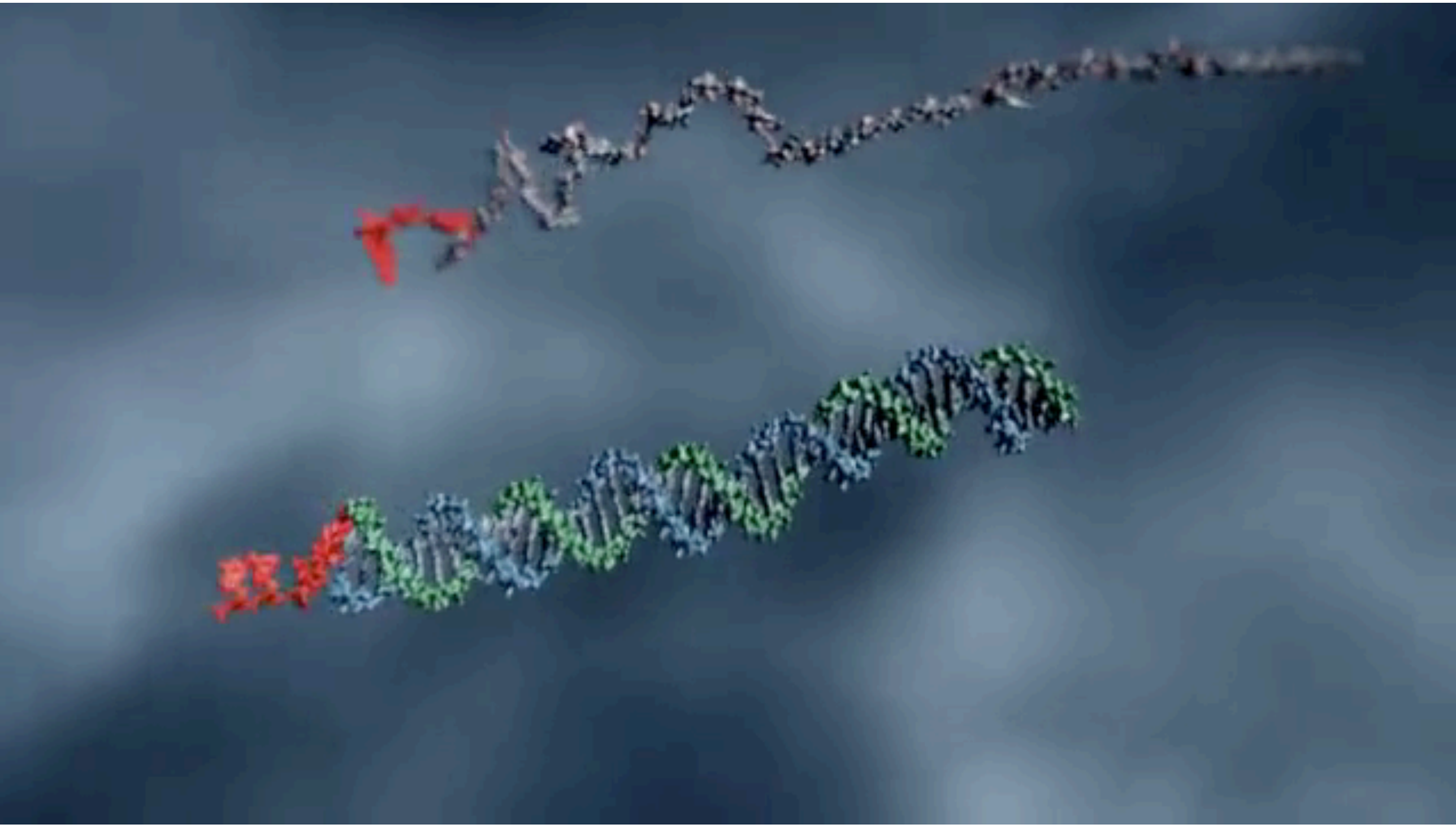

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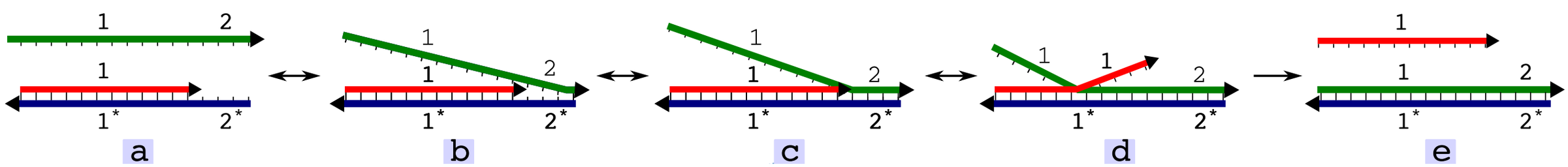
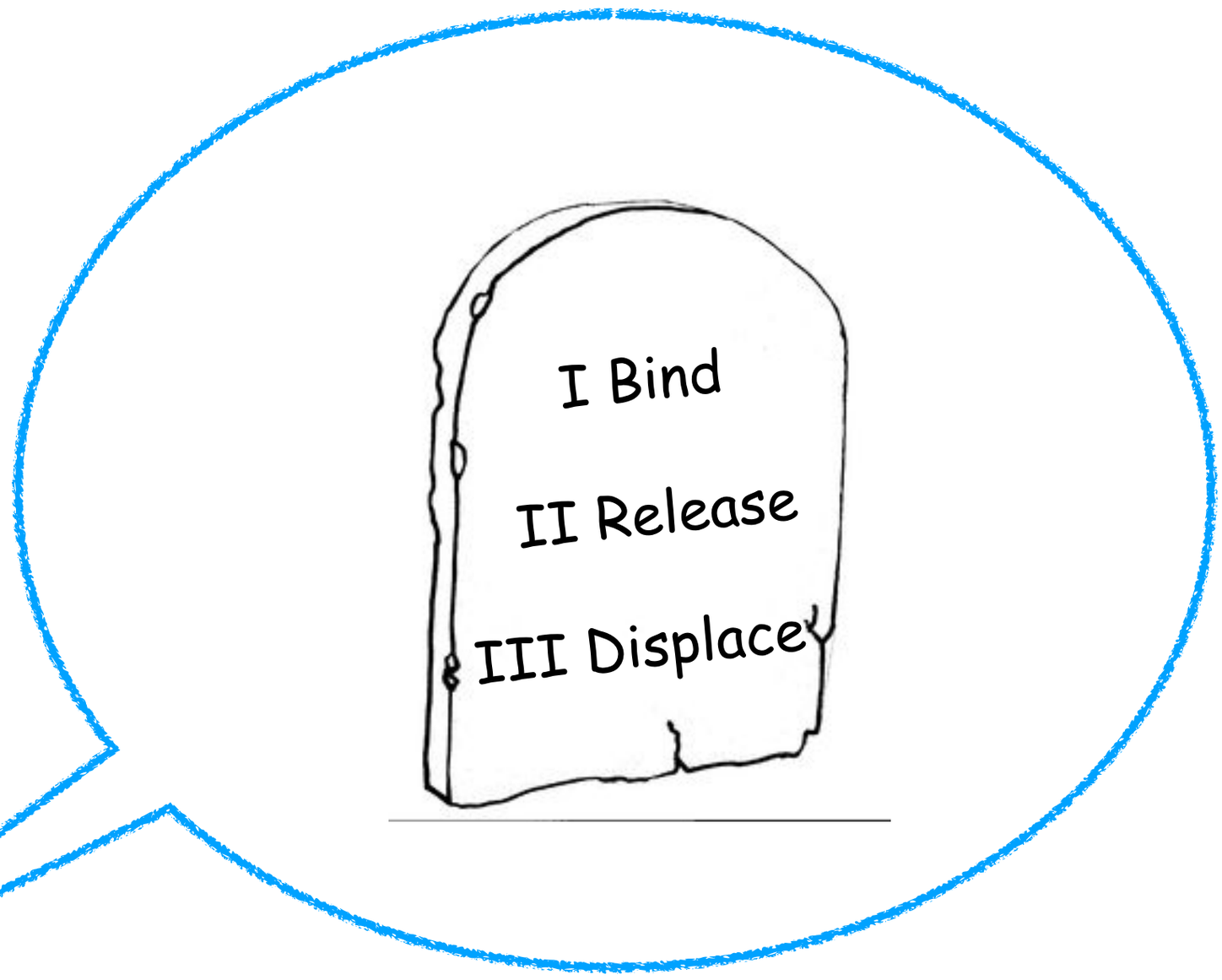


Adapted from Zhang & Seelig 2011

Strand Displacement Cascades

=

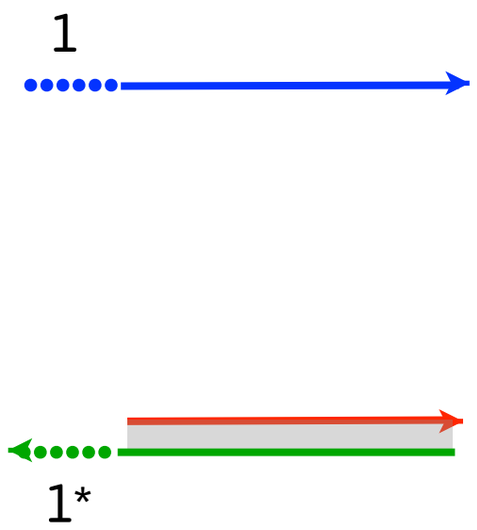
Three Rules



Domain level rules for DSD

Rule 1: ***Bind***

Example



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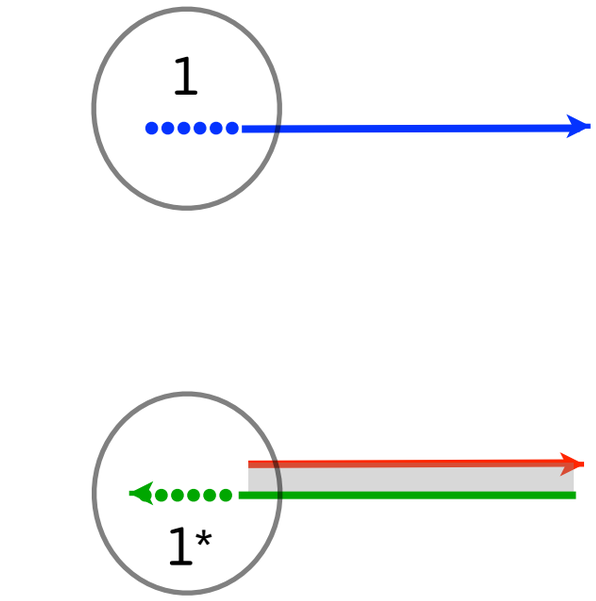

single-stranded
complementary
domains

Domain level rules for DSD

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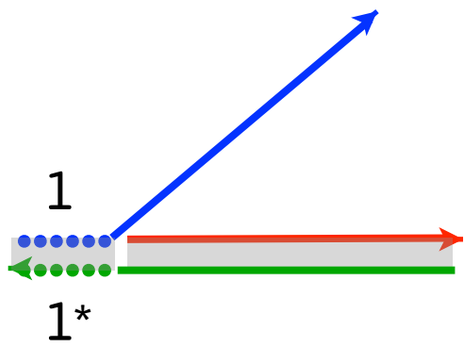
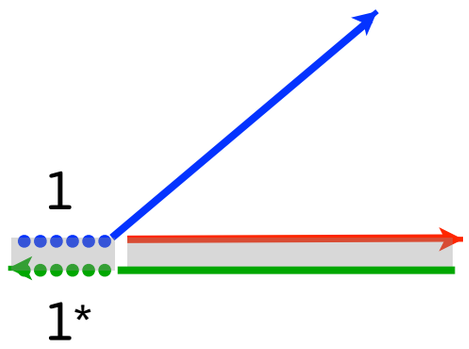


Domain level rules for DSD

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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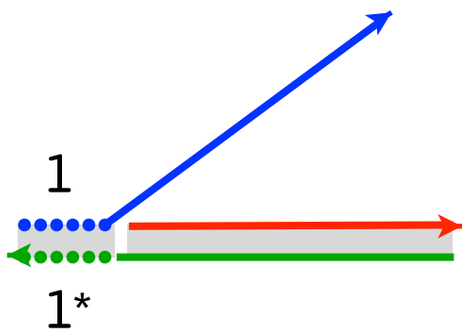
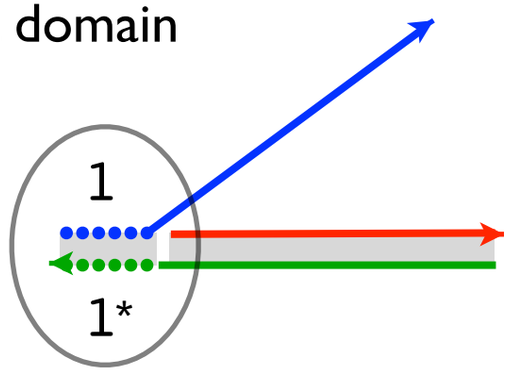


Domain level rules for DSD

Rule 2: ***Release***

Example

blue strand bound by only
a short domain



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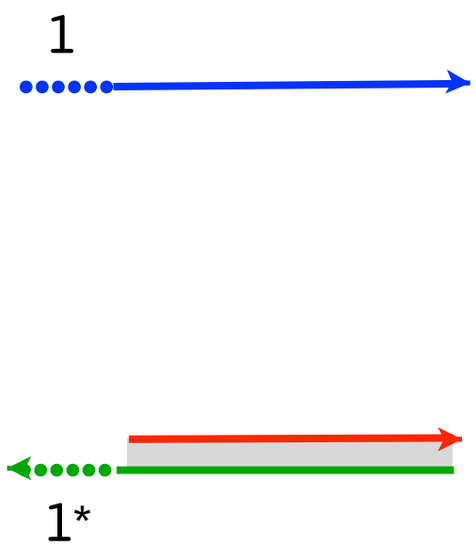
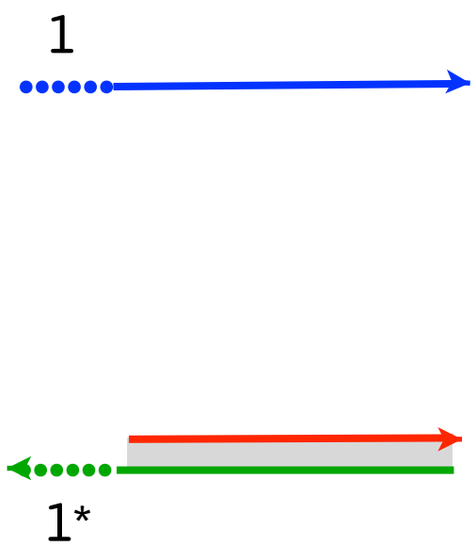


Domain level rules for DSD

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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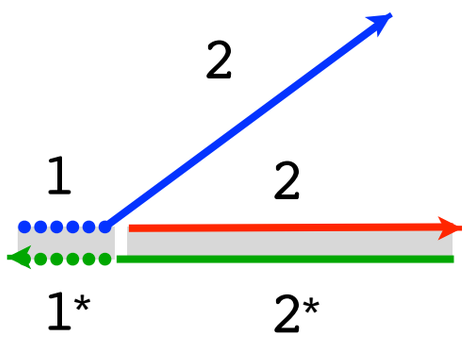

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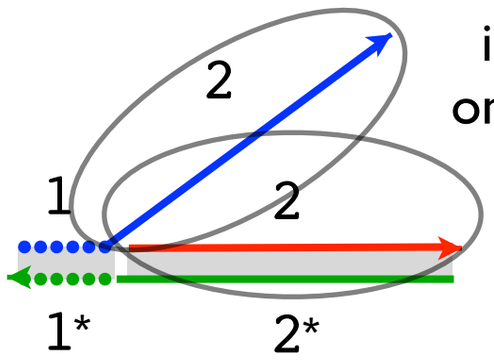

identical domains,
one bound, one free

Domain level rules for DSD

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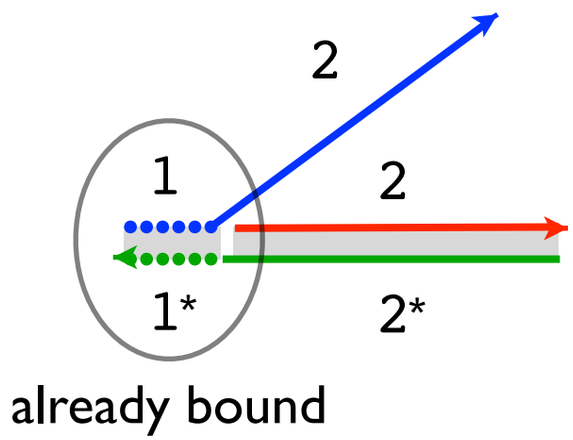

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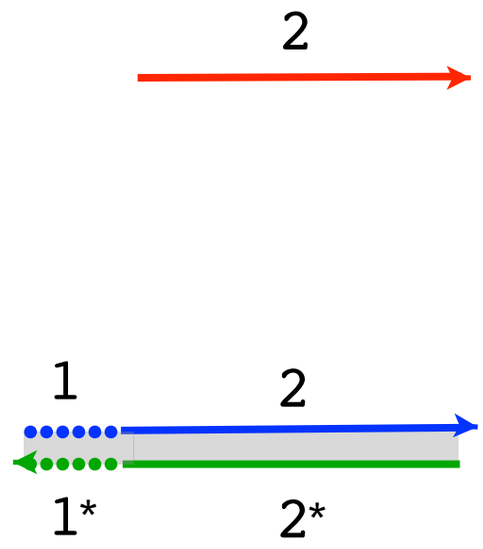
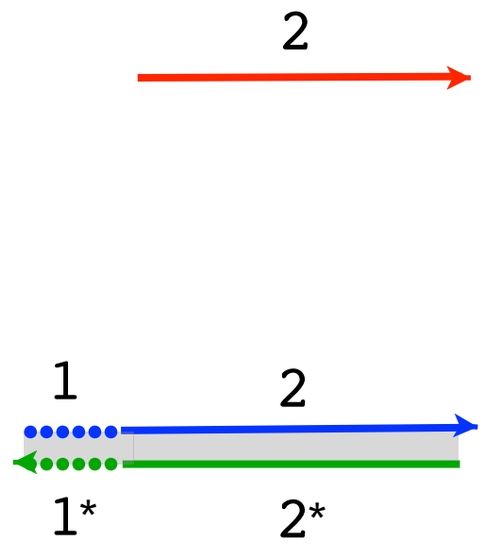


Domain level rules for DSD

Rule 3: ***Displace***

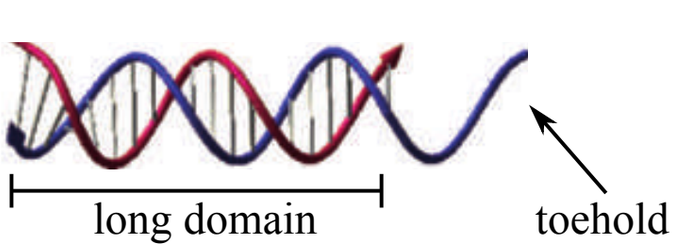
A domain can **displace** an identical domain of another strand,
if neighboring domains are already bound

Example



Why do we use toeholds?

Adapted from Zhang & Seelig 2011



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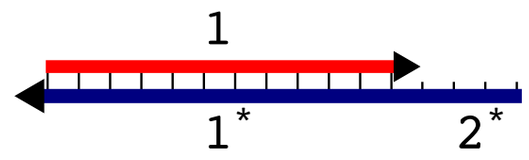

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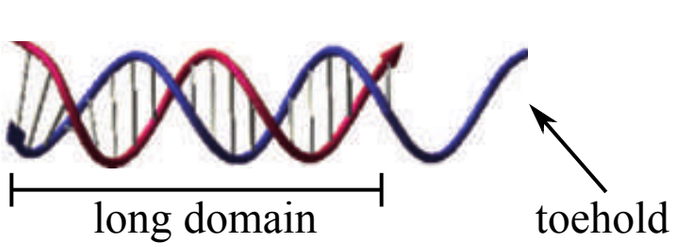

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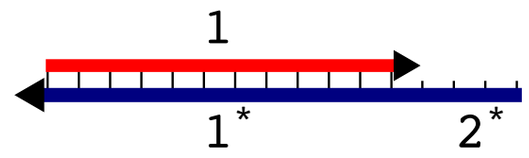
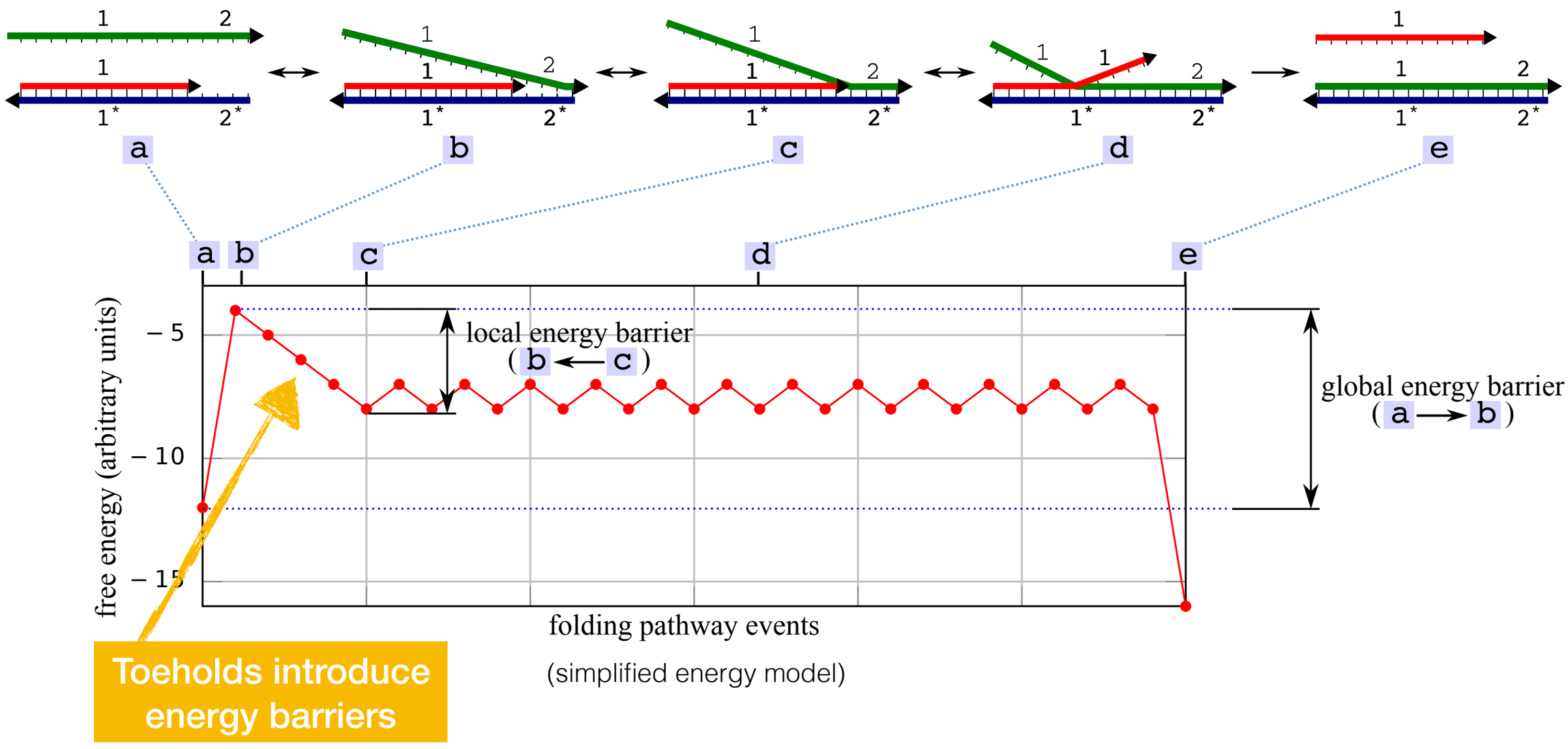


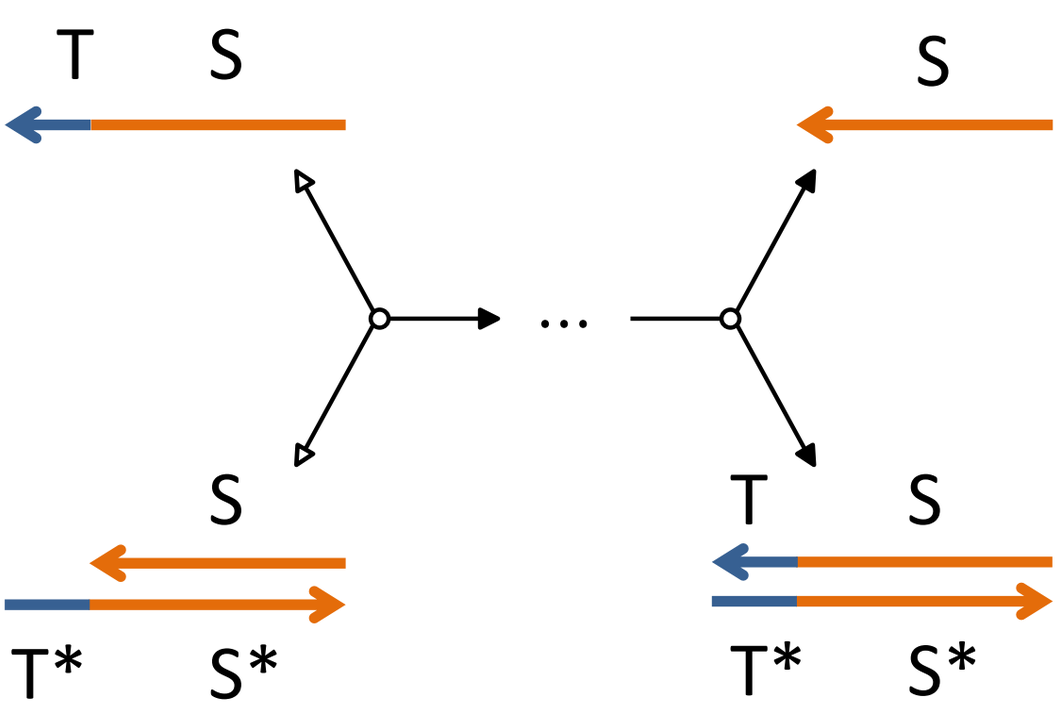


Toeholds introduce energy barriers

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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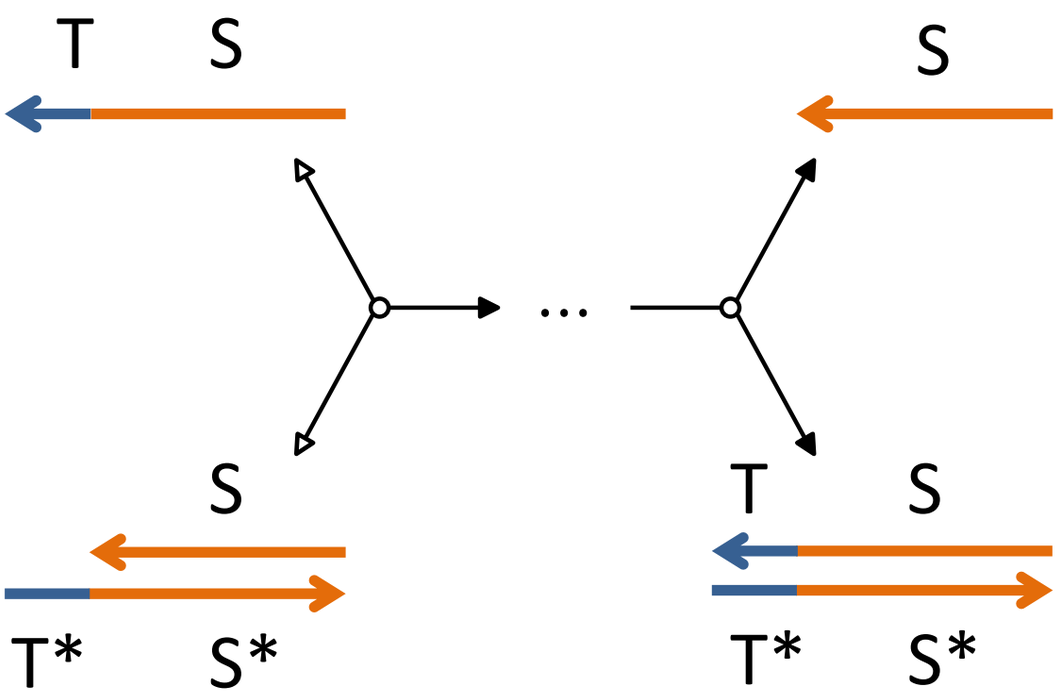

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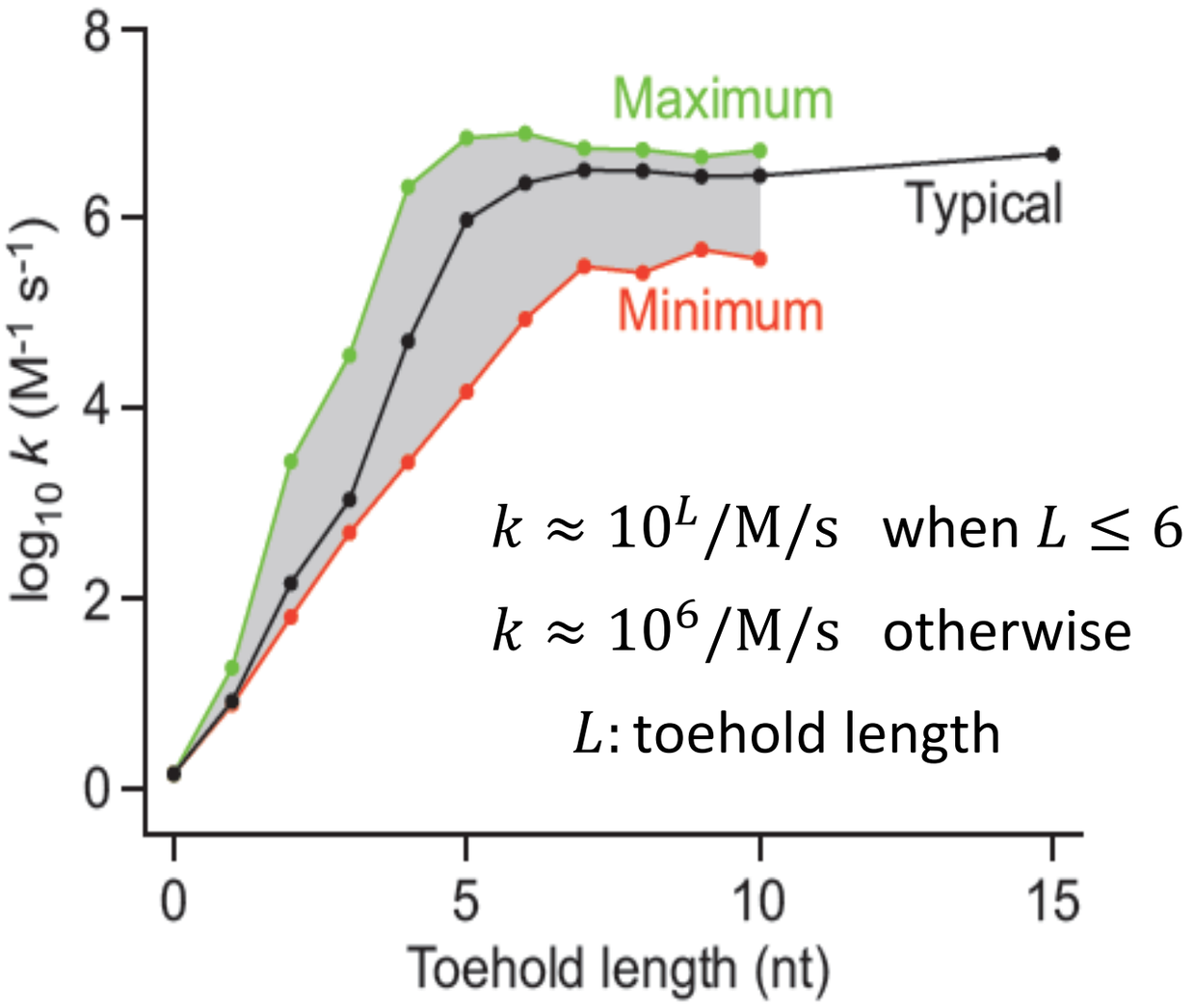

Zhang and Seelig, *Nature Chemistry* 2011

Kinetics of toehold-mediated strand displacement

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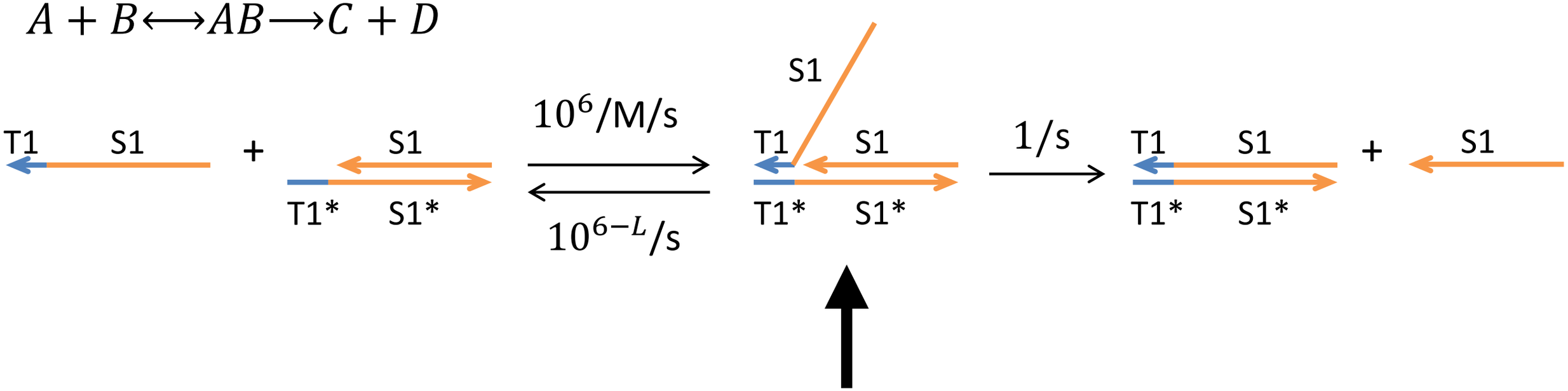

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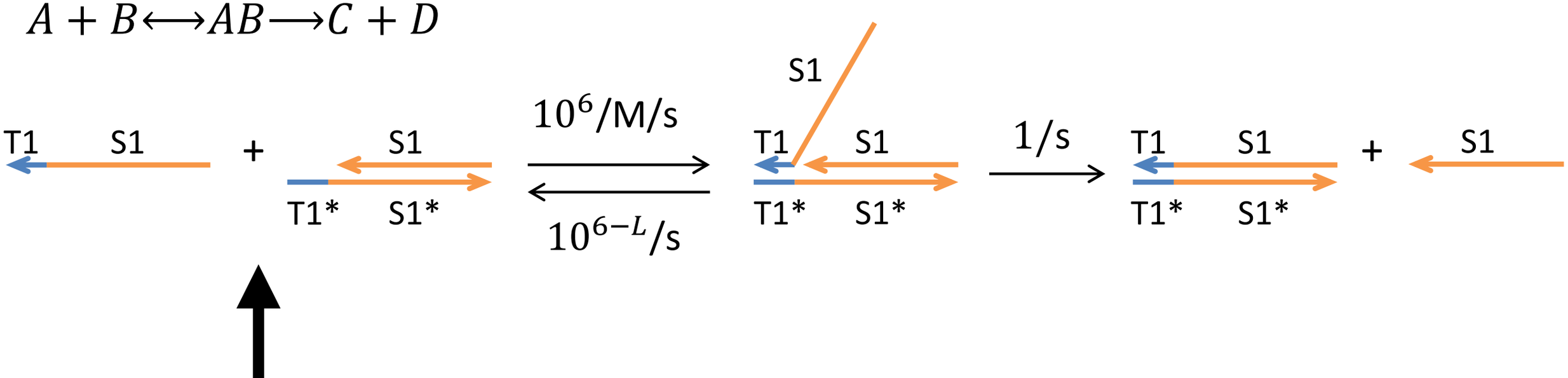

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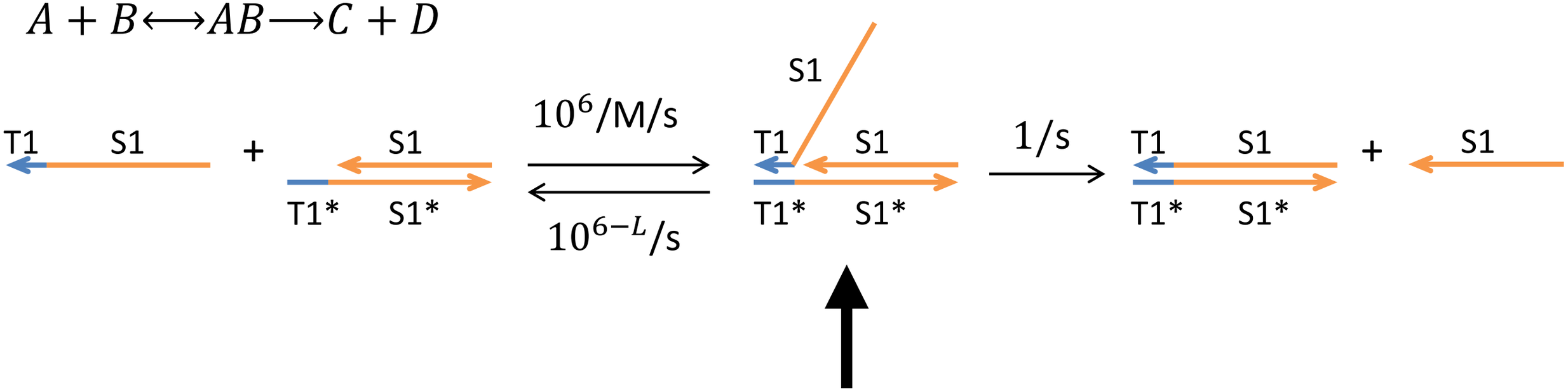

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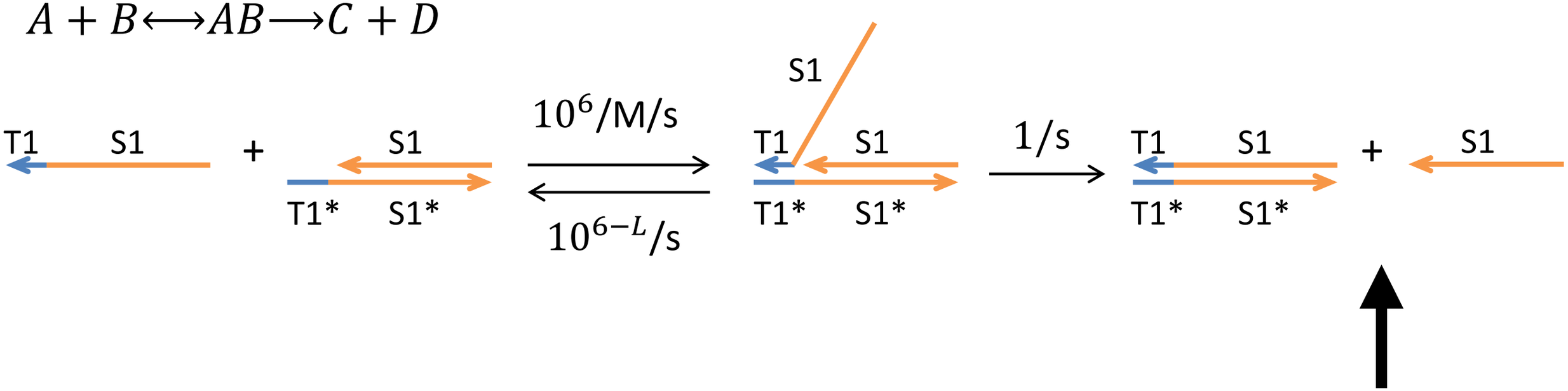

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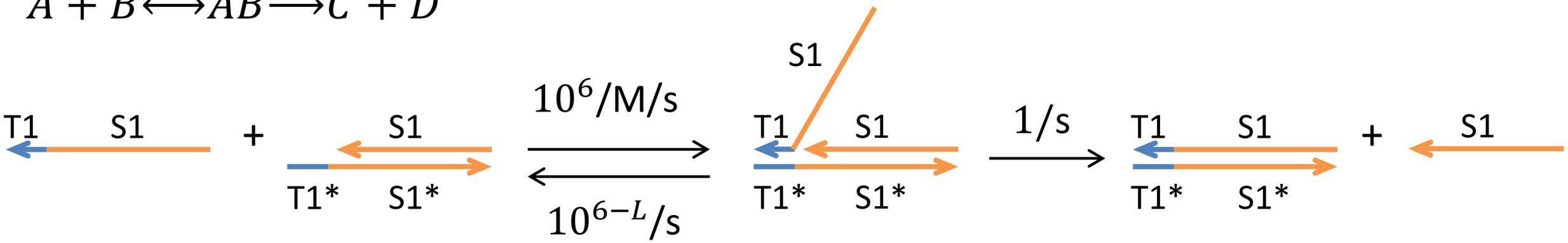

simplify: $A + B \xrightarrow{k_{eff}} C + D$ $\quad k_{eff} = ?$

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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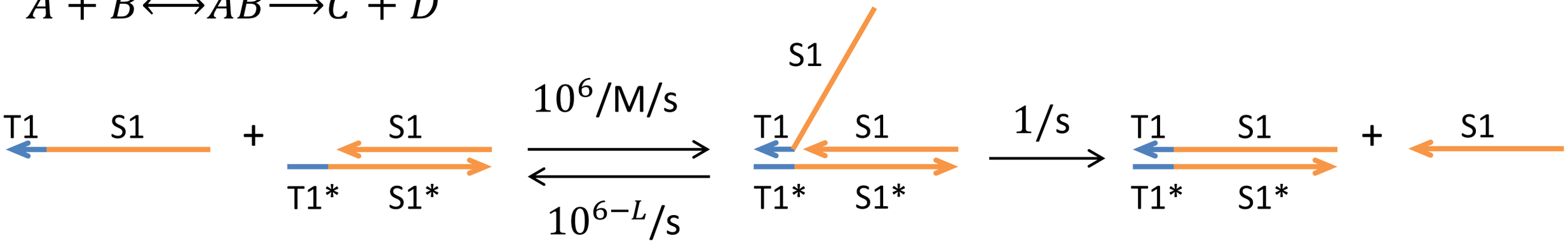

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Strand displacement in the lab

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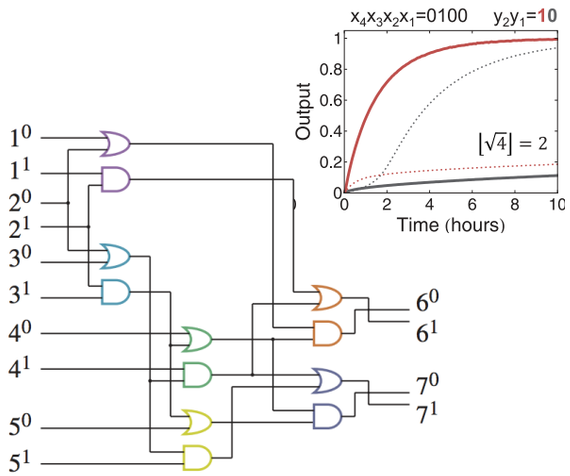
molecular
logic circuits



- Large autonomous biochemical networks built from scratch

  Qian, Winfree, *Science* 2011

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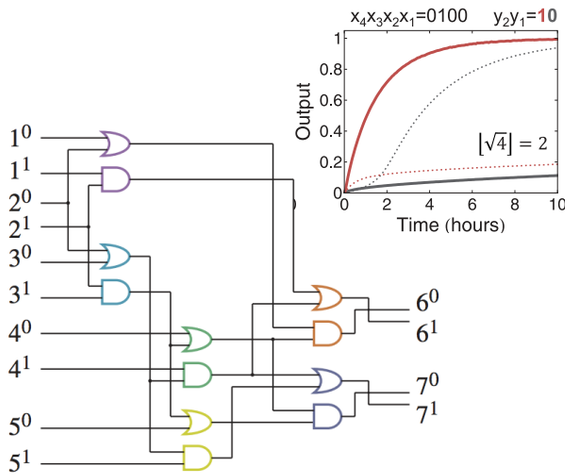

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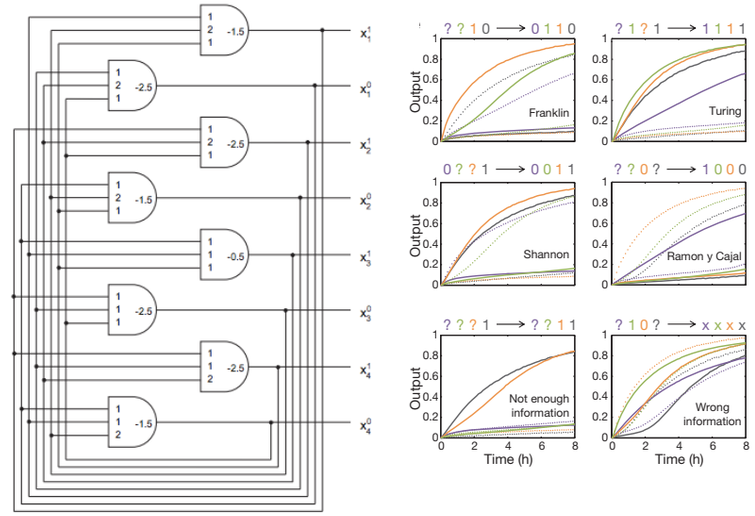
strand displacement cascades

molecular artificial neural networks



- Biochemical system doing inference

Qian, Winfree, Bruck *Nature* 2011

Strand displacement in the lab

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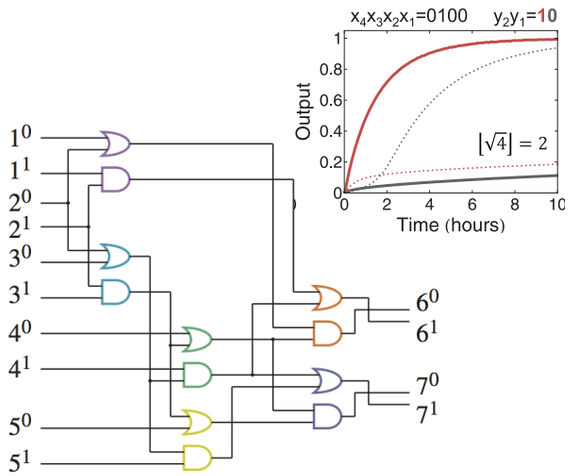

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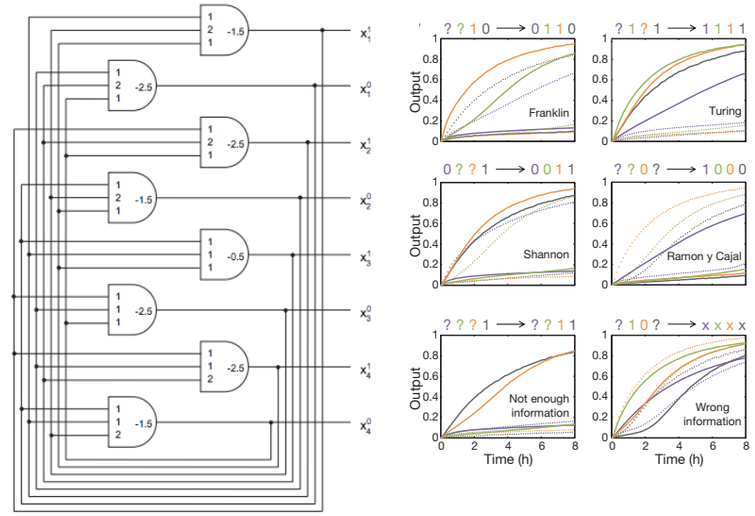

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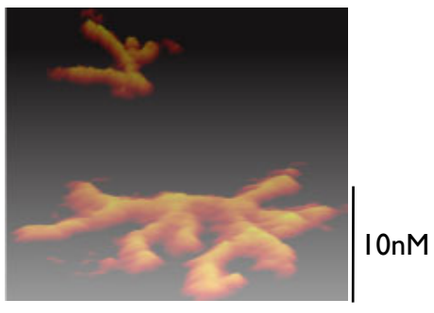
strand displacement cascades

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

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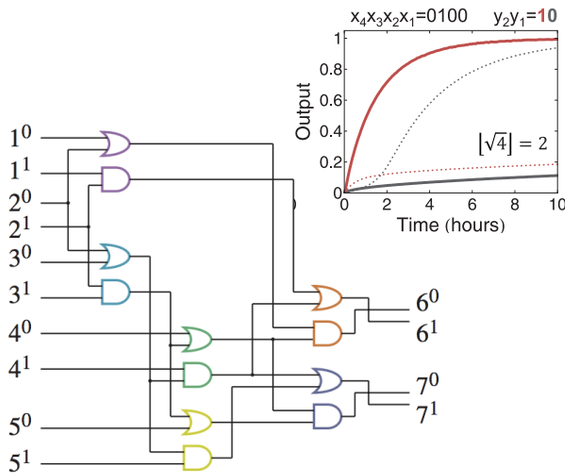

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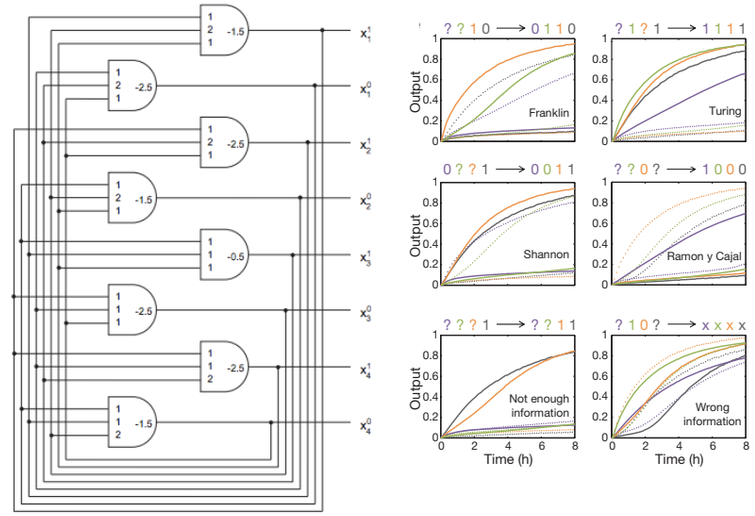

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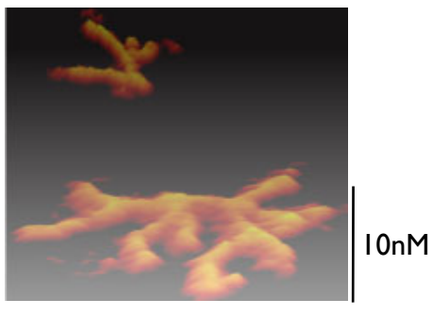

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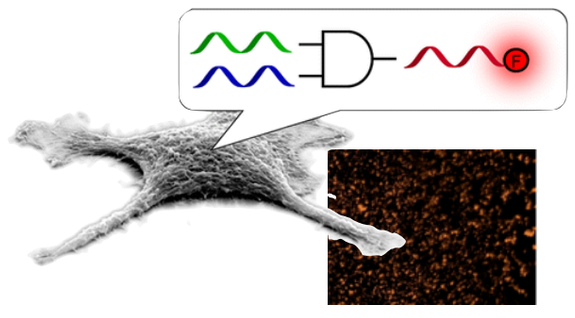
strand displacement in vivo

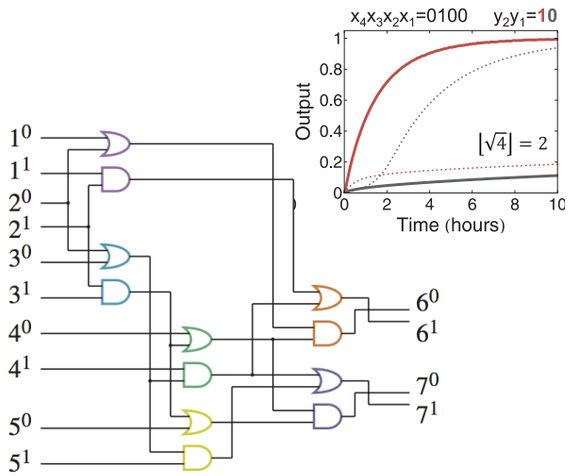


- Logic on biological signals

Hemphill, Deiters *J Am Chem Soc* 2013

Strand displacement in the lab

molecular logic circuits



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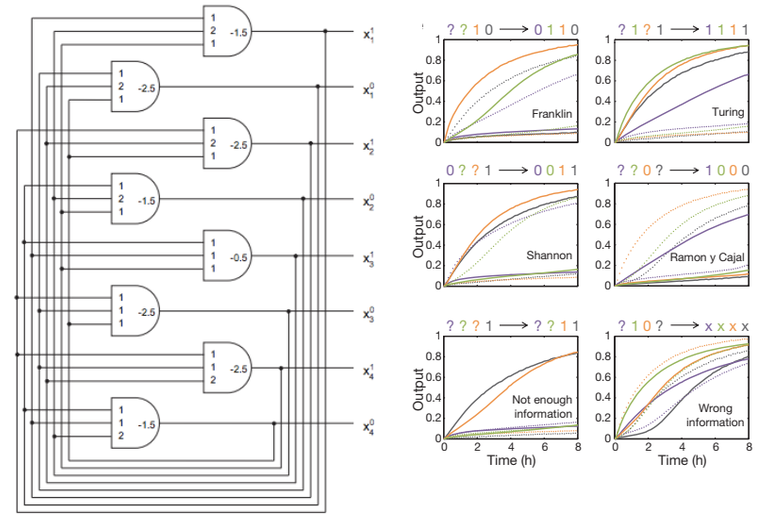
Qian, Winfree, *Science* 2011

molecular artificial neural networks

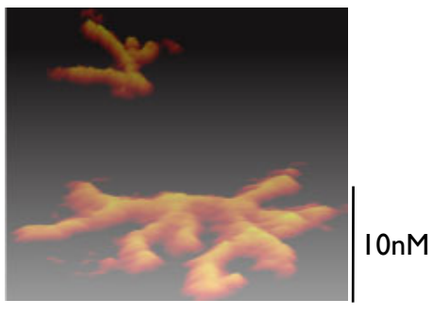


- Biochemical system doing inference

Qian, Winfree, Bruck *Nature* 2011

strand displacement cascades

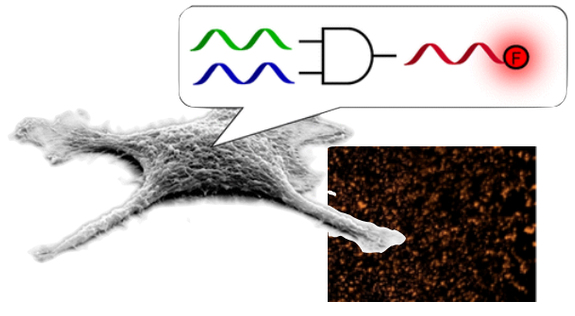
controlling assembly of nanoscale structures



- Prescribed nanoscale structures seen under atomic force microscope

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

strand displacement in vivo



- Logic on biological signals

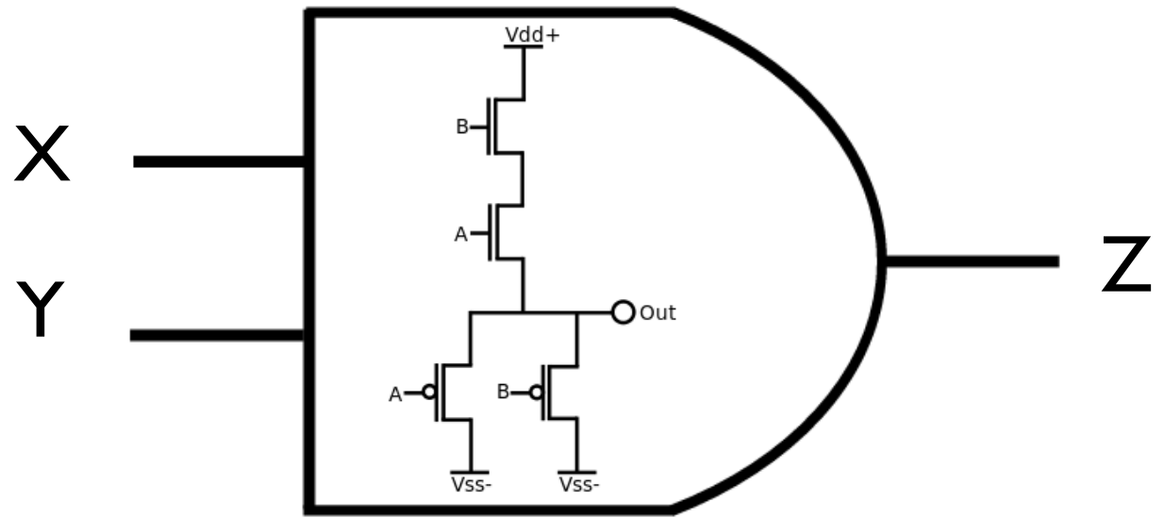
Hemphill, Deiters *J Am Chem Soc* 2013

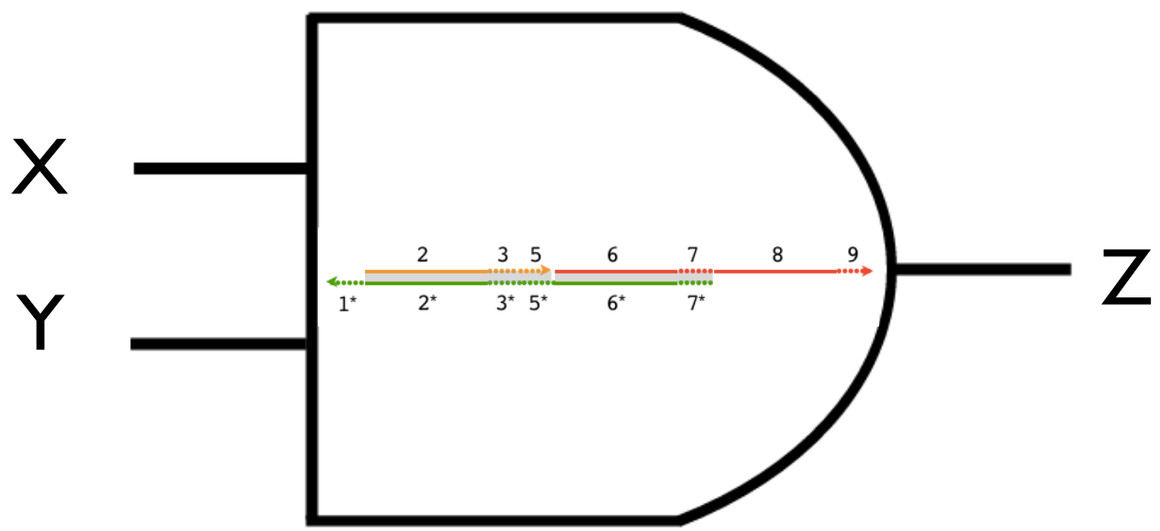
Tutorial Outline

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

AND gate

release Z if and only if X and Y are present

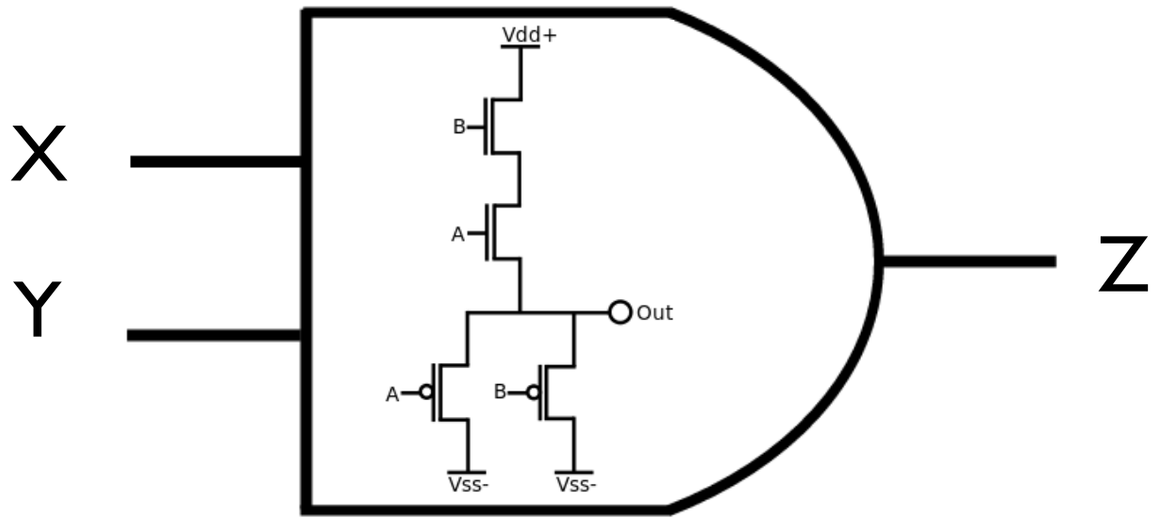




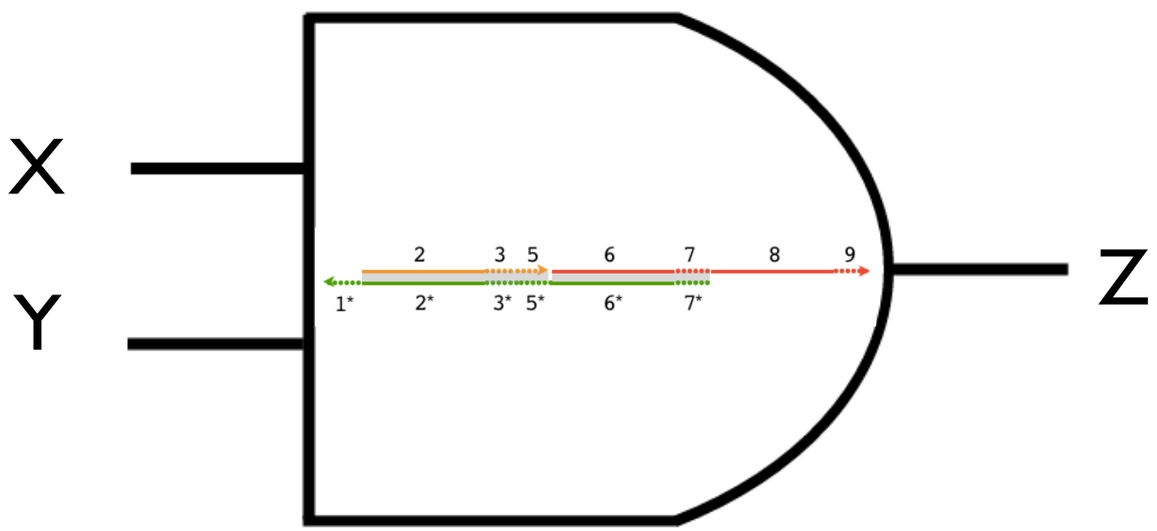
AND gate

release Z if and only if X and Y are present

voltages



strands

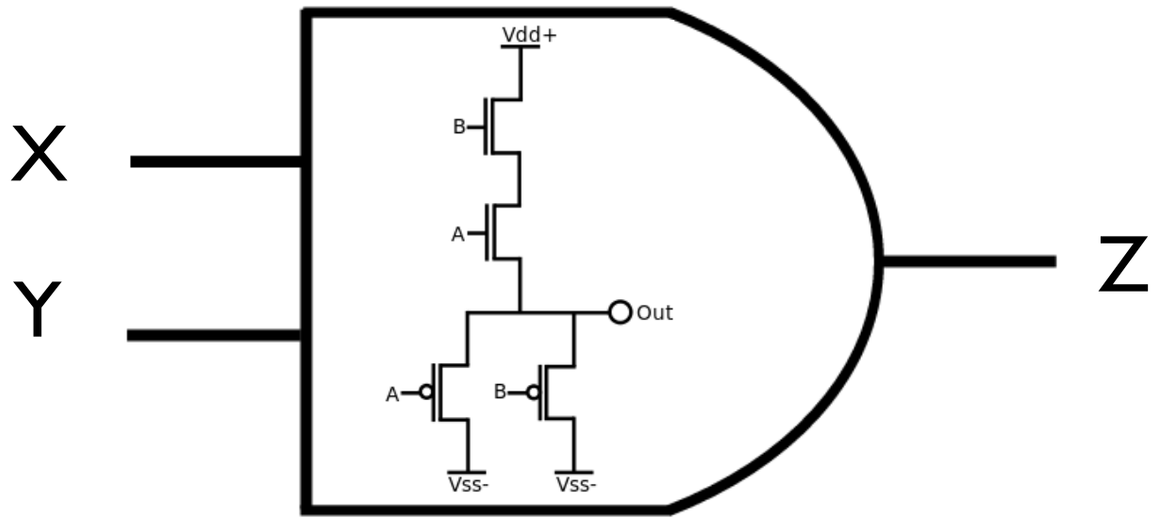


gate=complex

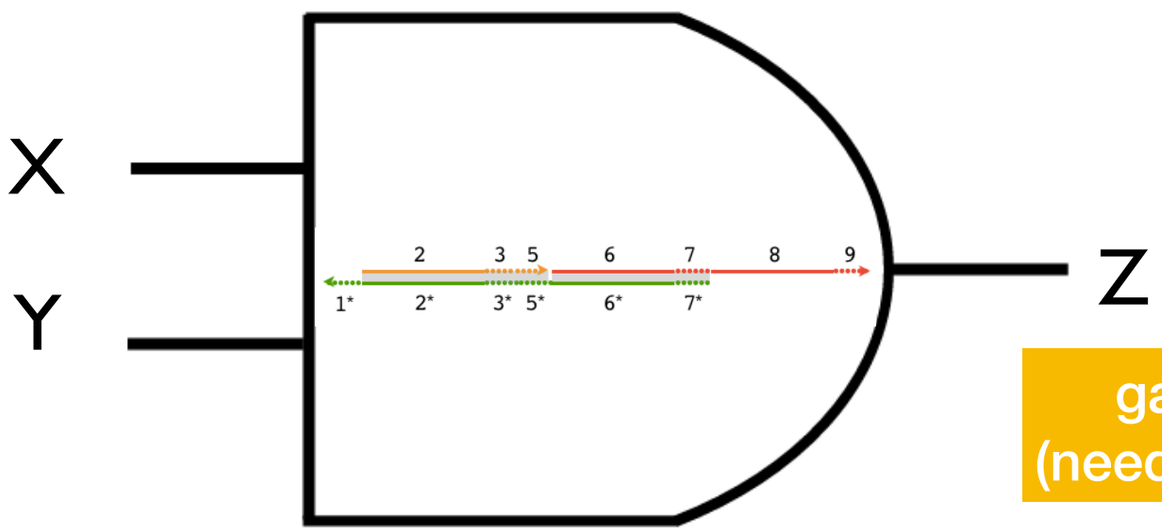
AND gate

release Z if and only if X and Y are present

voltages



strands



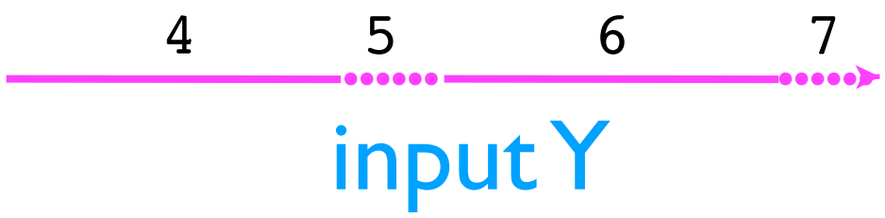
gates get consumed!
(need to have many copies)

gate=complex

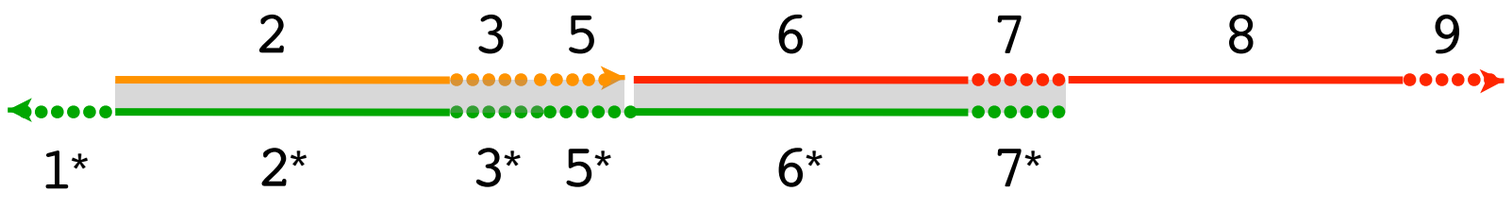
Strand Displacement Cascades Example: AND gate

release Z if and only if X and Y are present

input X



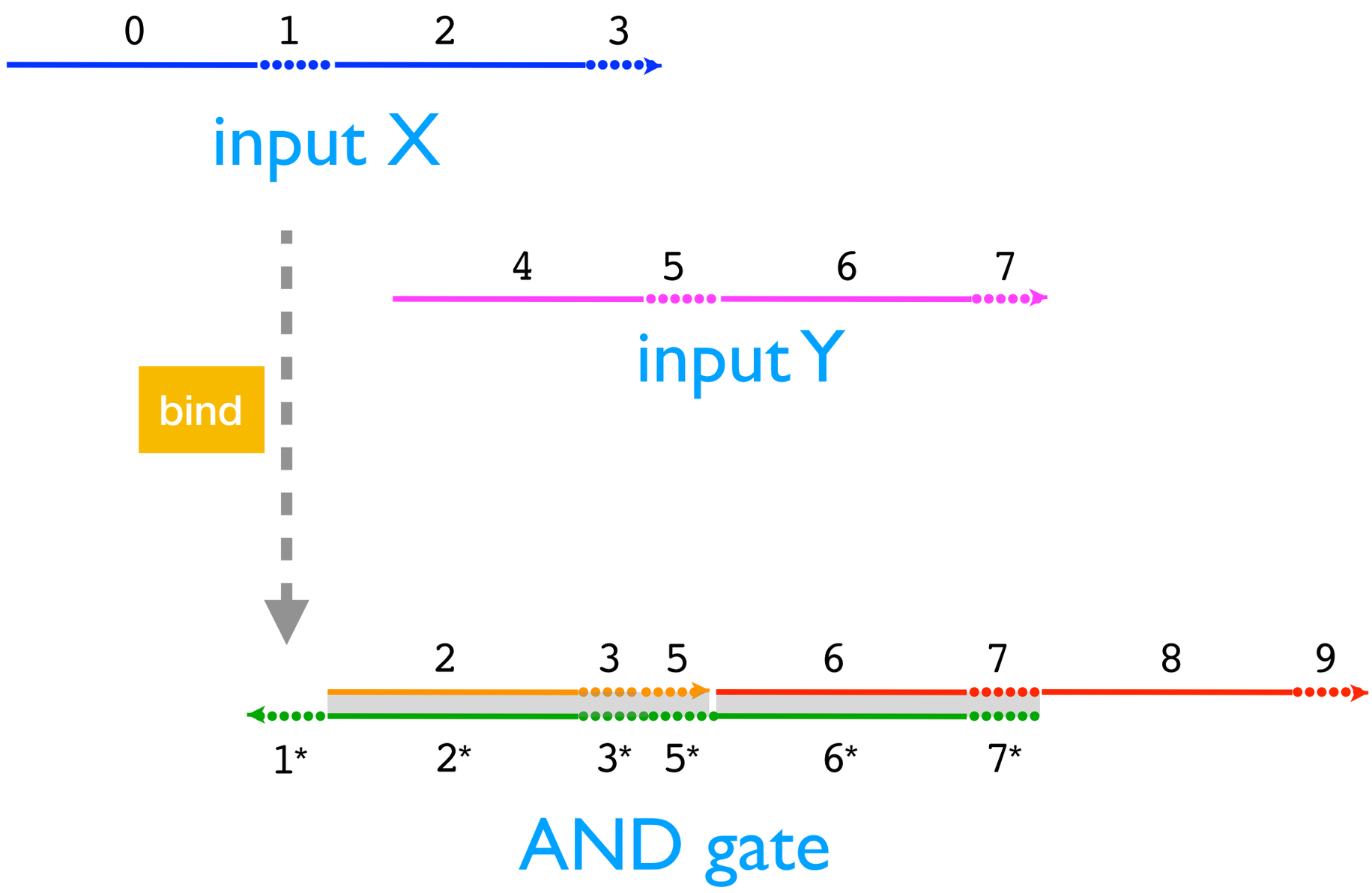
input Y



AND gate

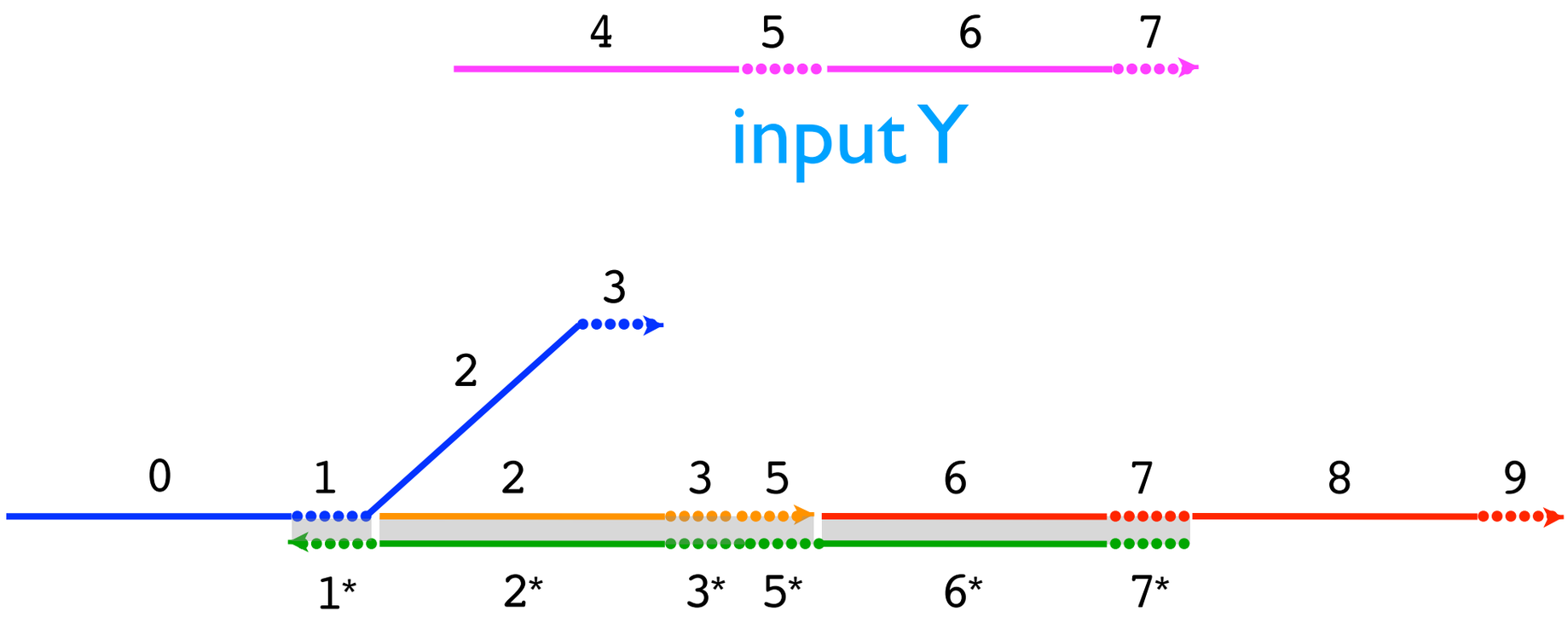
Strand Displacement Cascades Example: AND gate

release Z if and only if X and Y are present



Strand Displacement Cascades Example: AND gate

release Z if and only if X and Y are present



Strand Displacement Cascades Example: AND gate

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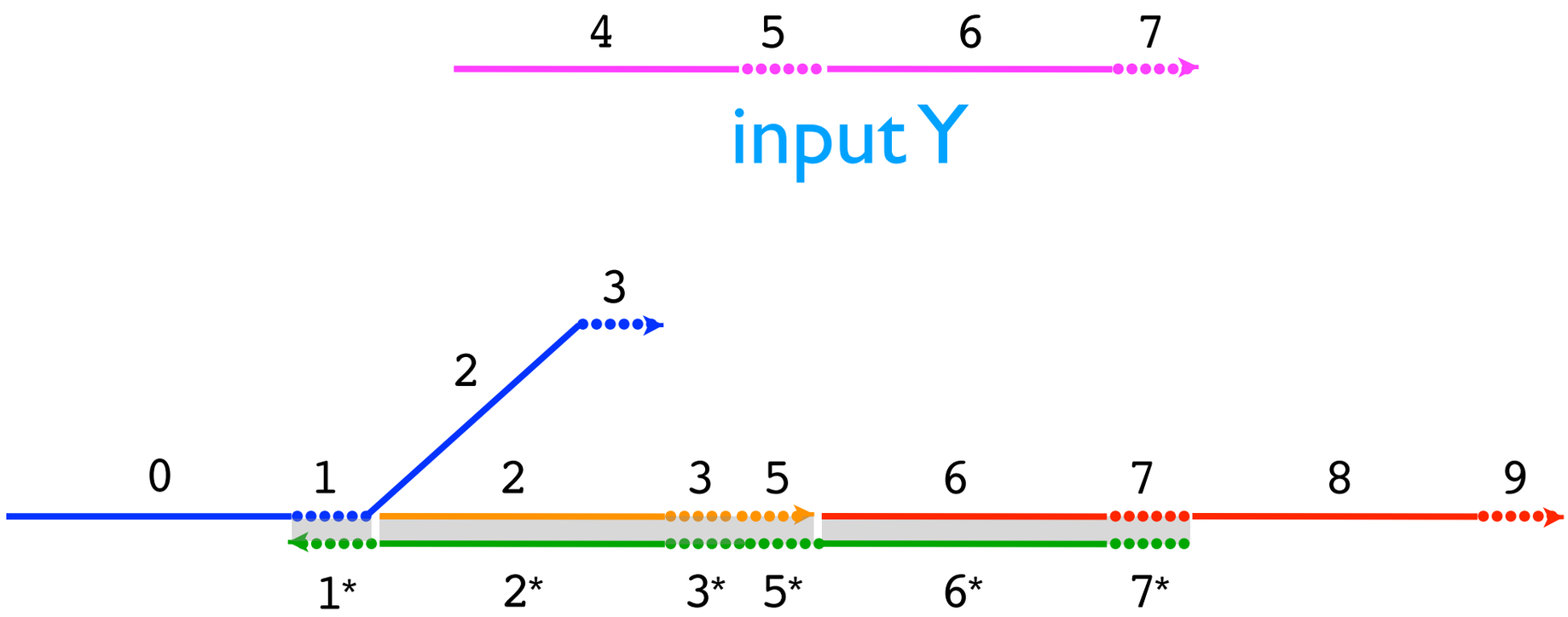

Strand Displacement Cascades Example: AND gate

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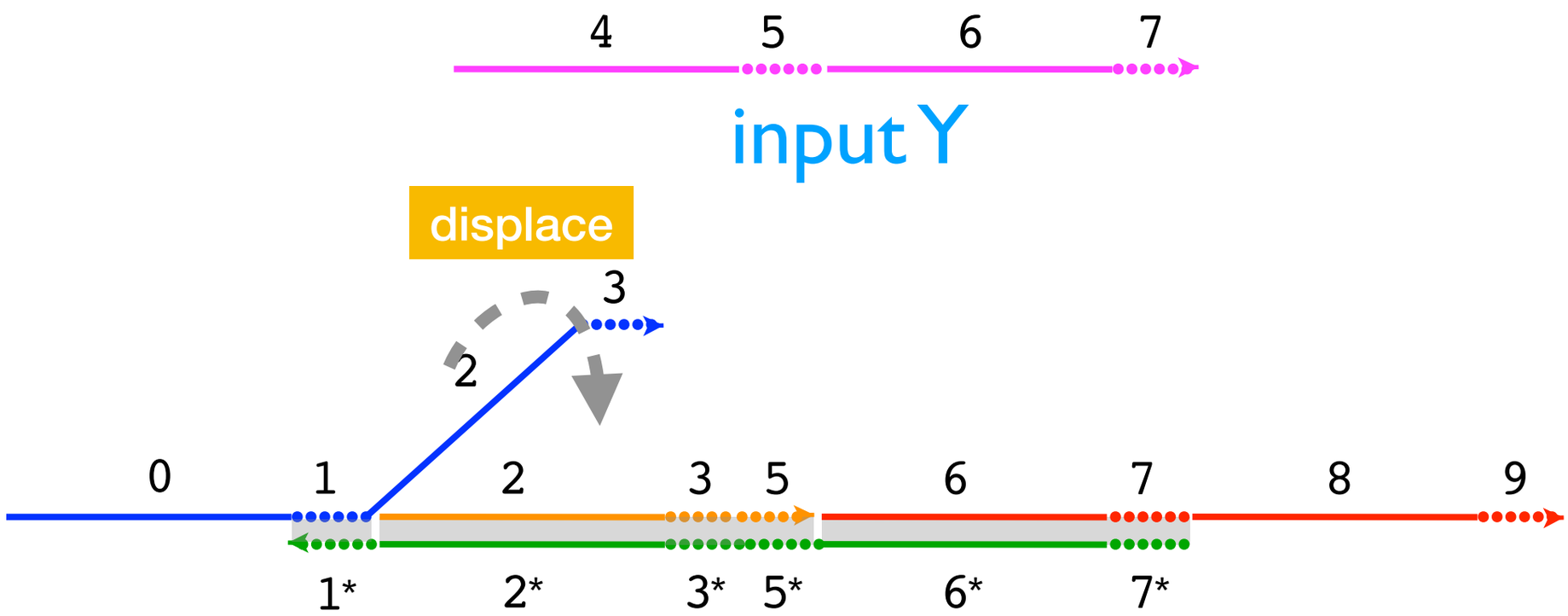
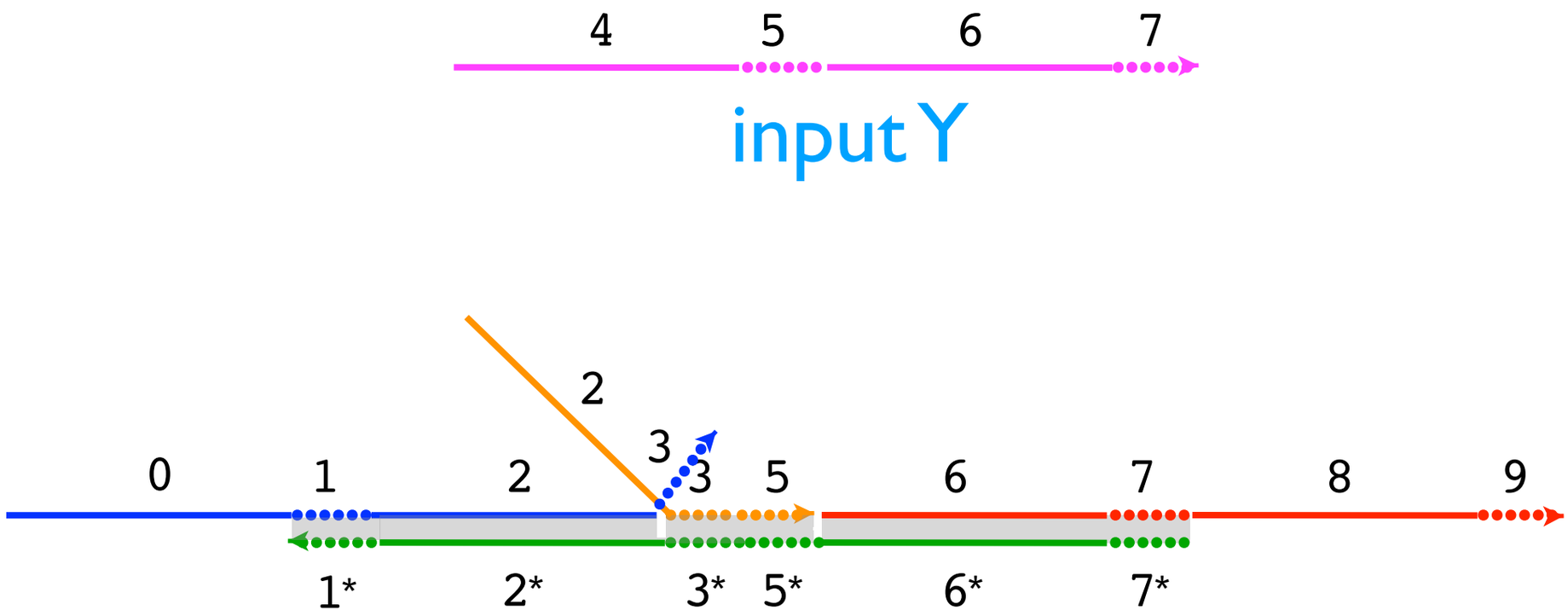


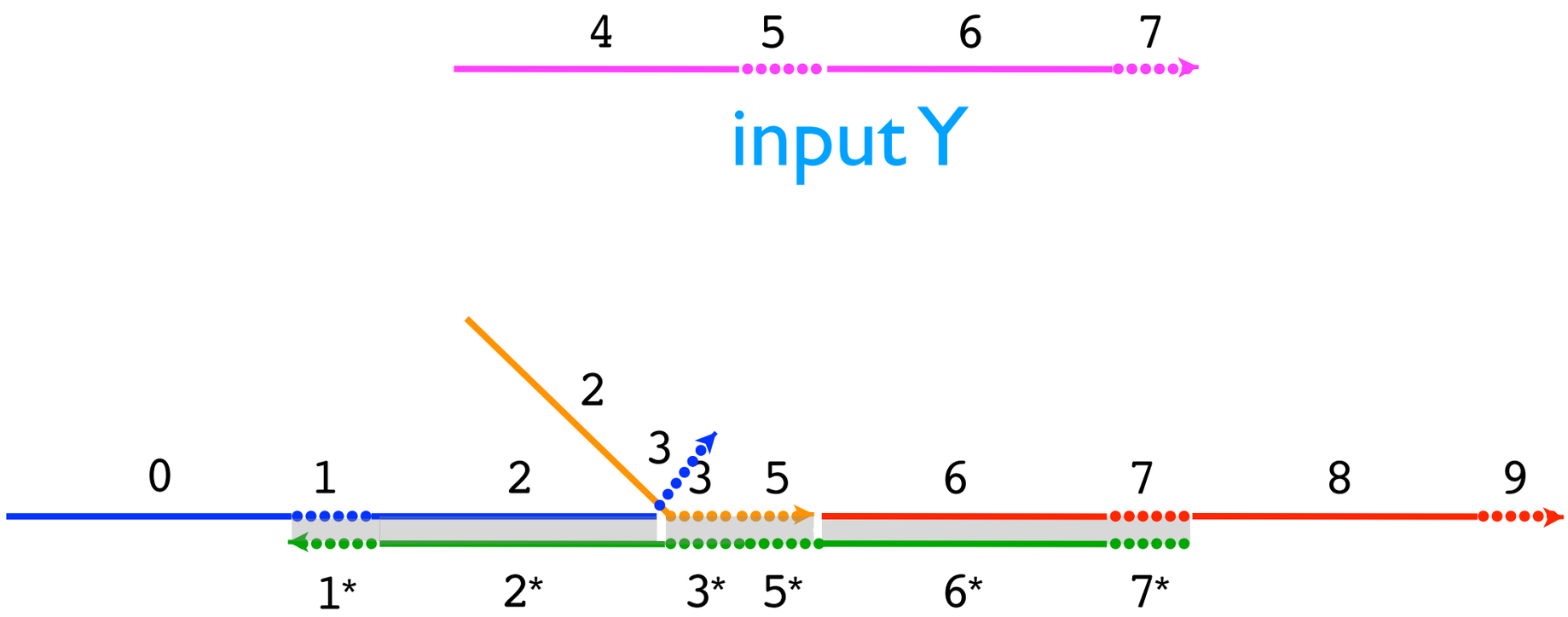
Strand Displacement Cascades Example: AND gate

release Z if and only if X and Y are present



Strand Displacement Cascades Example: AND gate

release Z if and only if X and Y are present



Strand Displacement Cascades Example: AND gate

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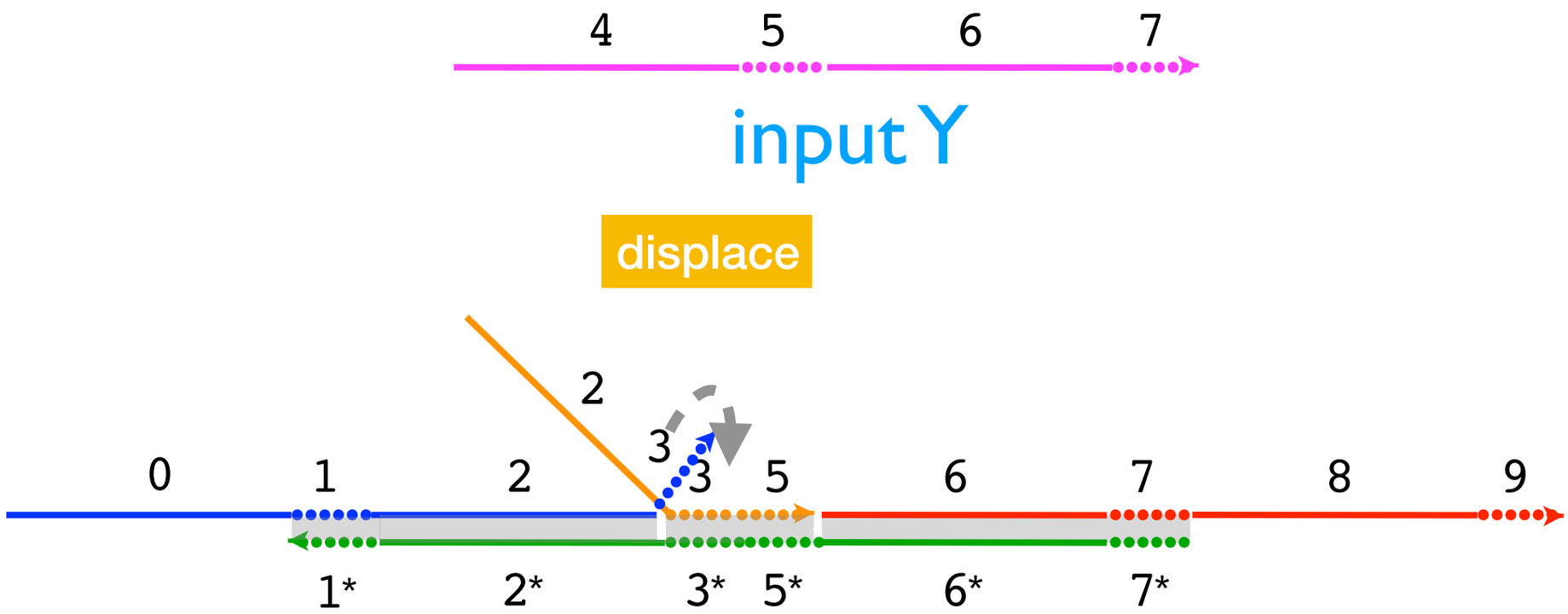
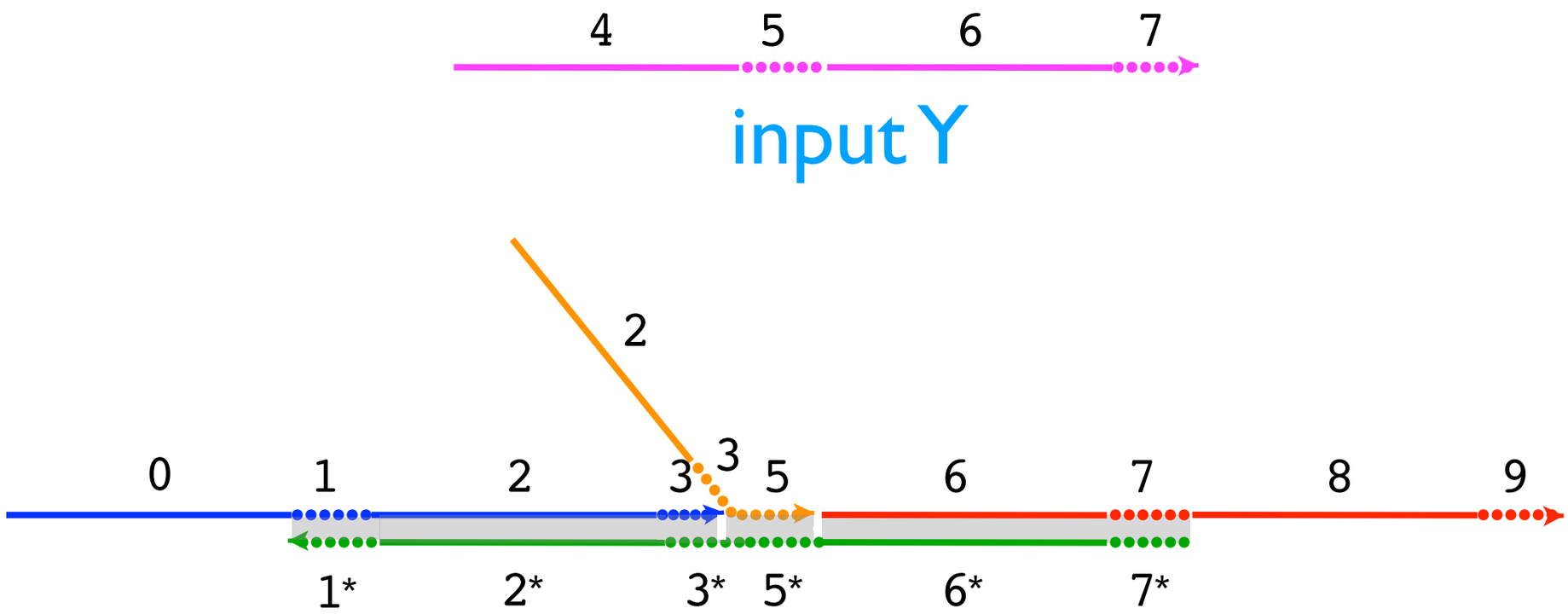


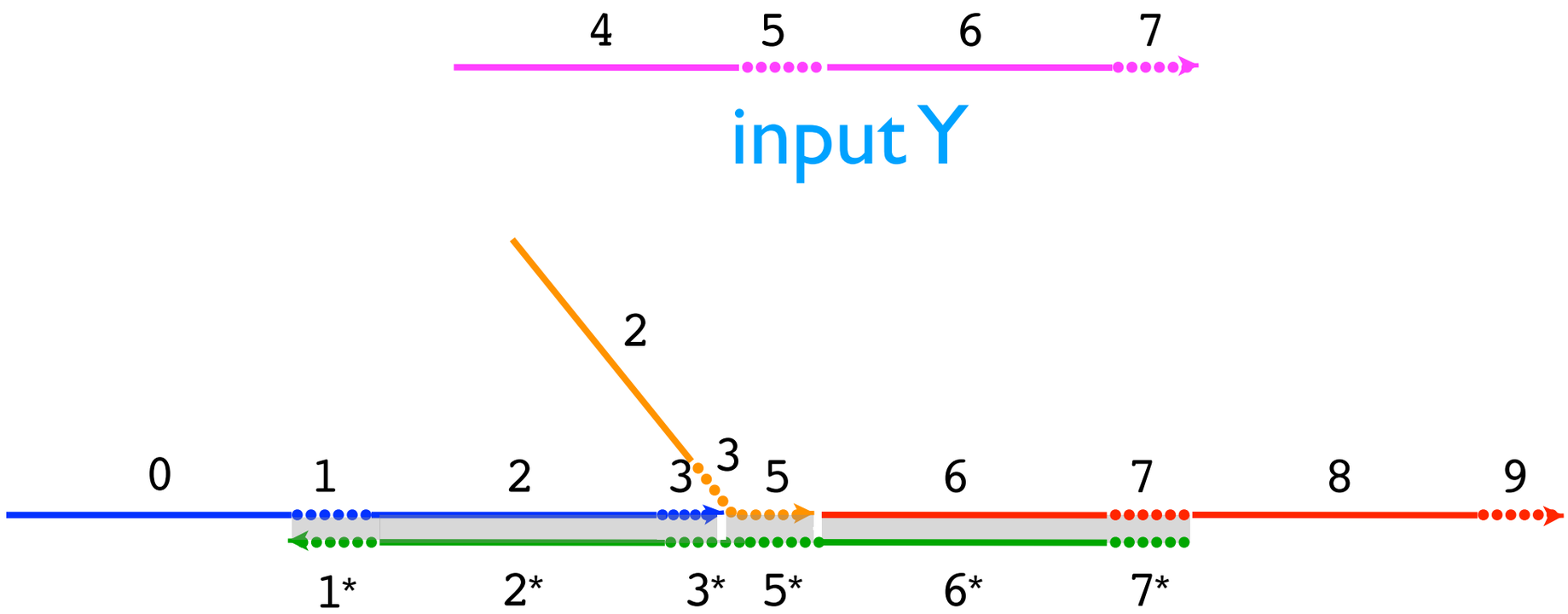
Strand Displacement Cascades Example: AND gate

release Z if and only if X and Y are present



Strand Displacement Cascades Example: AND gate

release Z if and only if X and Y are present



Strand Displacement Cascades Example: AND gate

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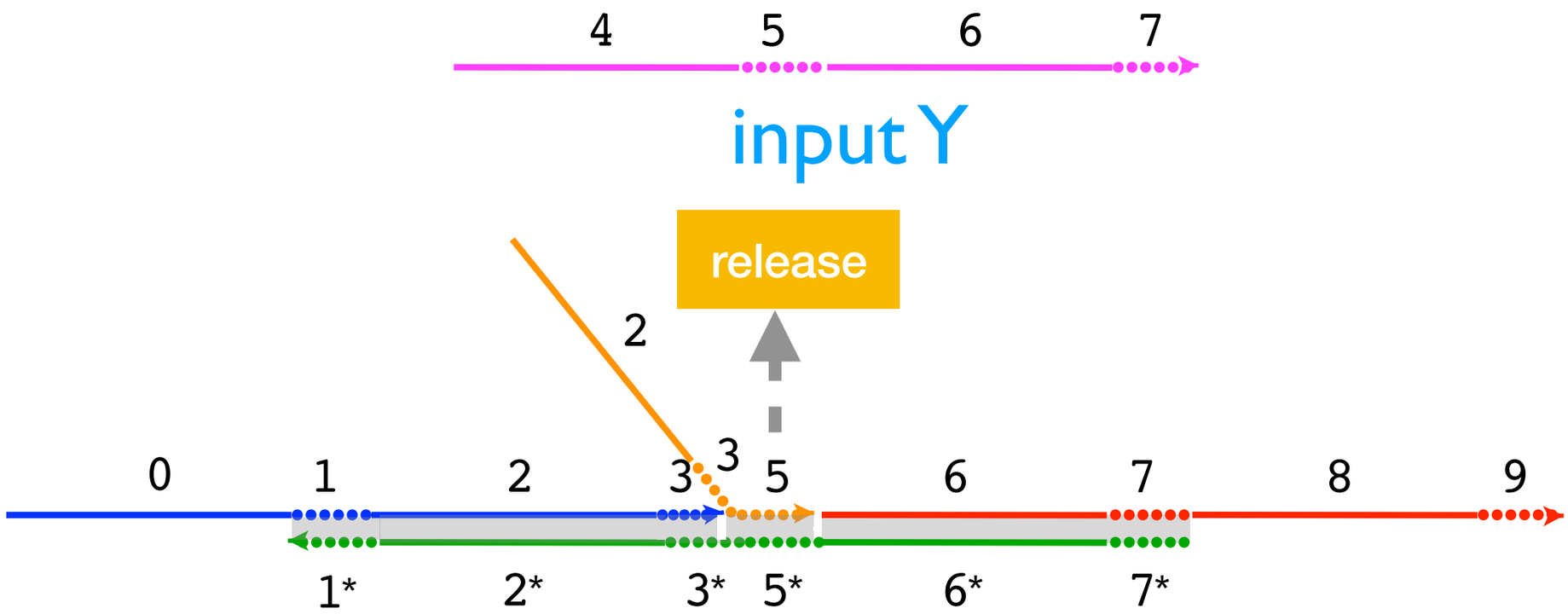
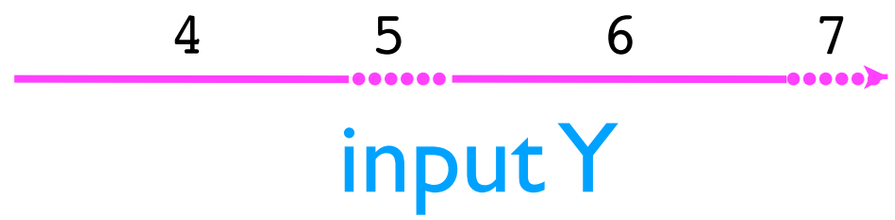
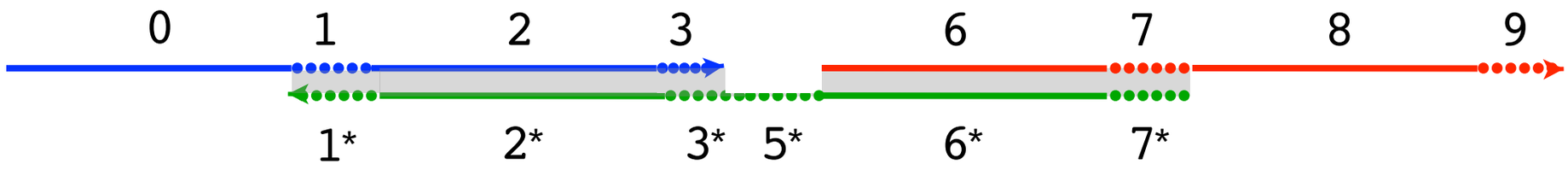


Strand Displacement Cascades Example: AND gate

release Z if and only if X and Y are present





Strand Displacement Cascades Example: AND gate

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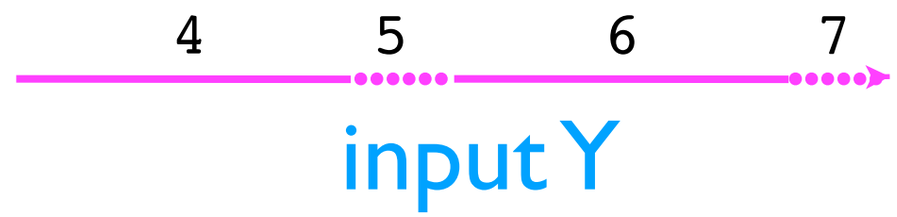

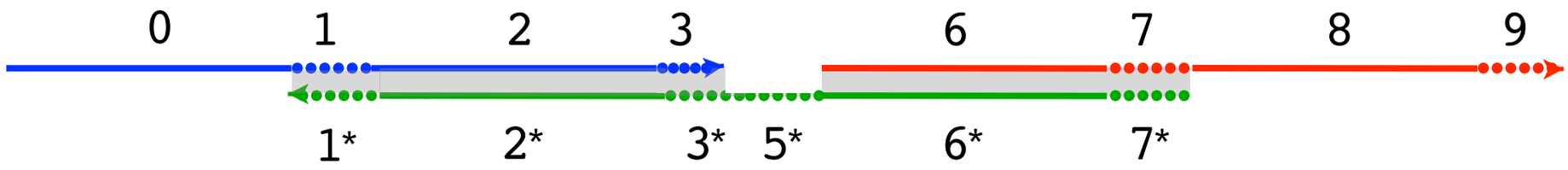

Strand Displacement Cascades Example: AND gate

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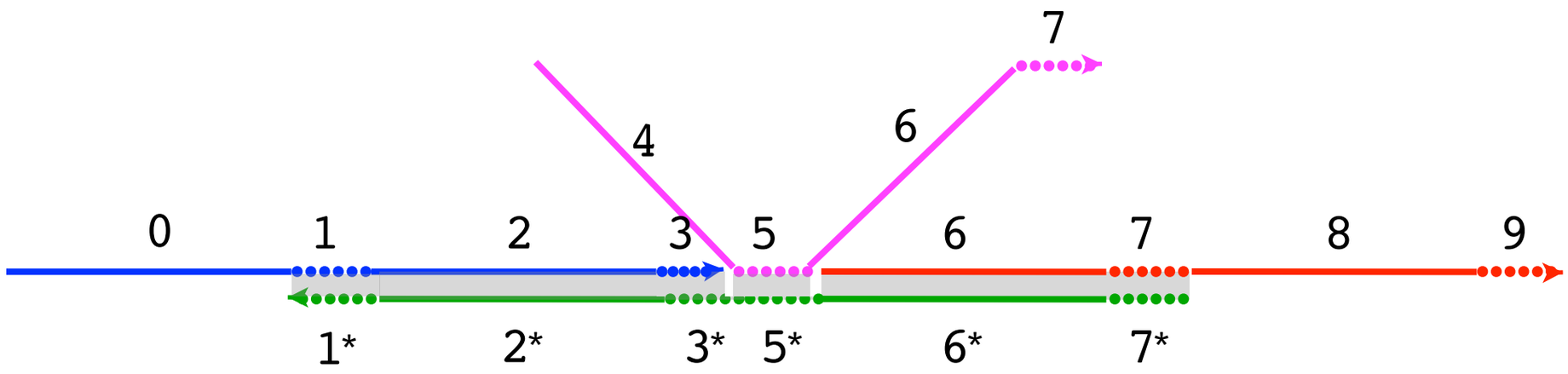

Strand Displacement Cascades Example: AND gate

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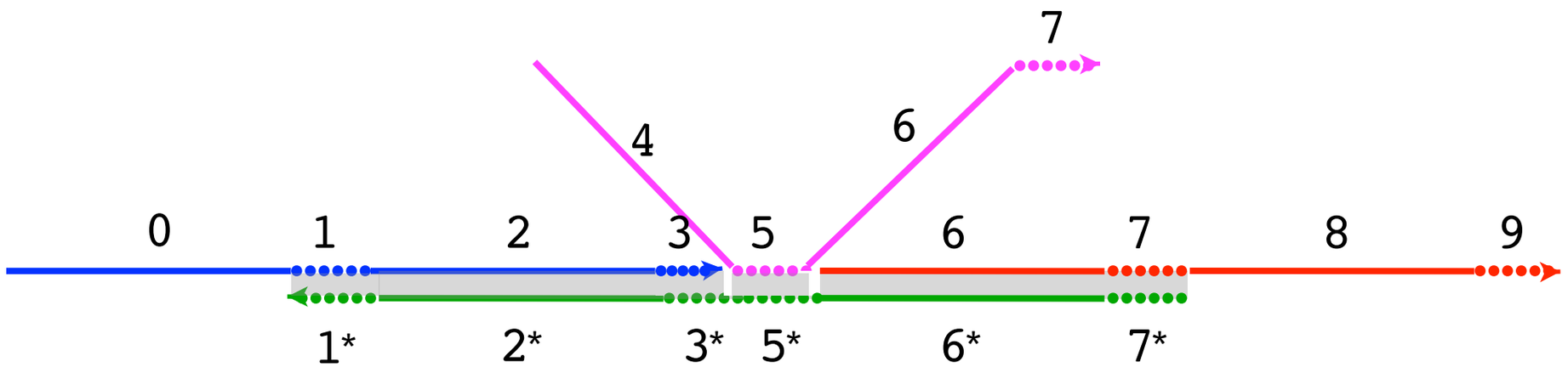

Strand Displacement Cascades Example: AND gate

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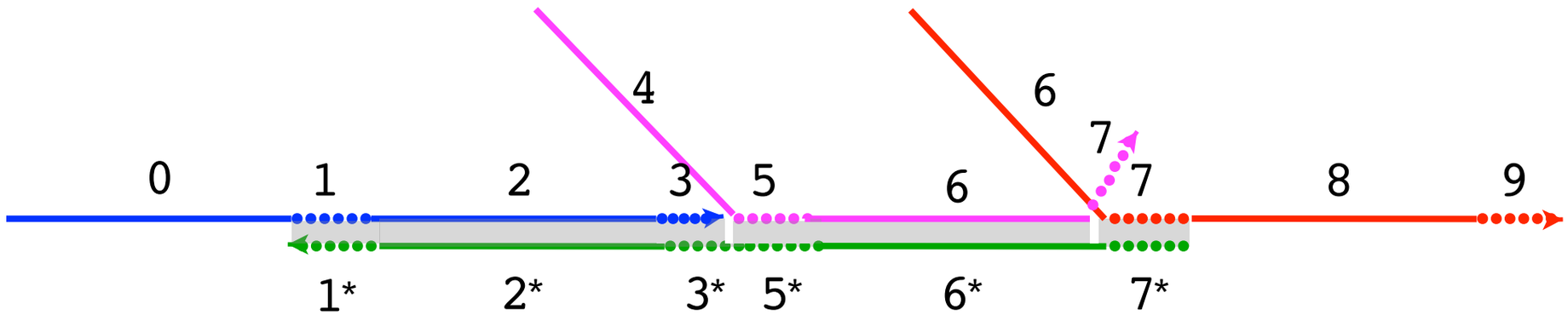

Strand Displacement Cascades Example: AND gate

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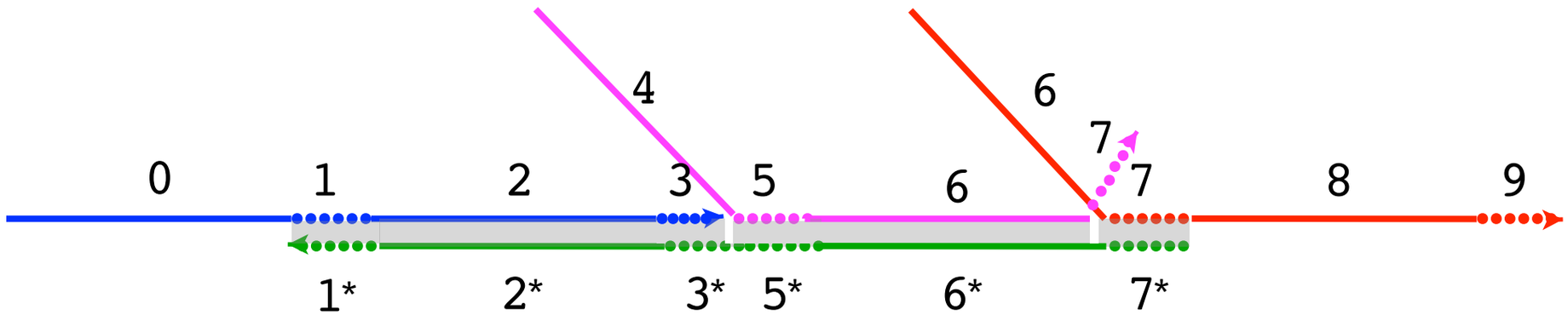
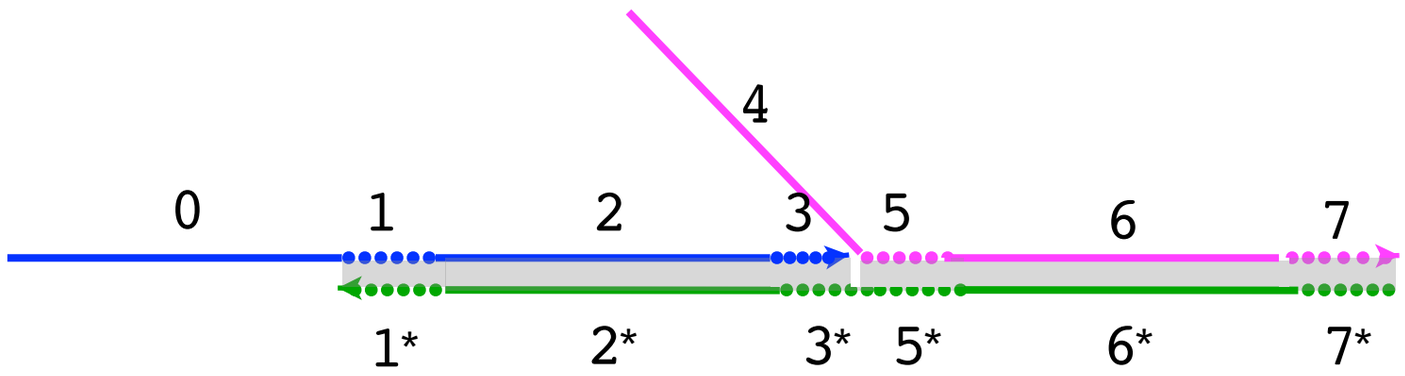


Strand Displacement Cascades Example: AND gate

release Z if and only if X and Y are present

waste

output Z



waste

Strand Displacement Cascades Example: AND gate

release Z if and only if X and Y are present

before

after

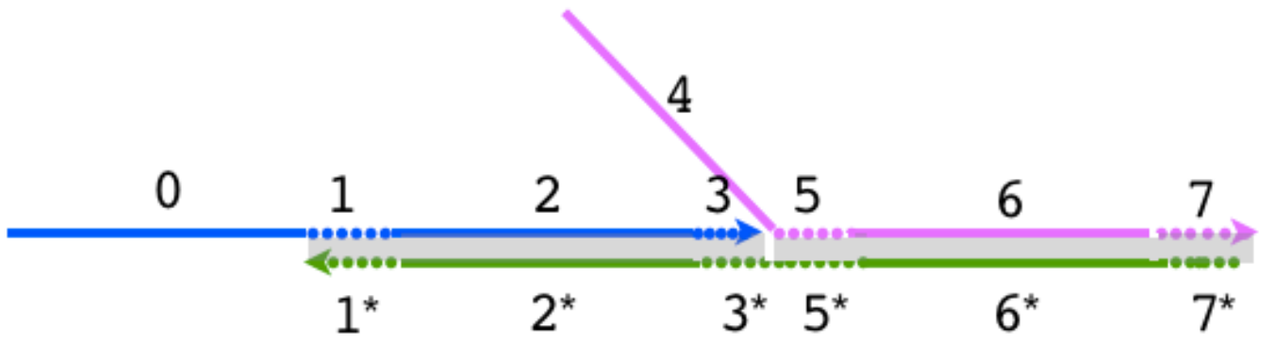
input X

output Z

input Y

waste

AND gate



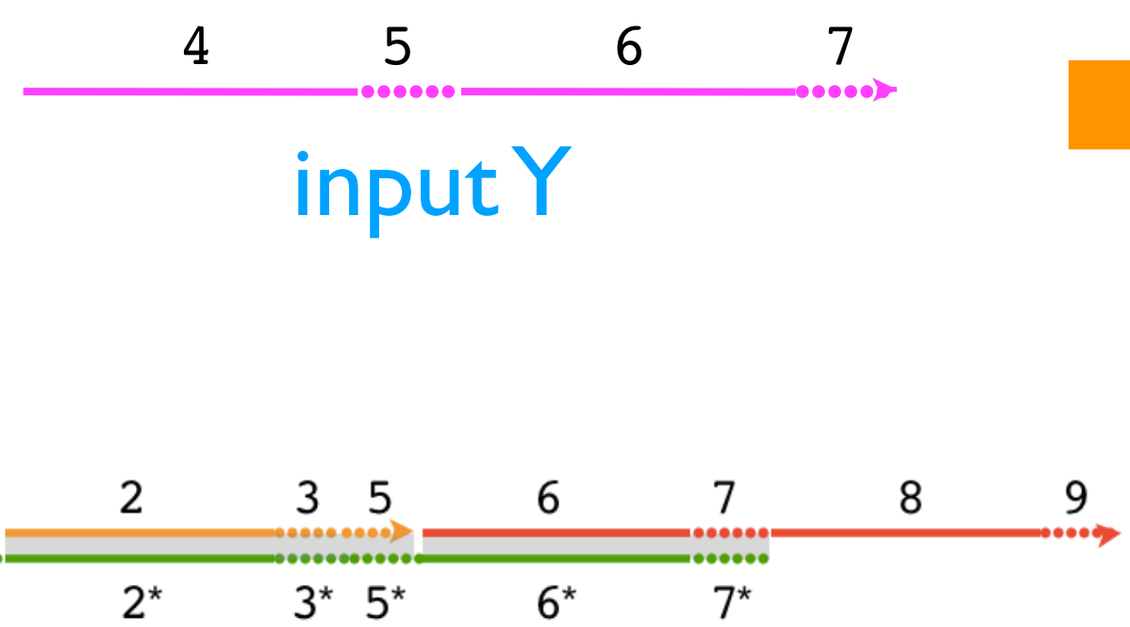
waste

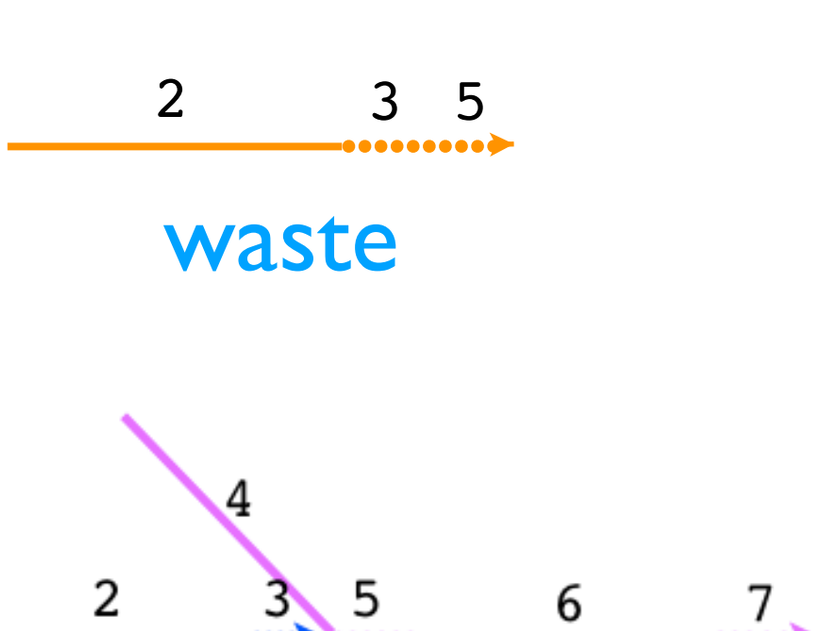
Strand Displacement Cascades Example: AND gate

release Z if and only if X and Y are present

before

after





Strand Displacement Cascades Example: AND gate

release Z if and only if X and Y are present

before

after

input X

Composable

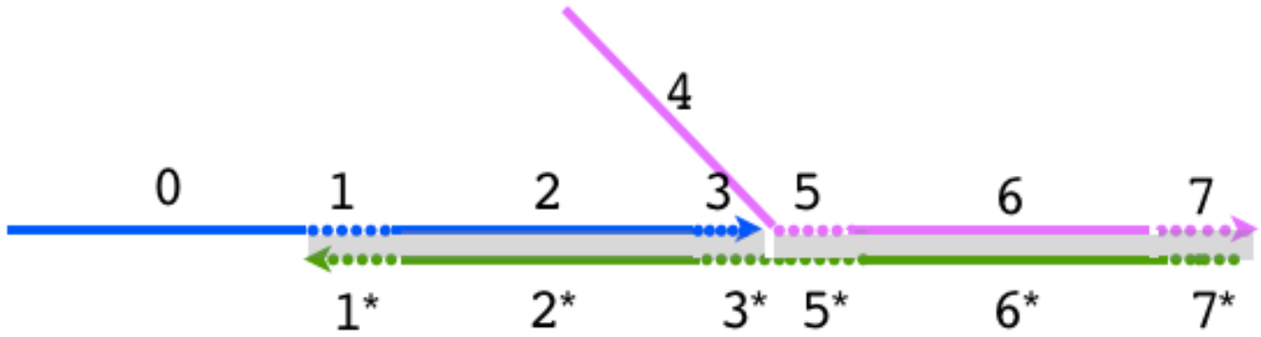
output Z

input Y

gate is “used up”

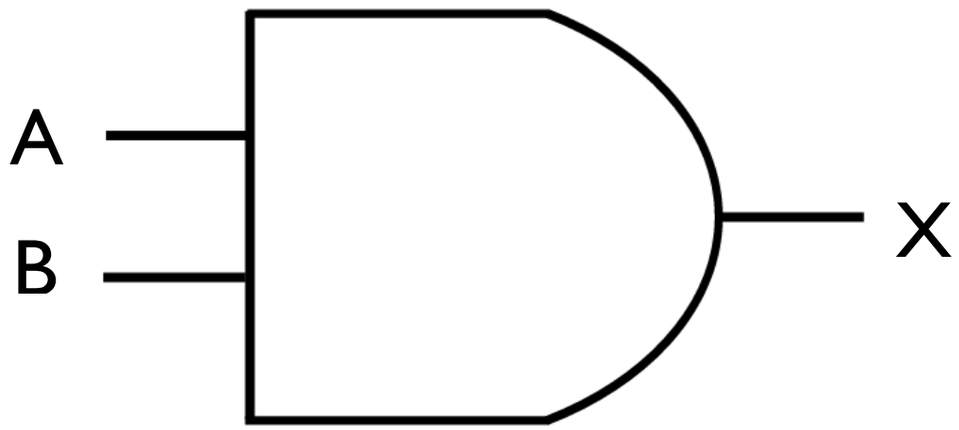
waste

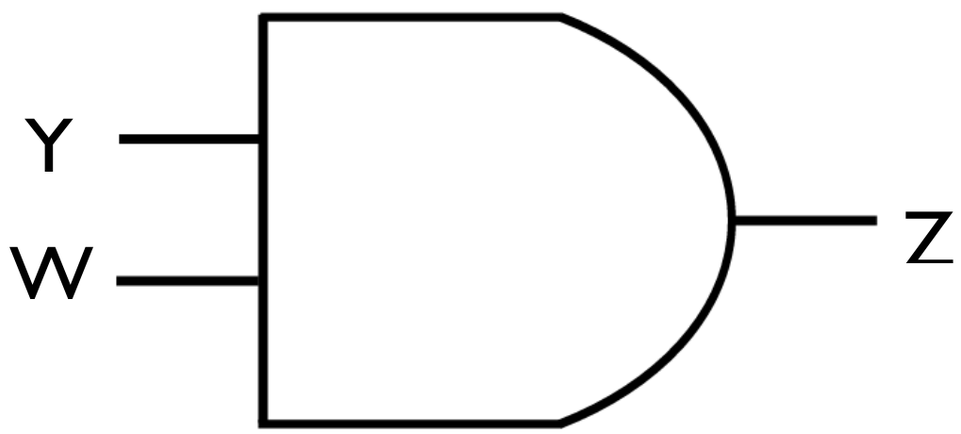
AND gate



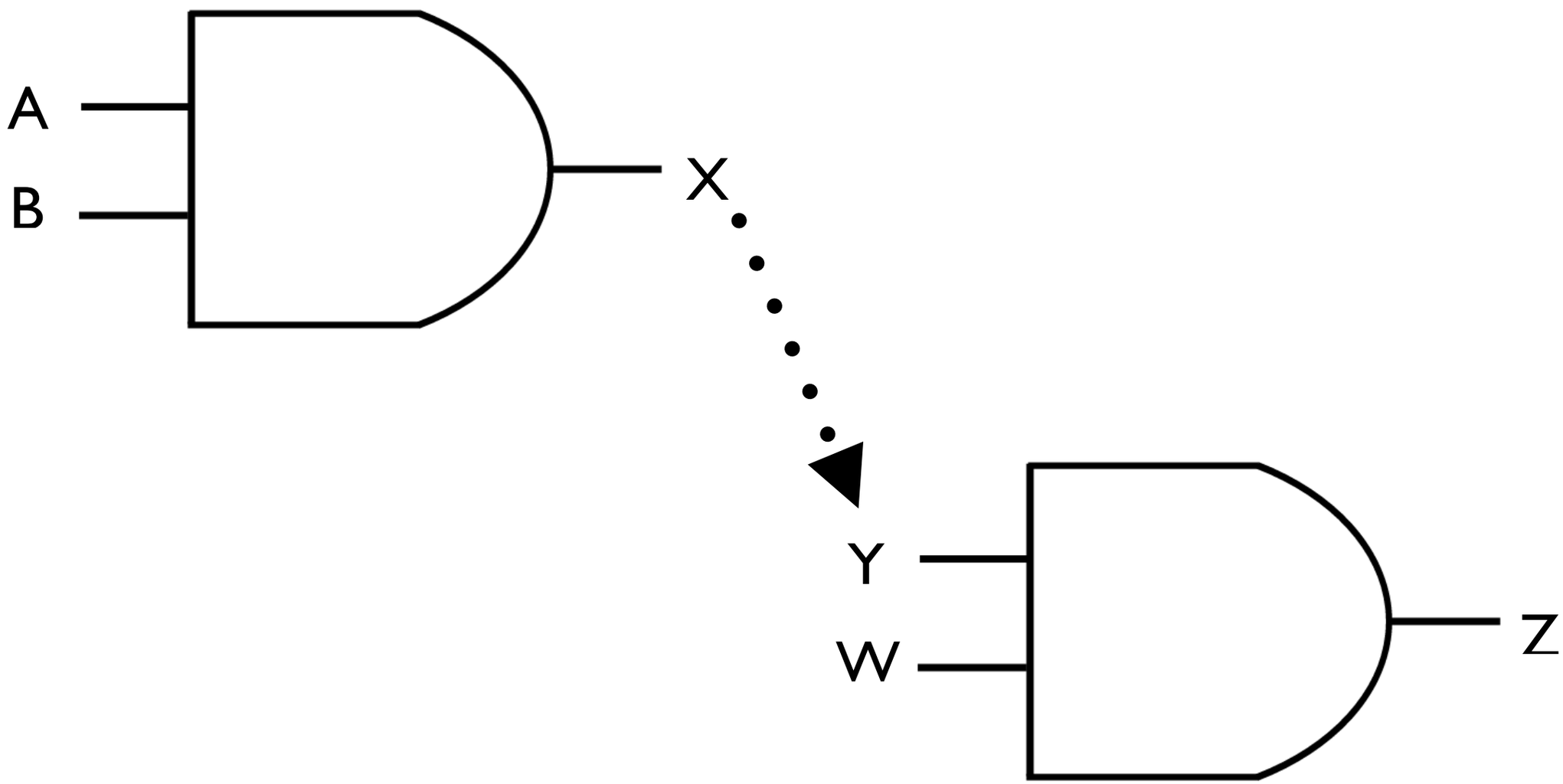
waste

Composing AND gates





Composing AND gates



We need a “wire”

Sequence Independence

Translator (a “wire”): X→Y

input X

output Y

Different coloring scheme to emphasize sequence (in)dependence!

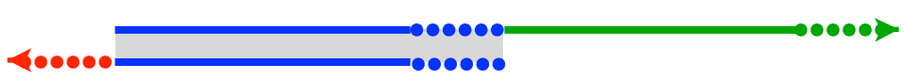
Sequence Independence

Translator (a “wire”): X→Y

input X

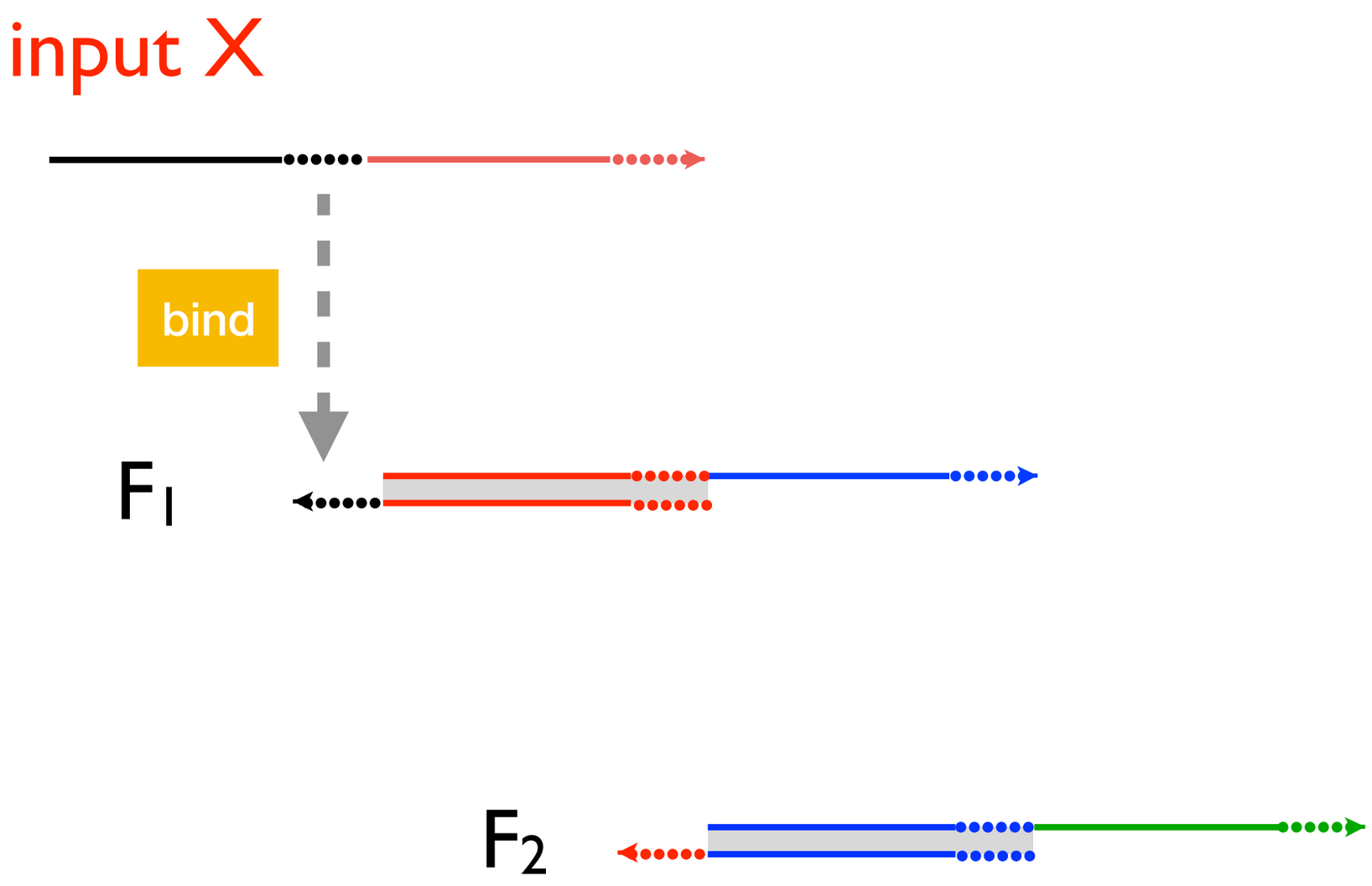
F_1

F_2



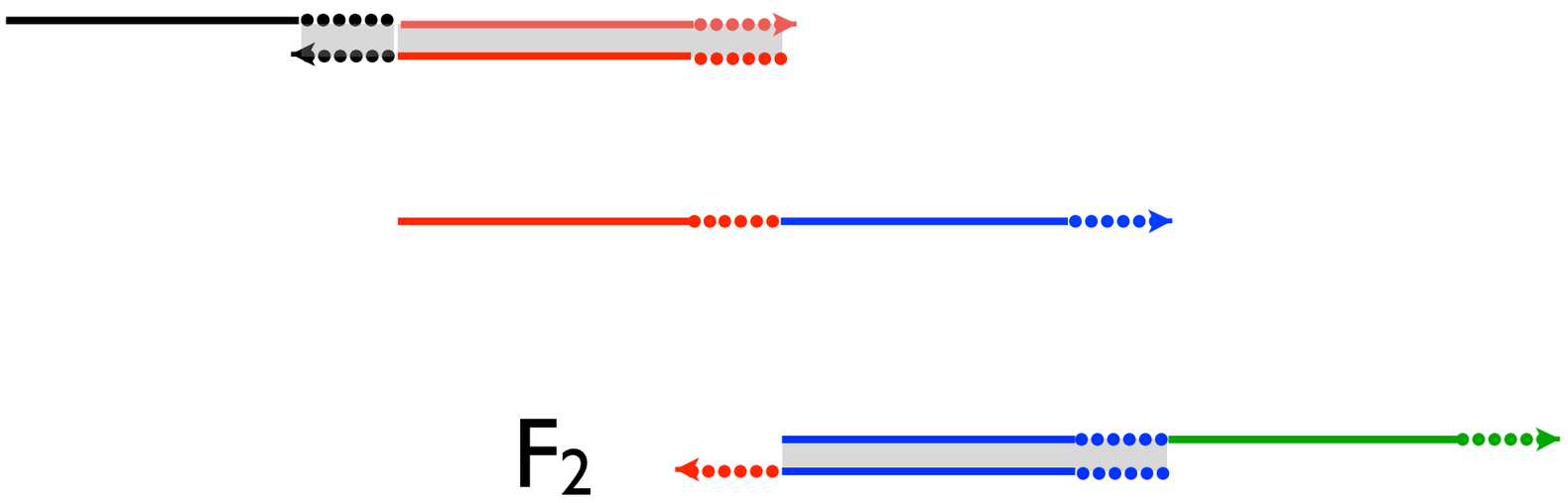
Sequence Independence

Translator (a “wire”): X→Y



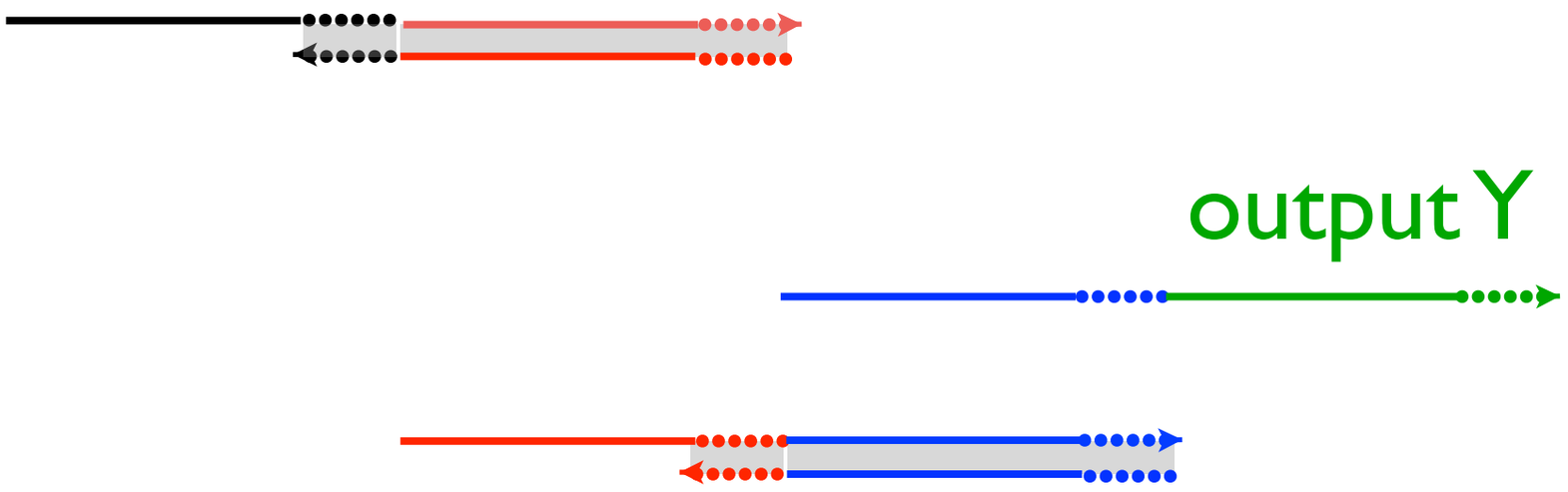
Sequence Independence

Translator (a “wire”): X→Y

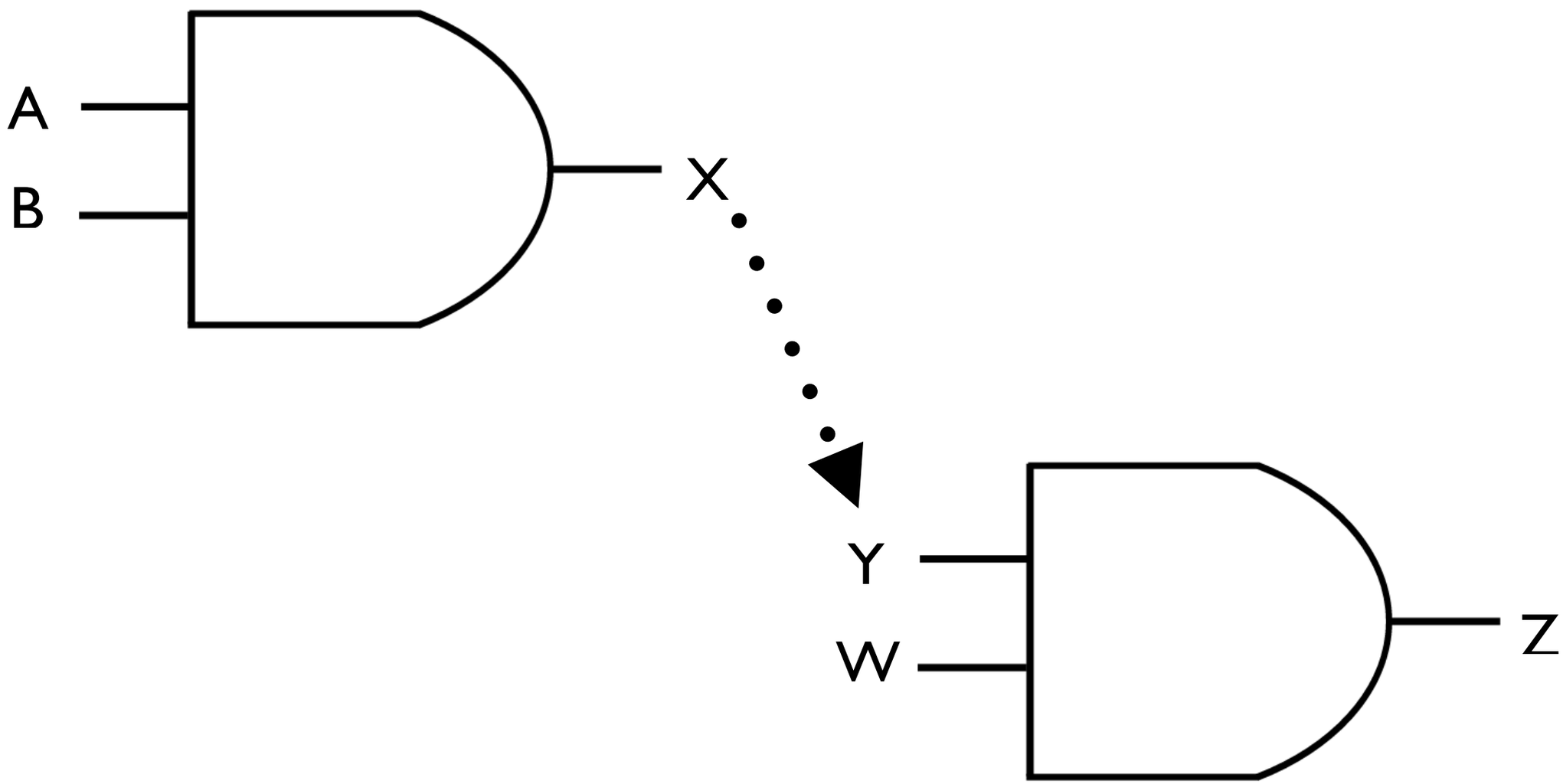


Sequence Independence

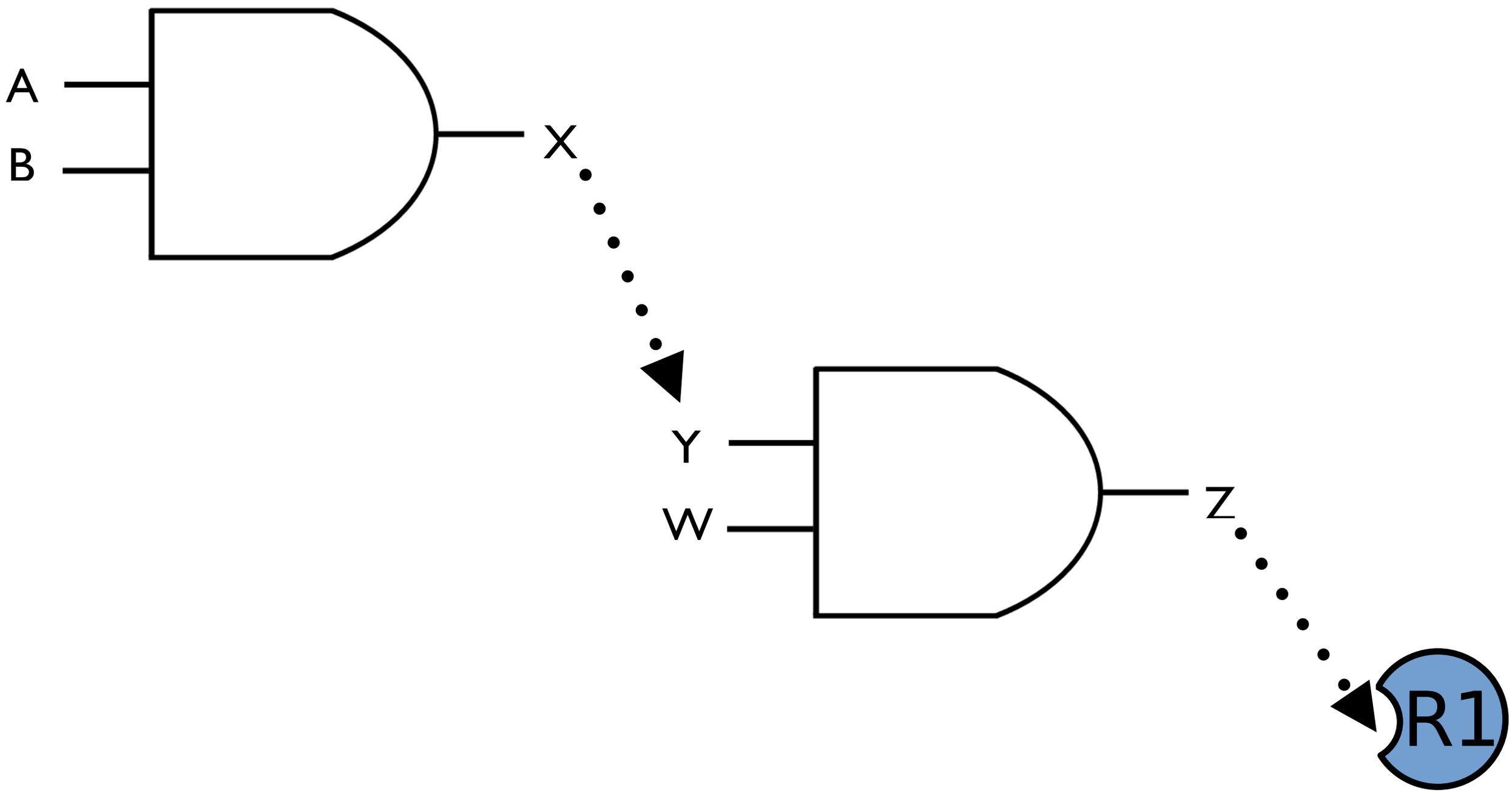
Translator (a “wire”): X→Y



Reading Output



Reading Output



FLUORESSENCE

Emission Peak 375-425nm	EX	EM
Calcein Blue	375	420
Cascade Blue®	400	420
DDS	341	414
Fast Blue	365	420
Fluoro-Gold™ (low pH)	323	408
Indo-1 (high calcium)	330	401
LysoSensor™ Blue (pH 5)	374	424
Mag-Indo-1	330	417
Pyrene	345	378

Emission Peak 425-455nm	EX	EM
4-Methylumbelliferone	360	449
7-Hydroxy-4-methylcoumarin (pH 9)	360	449
7-Amino-4-methylcoumarin	351	430
Alexa Fluor® 350	347	442
AMCA/AMCA-X	345	445
ANS	372	455
BFP (Blue Fluorescent Protein)	382	448
Calcofluor® White	350	440
Hoechst 33342 & 33258	352	449
Pacific Blue™	410	455
SITS	337	436
SpectrumBlue®	400	450

Emission Peak 455-490nm	EX	EM
1,5-IAEDANS	336	482
AmCyan	454	488
BOBO™-1	462	481
BO-PRO™-1	462	481
CFP (Cyan Fluorescent Protein)	434	477
Chromomycin A3	450	470
CPM	386	471
DAPI	358	461
Dansylchloride	380	475
Marina Blue®	365	460
POPO™-1	434	456
PO-PRO™	434	456
SpectrumAqua®	433	480
SYTO® 45	452	484
SYTOX® Blue	445	470

Emission Peak 490-510nm	EX	EM
Aniline Blue	370	509
Cy2®	489	506
DiO (DiOC(18) (3)	484	501
Fura-2 (high calcium)	335	505
GFP (enhanced)	488	508
GFP (sapphire)	395	508
LysoSensor™ Green (pH 5)	442	505
Mag-Fura-2	330	491
PBFI	334	504
Quinacrine Mustard	423	503
SYTO® 13	488	509
YO-PRO®-1	491	509
YOYO®-1	491	509
ZsGreen	492	505

Emission Peak 510-545nm	EX	EM
5-Carboxyfluorescein (5-FAM)	492	518
Acridine orange (+DNA)	500	526
Alexa Fluor® 430	434	540
Alexa Fluor® 488	495	519
BCECF (high pH)	503	528
BODIPY® FL	505	513
BTC	401/464	529
Calcein	494	517
Calcium Green-1™	506	531
Cascade Yellow™	402	545
CCF 2 (GeneBLAzer™)	402	520
Cl-NERF (low pH)	504	540
Dansyl cadaverine	335	518
DM-NERF (4.5-6.5 pH)	510	536
ELF®-97 alcohol	345	530
Eosin	524	544
Fluo-3	506	526
Fluo-4	494	516
Fluorescein (FITC)	494	518
Fluoro-Jade	475	525
FluoX®	494	519
Lucifer Yellow	428	536
LysoSensor™ Yellow/Blue (pH 4.2)	384	540
LysoTracker® Green	504	511
LysoTracker® Yellow	465	535
Magnesium Green™	506	531
Mithramycin	395	535
MitoTracker® Green	490	516
NBD (amine)	465	535
Oregon Green® 488	496	524
Oregon Green® 500	503	522
Oregon Green® 514	511	530
Qdot™ 525	UV	525
Rhodamine 110	496	520
Rhodamine 123	507	529
Rhodamine Green™	502	527
SBFI	334	525
Sodium Green™	507	535
SpectrumGreen®	497	524
SYTO® 11	508	527
SYTOX® Green	504	523
TO-PRO®-1	514	533
TOTO®-1	514	533
YFP (Yellow Fluorescent Protein)	513	527
ZsYellow	528	540

Emission Peak 545-570nm	EX	EM
TAMRA	542	568
Rhodamine 6G	525	555
6-JDE	525	555
Alexa Fluor® 532	531	554
Alexa Fluor® 555	553	568
ATTO-TAG™ CBQCA	465	560
Auramine O-Feulgen	460	550
BODIPY® 530/550	533	550
BODIPY® 558/568	558	568
DiI (DiIC(18)(3))	549	565
Erythrosin	529	554
Fluoro-Gold™ (high pH)	368	565
JOJO™-1	529	545
JO-PRO™-1	529	545
PKH26	551	567
Qdot™ 565	UV	565
Rhodamine Phalloidin	542	565
SNAFL®-2	525	546
Spectrum Gold®	530	555

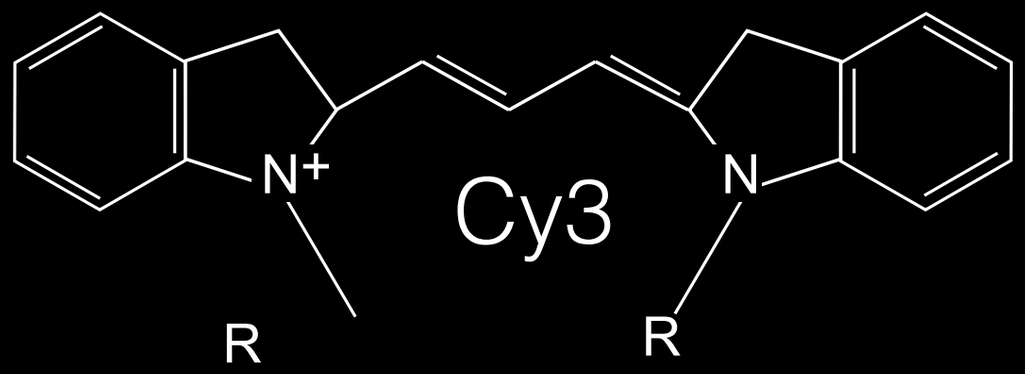
Emission Peak 570-585nm	EX	EM
Alexa Fluor® 546	556	573
BODIPY® 564/570	564	570
BODIPY® TMR	542	574
Calcium Orange™	549	576
CryptoLight CF-3™	545	576
CryptoLight CF-4™	555	580
Cy3®	550	570
Dispoxyl®	373	574
LOLO™-1	565	579
LO-PRO™-1	565	579
MitoTracker® Orange	551	576
POPO™-3	534	572
PO-PRO™-3	534	572
PyMPO	415	570
Pyronin Y	555	580
Qdot™ 585	UV	585
R-PE	488	575
Rhod-2	550	571
R-phycoerythrin (PE)	565	575
SYTOX® Orange	547	570

Emission Peak 585-595nm	EX	EM
ATTO-TAG™ FQ	486	591
BODIPY® 581/591	582	590
DiIe12 (Red Fluorescent Protein)	558	583
Lissamine rhodamine B	570	590
LysoTracker® Red	577	592
Resorufin	570	585
Rhodamine B	555	580
Rhodamine Red™	570	590
SpectrumOrange®	559	588
Tetramethylrhodamine (TRITC)	555	580

Emission Peak 595-605nm	EX	EM
5-ROX (carboxy-X-rhodamine)	574	602
Alexa Fluor® 568	579	604
AsRed	577	597
BOBO™-3	570	604
BO-PRO™-3	570	604
CryptoLight CF-5	566	597
Cy3.5®	581	596
Di-4 ANEPPS	488	605
Ethidium bromide	518	605
FM® 1-43	479	598
MitoTracker® Red	578	599
Qdot™ 605	UV	605
X-Rhodamine (XRITC)	580	605

Emission Peak 605-655nm	EX	EM
7-Aminoactinomycin D (7-AAD)	546	647
Acridine orange (+RNA)	460	650
Alexa Fluor® 594	591	618
Alexa Fluor® 633	632	647
BODIPY® 630/650-X	630	650
BODIPY® TR-X	589	617
Calcium Crimson™	590	615
C-phycocyanin	620	648
CryptoLight™ CF-1	575	641
CryptoLight™ CF-2	585	658
CryptoLight™ CF-6	566	615
CTC Formazan	450	630
Di-8 ANEPPS	468	635
DiA (4-Di-16-ASP)	491	613
Ethidium homodimer-1 (EthD-1)	528	617
Europium (III) Chloride	337	613
Fura Red™ (high calcium)	436	637
HcRed	591	613
Nile Red	549	628
PE-Alexa Fluor® 610	488	628
PE-Texas Red®	488	615
Propidium Iodide	536	617
Qdot™ 655	UV	655
RH 414	500	635
R-phycocyanin	618	642
SNAFL®-1 (high pH)	576	635
SNARF®-1 (high pH)	575	635
SpectrumRed®	587	612
SYTO® 17	621	634
Texas Red® / Texas Red®-X	595	615
YO-PRO®-3	612	631
YOYO®-3	612	631

Emission Peak 655-800nm	EX	EM
5-Carboxynaphthofluorescein	598	668
Alexa Fluor® 647	653	669
Alexa Fluor® 660	663	690
Alexa Fluor® 680	679	702
Alexa Fluor® 700	702	723
Alexa Fluor® 750	749	775
Allophycocyanin (APC)	650	660
APC	633/635	660
APC-Cy5.5	633/635	694
APC-Cy7	633/635	767
BODIPY® 650/665-X	650	665
Cy5®	649	670
Cy5.5®	675	694
Cy7®	743	767
DiD (DiIC (18)(5))	644	665
DiR (DiIC(18)(7))	750	779
PE-Alexa Fluor® 647	633/635	668
PE-Cy5	633/635	670
PE-Cy5.5	633/635	694
PE-Cy7	633/635	767
PerCP	488	675
PerCP/Cy5.5	488	710
SensiLight™ P1	545	666
SensiLight™ P3	614	662
SpectrumFRed®	655	675
TO-PRO®-3	642	660
TO-PRO®-5	748	768
TOTO®-3	642	660



FLUORESSENCE

Emission Peak 375-425nm	EX	EM
Calcein Blue	375	420
Cascade Blue®	400	420
DDS	341	414
Fast Blue	365	420
Fluoro-Gold™ (low pH)	323	408
Indo-1 (high calcium)	330	401
LysoSensor™ Blue (pH 5)	374	424
Mag-Indo-1	330	417
Pyrene	345	378

Emission Peak 425-455nm	EX	EM
4-Methylumbelliferone	360	449
7-Hydroxy-4-methylcoumarin (pH 9)	360	449
7-Amino-4-methylcoumarin	351	430
Alexa Fluor® 350	347	442
AMCA/AMCA-X	345	445
ANS	372	455
BFP (Blue Fluorescent Protein)	382	448
Calcofluor® White	350	440
Hoechst 33342 & 33258	352	449
Pacific Blue™	410	455
SITS	337	436
SpectrumBlue®	400	450

Emission Peak 455-490nm	EX	EM
1,5-IAEDANS	336	482
AmCyan	454	488
BOBO™-1	462	481
BO-PRO™-1	462	481
CFP (Cyan Fluorescent Protein)	434	477
Chromomycin A3	450	470
CPM	386	471
DAPI	358	461
Dansylchloride	380	475
Marina Blue®	365	460
POPO™-1	434	456
PO-PRO™	434	456
SpectrumAqua®	433	480
SYTO® 45	452	484
SYTOX® Blue	445	470

Emission Peak 490-510nm	EX	EM
Aniline Blue	370	509
Cy2®	489	506
DiO (DiOC(18) (3)	484	501
Fura-2 (high calcium)	335	505
GFP (enhanced)	488	508
GFP (sapphire)	395	508
LysoSensor™ Green (pH 5)	442	505
Mag-Fura-2	330	491
PBFI	334	504
Quinacrine Mustard	423	503
SYTO® 13	488	509
YO-PRO®-1	491	509
YOYO®-1	491	509
ZsGreen	492	505

Emission Peak 510-545nm	EX	EM
5-Carboxyfluorescein (5-FAM)	492	518
Acridine orange (+DNA)	500	526
Alexa Fluor® 430	434	540
Alexa Fluor® 488	495	519
BCECF (high pH)	503	528
BODIPY® FL	505	513
BTC	401/464	529
Calcein	494	517
Calcium Green-1™	506	531
Cascade Yellow™	402	545
CCF 2 (GeneBLAzer™)	402	520
Cl-NERF (low pH)	504	540
Dansyl cadaverine	335	518
DM-NERF (4.5-6.5 pH)	510	536
ELF®-97 alcohol	345	530
Eosin	524	544
Fluo-3	506	526
Fluo-4	494	516
Fluorescein (FITC)	494	518
Fluoro-Jade	475	525
FluoX®	494	519
Lucifer Yellow	428	536
LysoSensor™ Yellow/Blue (pH 4.2)	384	540
LysoTracker® Green	504	511
LysoTracker® Yellow	465	535
Magnesium Green™	506	531
Mithramycin	395	535
MitoTracker® Green	490	516
NBD (amine)	465	535
Oregon Green® 488	496	524
Oregon Green® 500	503	522
Oregon Green® 514	511	530
Qdot™ 525	UV	525
Rhodamine 110	496	520
Rhodamine 123	507	529
Rhodamine Green™	502	527
SBFI	334	525
Sodium Green™	507	535
SpectrumGreen®	497	524
SYTO® 11	508	527
SYTOX® Green	504	523
TO-PRO®-1	514	533
TOTO®-1	514	533
YFP (Yellow Fluorescent Protein)	513	527
ZsYellow	528	540

Emission Peak 545-570nm	EX	EM
TAMRA	542	568
Rhodamine 6G	525	555
6-JDE	525	555
Alexa Fluor® 532	531	554
Alexa Fluor® 555	553	568
ATTO-TAG™ CBQCA	465	560
Auramine O-Feulgen	460	550
BODIPY® 530/550	533	550
BODIPY® 558/568	558	568
DiI (DiIC(18)(3))	549	565
Erythrosin	529	554
Fluoro-Gold™ (high pH)	368	565
JOJO™-1	529	545
JO-PRO™-1	529	545
PKH26	551	567
Qdot™ 565	UV	565
Rhodamine Phalloidin	542	565
SNAFL®-2	525	546
Spectrum Gold®	530	555

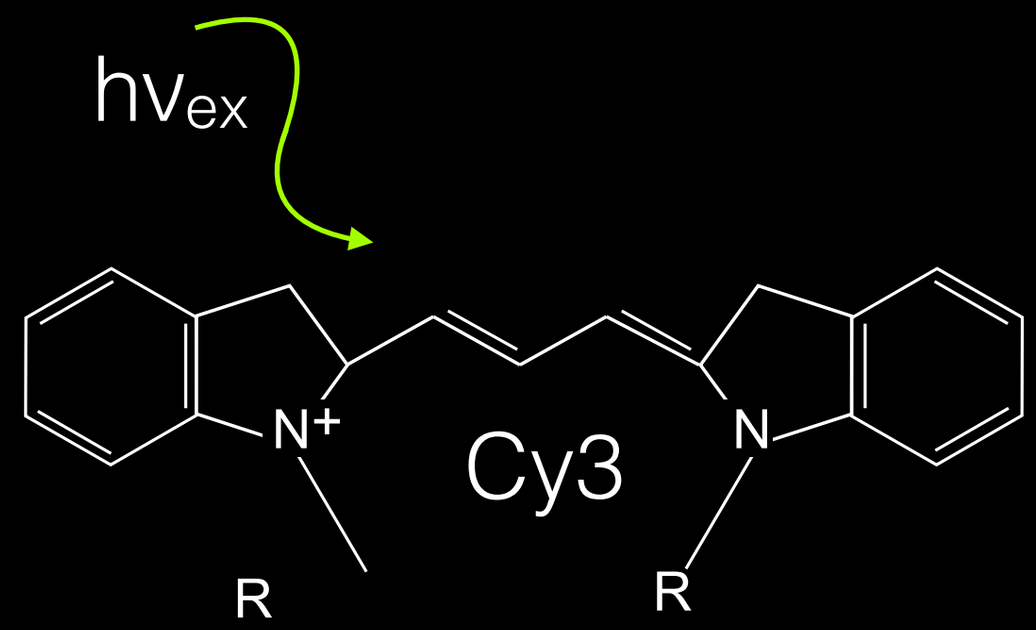
Emission Peak 570-585nm	EX	EM
Alexa Fluor® 546	556	573
BODIPY® 564/570	564	570
BODIPY® TMR	542	574
Calcium Orange™	549	576
CryptoLight CF-3™	545	576
CryptoLight CF-4™	555	580
Cy3®	550	570
Dispoxyl®	373	574
LOLO™-1	565	579
LO-PRO™-1	565	579
MitoTracker® Orange	551	576
POPO™-3	534	572
PO-PRO™-3	534	572
PyMPO	415	570
Pyronin Y	555	580
Qdot™ 585	UV	585
R-PE	488	575
Rhod-2	550	571
R-phycoerythrin (PE)	565	575
SYTOX® Orange	547	570

Emission Peak 585-595nm	EX	EM
ATTO-TAG™ FQ	486	591
BODIPY® 581/591	582	590
DiIe12 (Red Fluorescent Protein)	558	583
Lissamine rhodamine B	570	590
LysoTracker® Red	577	592
Resorufin	570	585
Rhodamine B	555	580
Rhodamine Red™	570	590
SpectrumOrange®	559	588
Tetramethylrhodamine (TRITC)	555	580

Emission Peak 595-605nm	EX	EM
5-ROX (carboxy-X-rhodamine)	574	602
Alexa Fluor® 568	579	604
AsRed	577	597
BOBO™-3	570	604
BO-PRO™-3	570	604
CryptoLight CF-5	566	597
Cy3.5®	581	596
Di-4 ANEPPS	488	605
Ethidium bromide	518	605
FM® 1-43	479	598
MitoTracker® Red	578	599
Qdot™ 605	UV	605
X-Rhodamine (XRITC)	580	605

Emission Peak 605-655nm	EX	EM
7-Aminoactinomycin D (7-AAD)	546	647
Acridine orange (+RNA)	460	650
Alexa Fluor® 594	591	618
Alexa Fluor® 633	632	647
BODIPY® 630/650-X	630	650
BODIPY® TR-X	589	617
Calcium Crimson™	590	615
C-phycocyanin	620	648
CryptoLight™ CF-1	575	641
CryptoLight™ CF-2	585	658
CryptoLight™ CF-6	566	615
CTC Formazan	450	630
Di-8 ANEPPS	468	635
DiA (4-Di-16-ASP)	491	613
Ethidium homodimer-1 (EthD-1)	528	617
Europium (III) Chloride	337	613
Fura Red™ (high calcium)	436	637
HcRed	591	613
Nile Red	549	628
PE-Alexa Fluor® 610	488	628
PE-Texas Red®	488	615
Propidium Iodide	536	617
Qdot™ 655	UV	655
RH 414	500	635
R-phycocyanin	618	642
SNAFL®-1 (high pH)	576	635
SNARF®-1 (high pH)	575	635
SpectrumRed®	587	612
SYTO® 17	621	634
Texas Red® / Texas Red®-X	595	615
YO-PRO®-3	612	631
YOYO®-3	612	631

Emission Peak 655-800nm	EX	EM
5-Carboxynaphthofluorescein	598	668
Alexa Fluor® 647	653	669
Alexa Fluor® 660	663	690
Alexa Fluor® 680	679	702
Alexa Fluor® 700	702	723
Alexa Fluor® 750	749	775
Allophycocyanin (APC)	650	660
APC	633/635	660
APC-Cy5.5	633/635	694
APC-Cy7	633/635	767
BODIPY® 650/665-X	650	665
Cy5®	649	670
Cy5.5®	675	694
Cy7®	743	767
DiD (DiIC (18)(5))	644	665
DiR (DiIC(18)(7))	750	779
PE-Alexa Fluor® 647	633/635	668
PE-Cy5	633/635	670
PE-Cy5.5	633/635	694
PE-Cy7	633/635	767
PerCP	488	675
PerCP/Cy5.5	488	710
SensiLight™ P1	545	666
SensiLight™ P3	614	662
SpectrumFRed®	655	675
TO-PRO®-3	642	660
TO-PRO®-5	748	768
TOTO®-3	642	660



FLUORESSENCE

Emission Peak 375-425nm	EX	EM
Calcein Blue	375	420
Cascade Blue®	400	420
DIDS	341	414
Fast Blue	365	420
Fluoro-Gold™ (low pH)	323	408
Indo-1 (high calcium)	330	401
LysoSensor™ Blue (pH 5)	374	424
Mag-Indo-1	330	417
Pyrene	345	378

Emission Peak 425-455nm	EX	EM
4-Methylumbelliferone	360	449
7-Hydroxy-4-methylcoumarin (pH 9)	360	449
7-Amino-4-methylcoumarin	351	430
Alexa Fluor® 350	347	442
AMCA/AMCA-X	345	445
ANS	372	455
BFP (Blue Fluorescent Protein)	382	448
Calcofluor® White	350	440
Hoechst 33342 & 33258	352	449
Pacific Blue™	410	455
SITS	337	436
SpectrumBlue®	400	450

Emission Peak 455-490nm	EX	EM
1,5-IAEDANS	336	482
AmCyan	454	488
BOBO™-1	462	481
BO-PRO™-1	462	481
CFP (Cyan Fluorescent Protein)	434	477
Chromomycin A3	450	470
CPM	386	471
DAPI	358	461
Dansylchloride	380	475
Marina Blue®	365	460
POPO™-1	434	456
PO-PRO™	434	456
SpectrumAqua®	433	480
SYTO® 45	452	484
SYTOX® Blue	445	470

Emission Peak 490-510nm	EX	EM
Aniline Blue	370	509
Cy2®	489	506
DiO (DiOC(18) (3)	484	501
Fura-2 (high calcium)	335	505
GFP (enhanced)	488	508
GFP (sapphire)	395	508
LysoSensor™ Green (pH 5)	442	505
Mag-Fura-2	330	491
PBFI	334	504
Quinacrine Mustard	423	503
SYTO® 13	488	509
YO-PRO®-1	491	509
YOYO®-1	491	509
ZsGreen	492	505

Emission Peak 510-545nm	EX	EM
5-Carboxyfluorescein (5-FAM)	492	518
Acridine orange (+DNA)	500	526
Alexa Fluor® 430	434	540
Alexa Fluor® 488	495	519
BCECF (high pH)	503	528
BODIPY® FL	505	513
BTC	401/464	529
Calcein	494	517
Calcium Green-1™	506	531
Cascade Yellow™	402	545
CCF2 (GeneBLAzer™)	402	520
Cl-NERF (low pH)	504	540
Dansyl cadaverine	335	518
DM-NERF (4.5-6.5 pH)	510	536
ELF®-97 alcohol	345	530
Eosin	524	544
Fluo-3	506	526
Fluo-4	494	516
Fluorescein (FITC)	494	518
Fluoro-Jade	475	525
FluoX®	494	519
Lucifer Yellow	428	536
LysoSensor™ Yellow/Blue (pH 4.2)	384	540
LysoTracker® Green	504	511
LysoTracker® Yellow	465	535
Magnesium Green™	506	531
Mithramycin	395	535
MitoTracker® Green	490	516
NBD (amine)	465	535
Oregon Green® 488	496	524
Oregon Green® 500	503	522
Oregon Green® 514	511	530
Qdot™ 525	UV	525
Rhodamine 110	496	520
Rhodamine 123	507	529
Rhodamine Green™	502	527
SBFI	334	525
Sodium Green™	507	535
SpectrumGreen®	497	524
SYTO® 11	508	527
SYTOX® Green	504	523
TO-PRO®-1	514	533
TOTO®-1	514	533
YFP (Yellow Fluorescent Protein)	513	527
ZsYellow	528	540

Emission Peak 545-570nm	EX	EM
TAMRA	542	568
Rhodamine 6G	525	555
6-JDE	525	555
Alexa Fluor® 532	531	554
Alexa Fluor® 555	553	568
ATTO-TAG™ CBQCA	465	560
Auramine O-Feulgen	460	550
BODIPY® 530/550	533	550
BODIPY® 558/568	558	568
DiI (DiIC(18)(3))	549	565
Erythrosin	529	554
Fluoro-Gold™ (high pH)	368	565
JOJO™-1	529	545
JO-PRO™-1	529	545
PKH26	551	567
Qdot™ 565	UV	565
Rhodamine Phalloidin	542	565
SNAFL®-2	525	546
Spectrum Gold®	530	555

Emission Peak 570-585nm	EX	EM
Alexa Fluor® 546	556	573
BODIPY® 564/570	564	570
BODIPY® TMR	542	574
Calcium Orange™	549	576
CryptoLight CF-3™	545	576
CryptoLight CF-4™	555	580
Cy3®	550	570
Dispoxyl®	373	574
LOLO™-1	565	579
LO-PRO™-1	565	579
MitoTracker® Orange	551	576
POPO™-3	534	572
PO-PRO™-3	534	572
PyMPO	415	570
Pyronin Y	555	580
Qdot™ 585	UV	585
R-PE	488	575
Rhod-2	550	571
R-phycoerythrin (PE)	565	575
SYTOX® Orange	547	570

Emission Peak 585-595nm	EX	EM
ATTO-TAG™ FQ	486	591
BODIPY® 581/591	582	590
DiIe12 (Red Fluorescent Protein)	558	583
Lissamine rhodamine B	570	590
LysoTracker® Red	577	592
Resorufin	570	585
Rhodamine B	555	580
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SpectrumOrange®	559	588
Tetramethylrhodamine (TRITC)	555	580

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5-ROX (carboxy-X-rhodamine)	574	602
Alexa Fluor® 568	579	604
AsRed	577	597
BOBO™-3	570	604
BO-PRO™-3	570	604
CryptoLight CF-5	566	597
Cy3.5®	581	596
Di-4 ANEPPS	488	605
Ethidium bromide	518	605
FM® 1-43	479	598
MitoTracker® Red	578	599
Qdot™ 605	UV	605
X-Rhodamine (XRITC)	580	605

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BODIPY® TR-X	589	617
Calcium Crimson™	590	615
C-phycocyanin	620	648
CryptoLight™ CF-1	575	641
CryptoLight™ CF-2	585	658
CryptoLight™ CF-6	566	615
CTC Formazan	450	630
Di-8 ANEPPS	468	635
DiA (4-Di-16-ASP)	491	613
Ethidium homodimer-1 (EthD-1)	528	617
Europium (III) Chloride	337	613
Fura Red™ (high calcium)	436	637
HcRed	591	613
Nile Red	549	628
PE-Alexa Fluor® 610	488	628
PE-Texas Red®	488	615
Propidium Iodide	536	617
Qdot™ 655	UV	655
RH 414	500	635
R-phycocyanin	618	642
SNAFL®-1 (high pH)	576	635
SNARF®-1 (high pH)	575	635
SpectrumRed®	587	612
SYTO® 17	621	634
Texas Red® / Texas Red®-X	595	615
YO-PRO®-3	612	631
YOYO®-3	612	631

Emission Peak 655-800nm	EX	EM
5-Carboxynaphthofluorescein	598	668
Alexa Fluor® 647	653	669
Alexa Fluor® 660	663	690
Alexa Fluor® 680	679	702
Alexa Fluor® 700	702	723
Alexa Fluor® 750	749	775
Allophycocyanin (APC)	650	660
APC	633/635	660
APC-Cy5.5	633/635	694
APC-Cy7	633/635	767
BODIPY® 650/665-X	650	665
Cy5®	649	670
Cy5.5®	675	694
Cy7®	743	767
DiD (DiIC (18)(5))	644	665
DiR (DiIC(18)(7))	750	779
PE-Alexa Fluor® 647	633/635	668
PE-Cy5	633/635	670
PE-Cy5.5	633/635	694
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PerCP/Cy5.5	488	710
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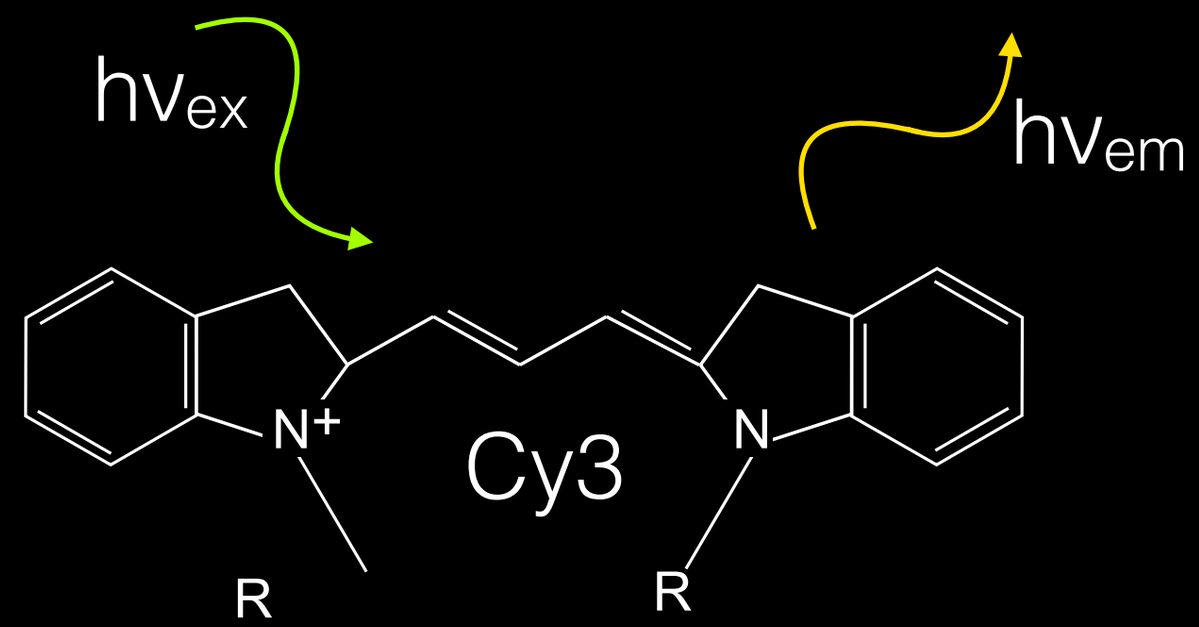
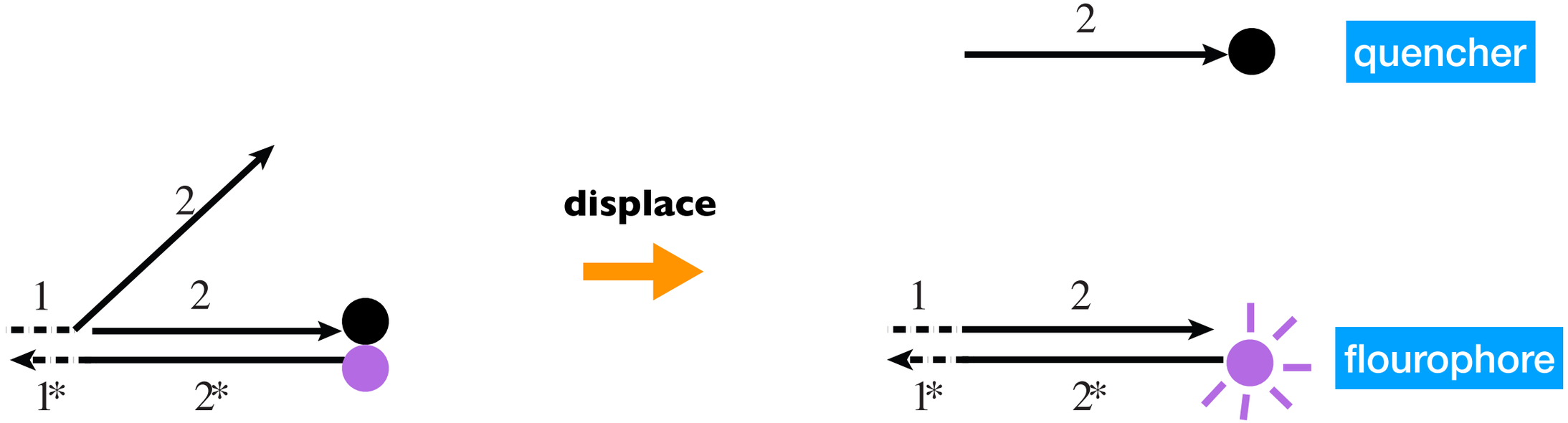
SpectrumFRed®	655	675
TO-PRO®-3	642	660
TO-PRO®-5	748	768
TOTO®-3	642	660

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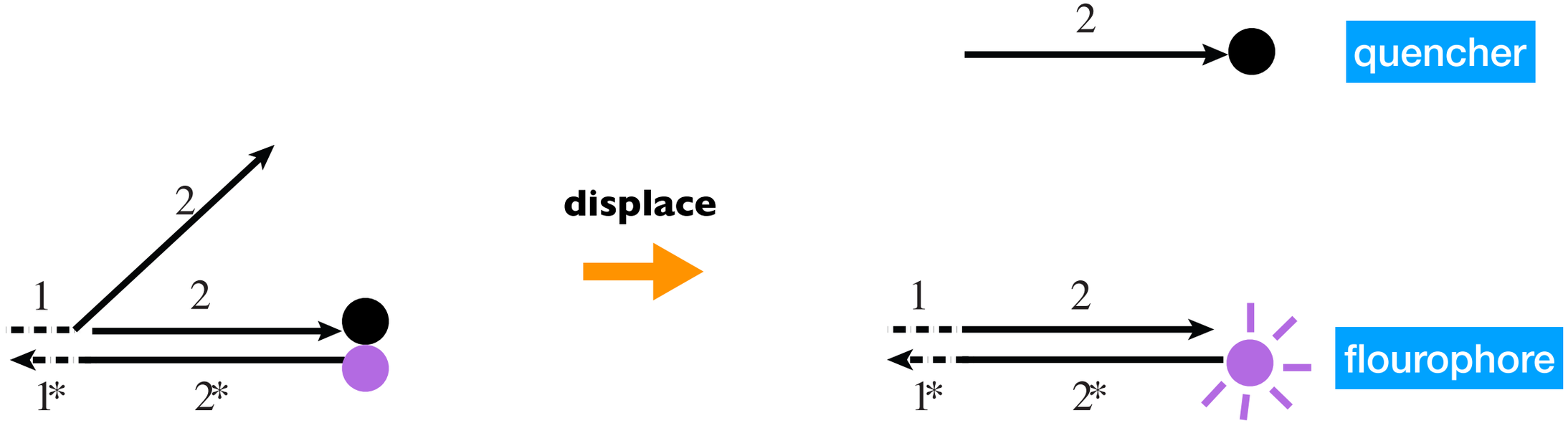
flourophore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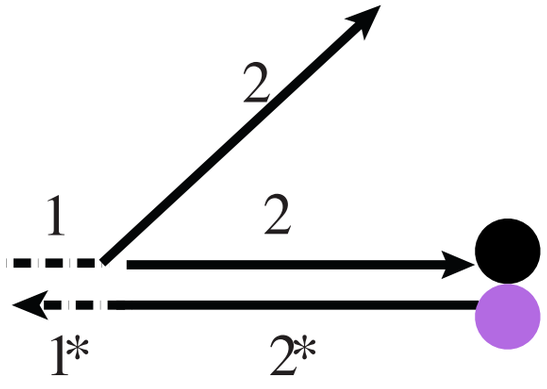
CYTATION 5



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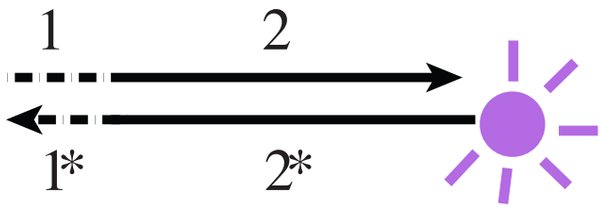
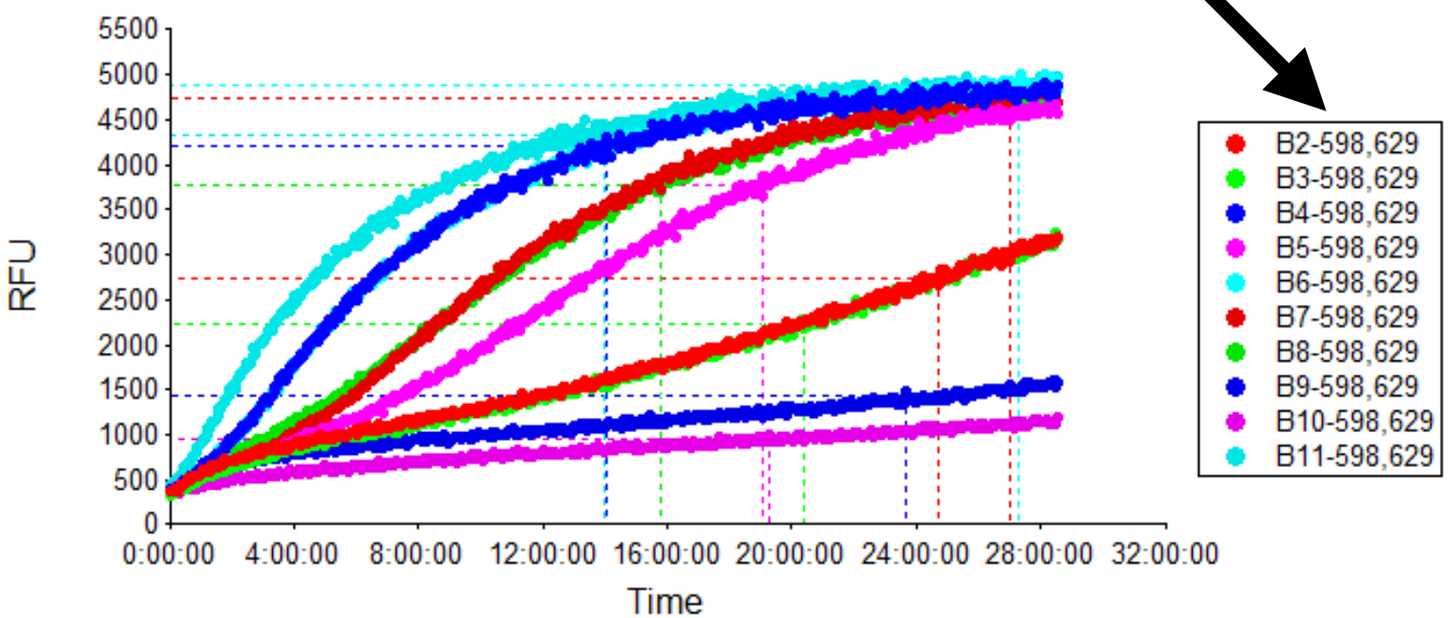


flourophore

read many different samples



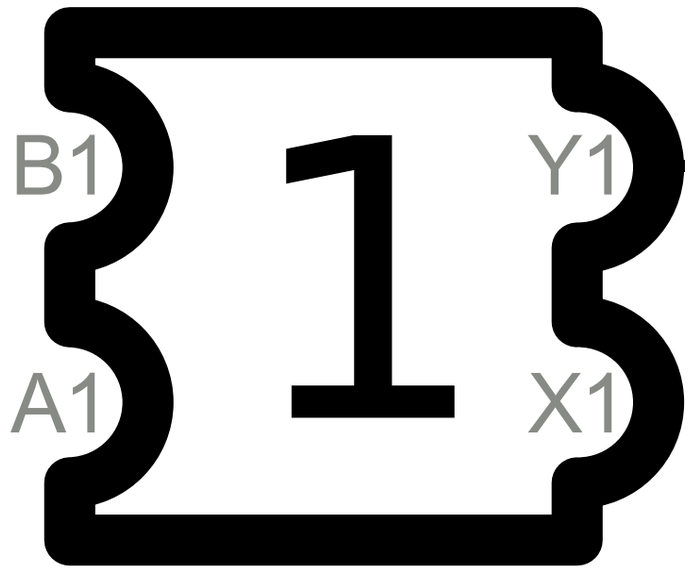
A reaction gate

$$A_1 + B_1 \rightarrow X_1 + Y_1$$

This *universal component*
can realize a number of logic gates

A reaction gate

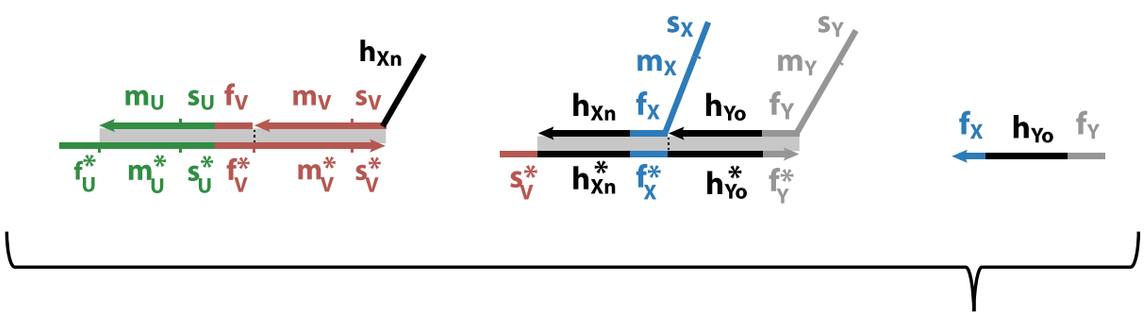
$$A_1 + B_1 \rightarrow X_1 + Y_1$$

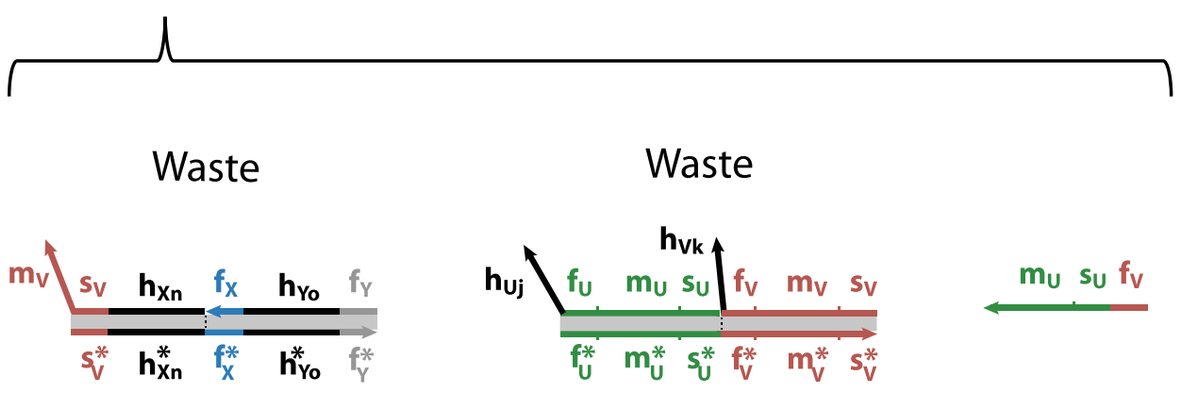


This *universal component*
can realize a number of logic gates

To implement this:

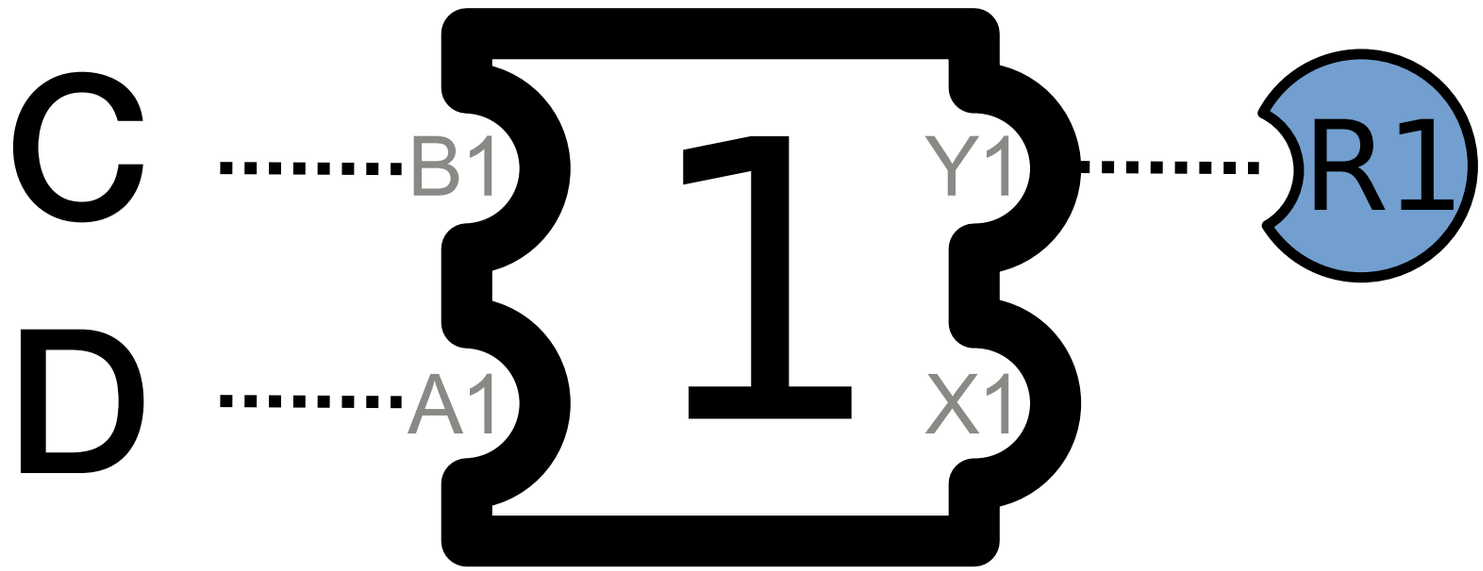
We start with large excess of DNA complexes (fuels) that mediate the reaction:





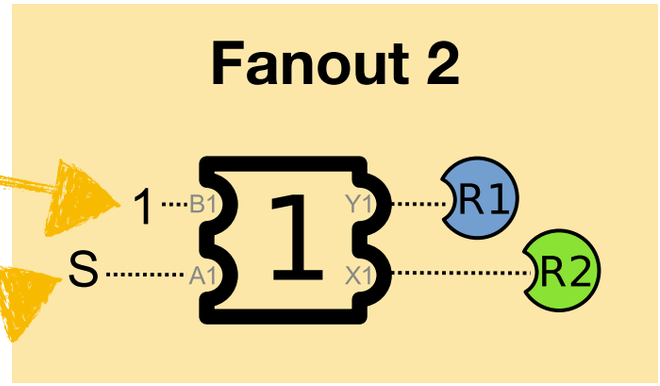
adopted from Srinivas et al, Science, 2017

AND gate



Signal Fanout Gates

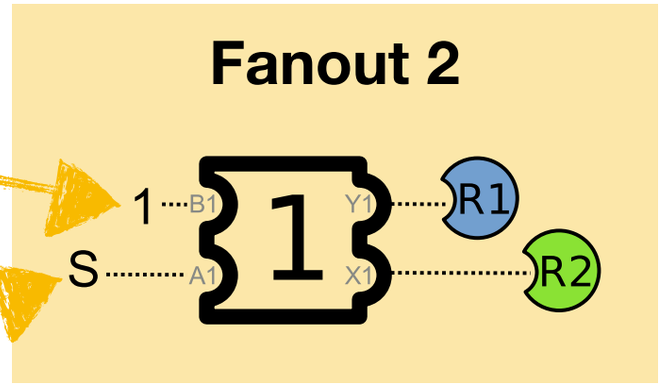
5’-GCACTTTTACATTTACATTACATTTAC-3’
fuel (always present)



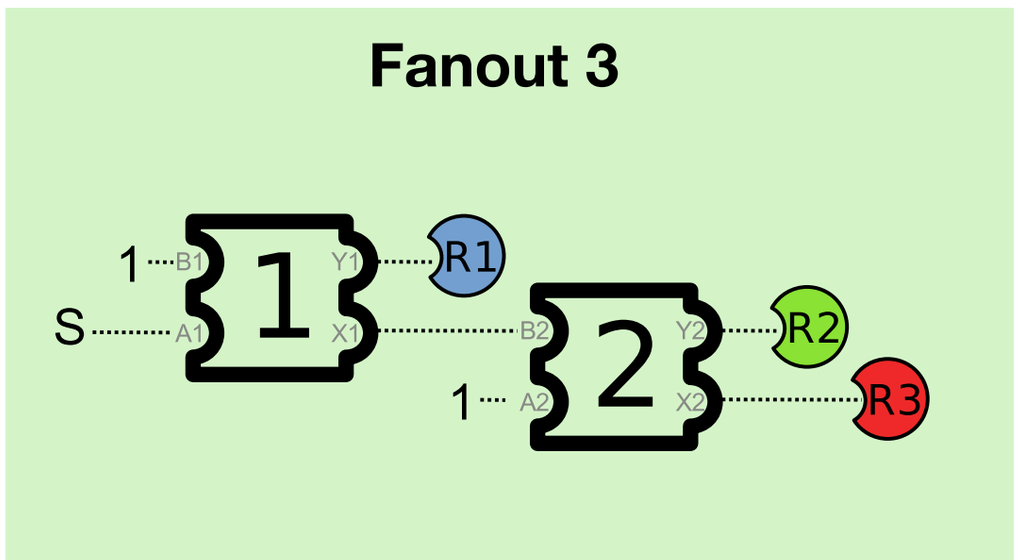
5’-ACAGATCACCAGATCATTATCAGAG-3’
strand representing signal S

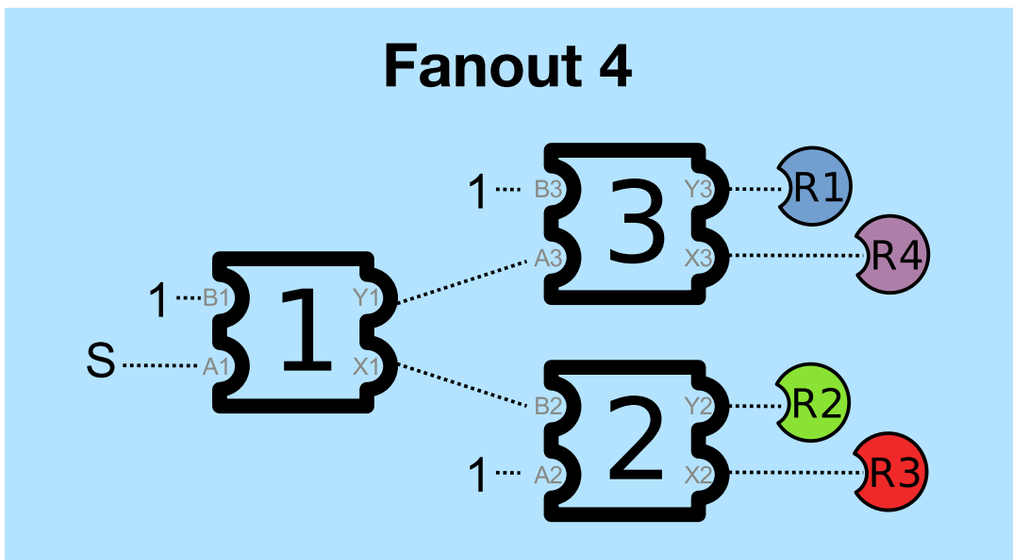
Signal Fanout Gates

5’-GCACTTTTACATTTACATTACATTTAC-3’
fuel (always present)

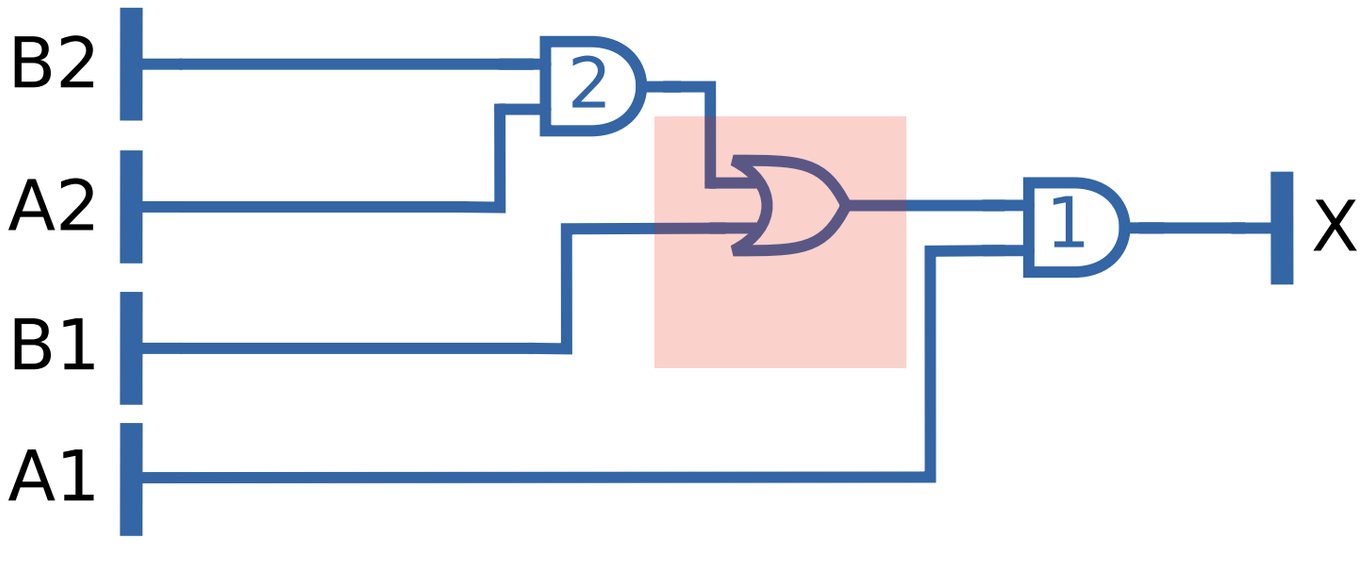


5’-ACAGATCACCAGATCATTATCAGAG-3’
strand representing signal S



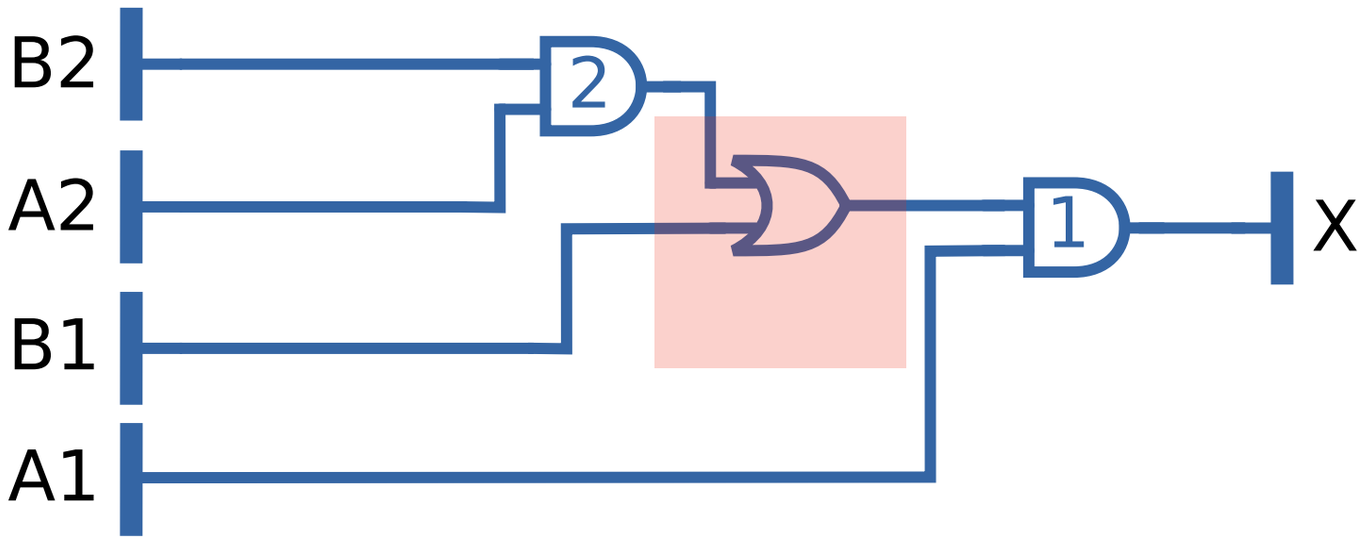


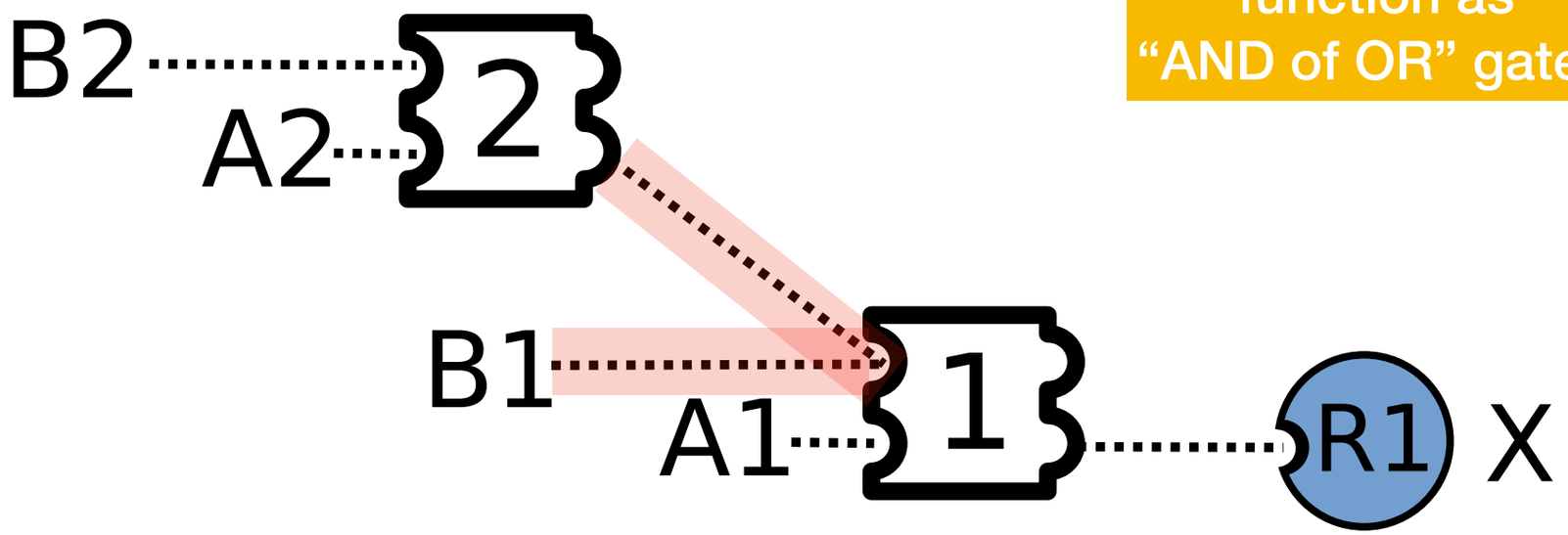
Handling OR gates



AND(A1, OR(B1, AND(A2, B2)))

Handling OR gates

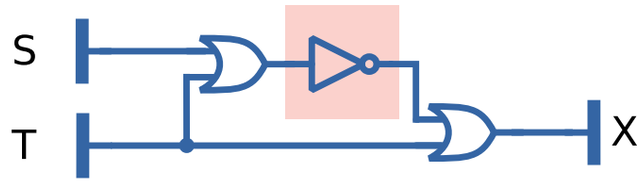




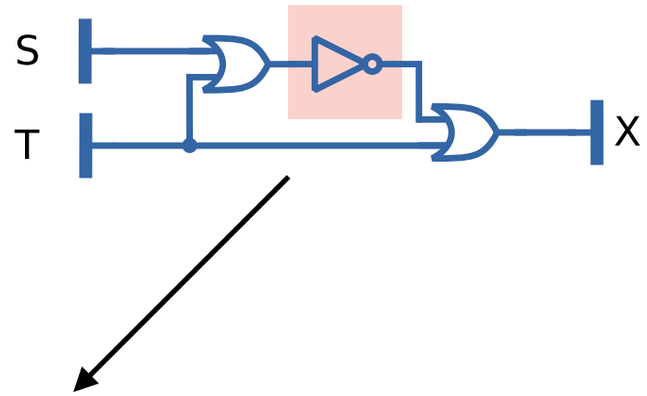
Reaction gates function as “AND of OR” gates

AND(A1, OR(B1, AND(A2, B2)))

Handling NOT gates



Handling NOT gates



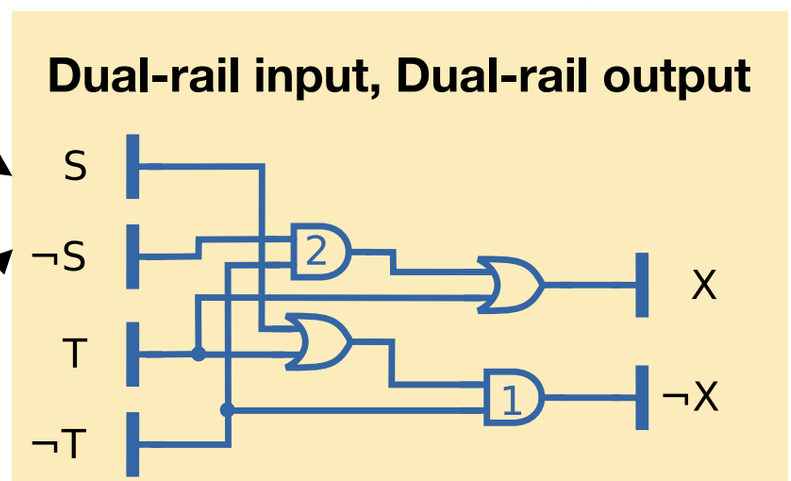
5′-CCCCCCC-3′

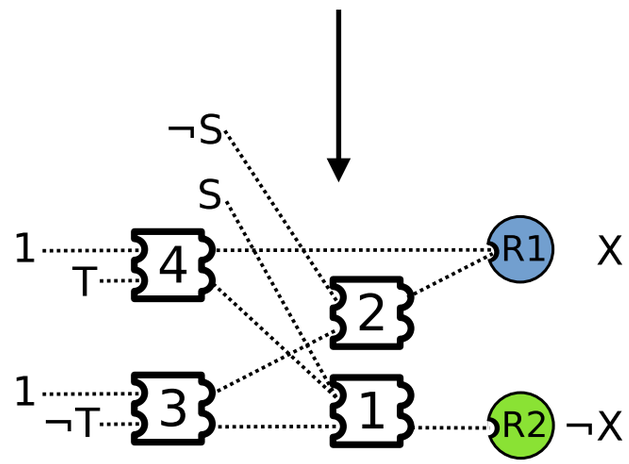
S

5′-TTTTTTT-3′

¬S

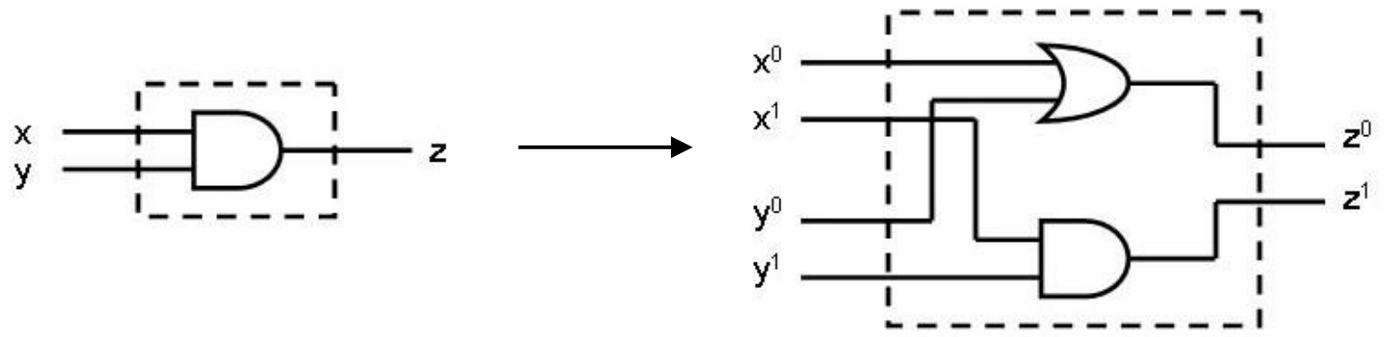
Dual-rail input, Dual-rail output



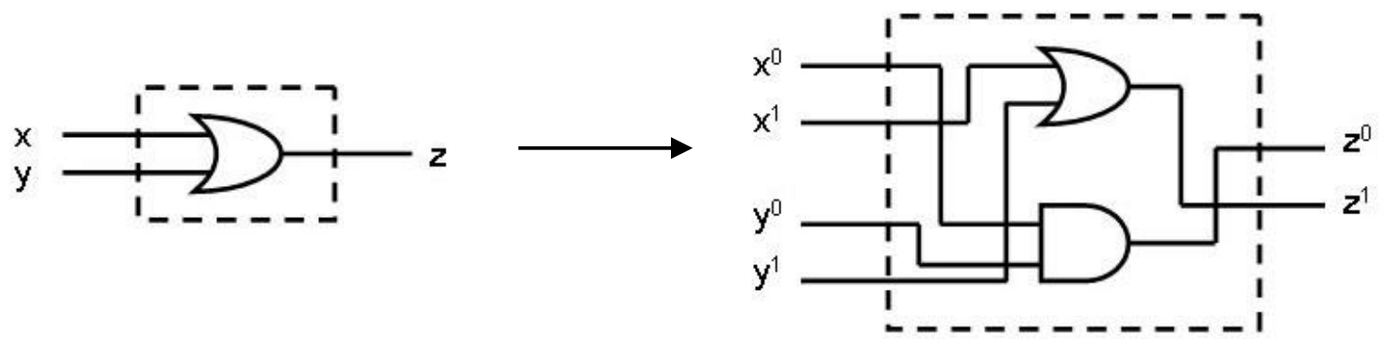


Dual rail logic

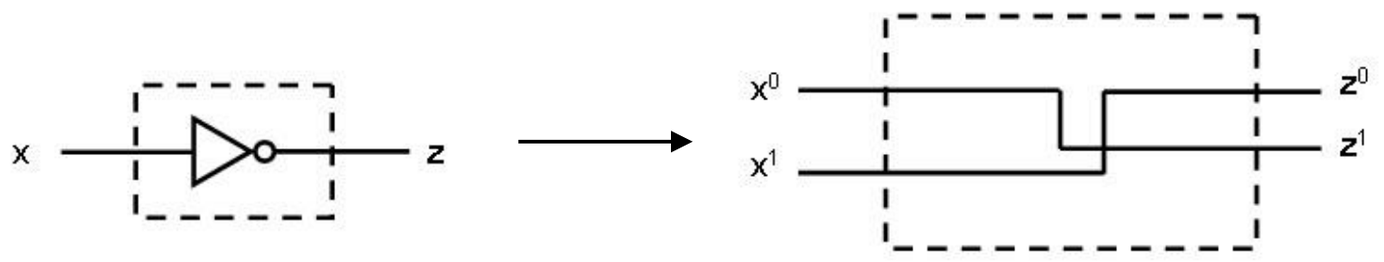
AND



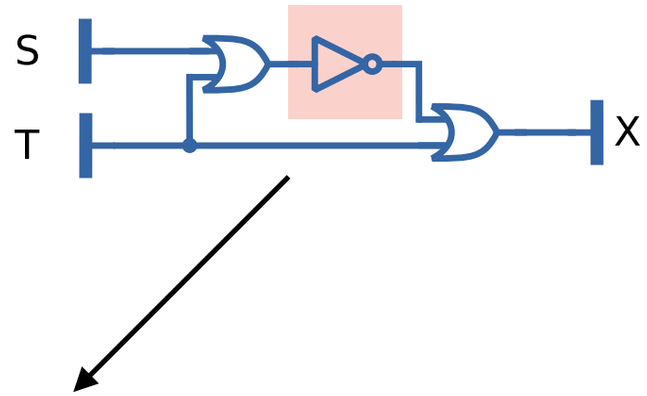
OR



NOT



Handling NOT gates



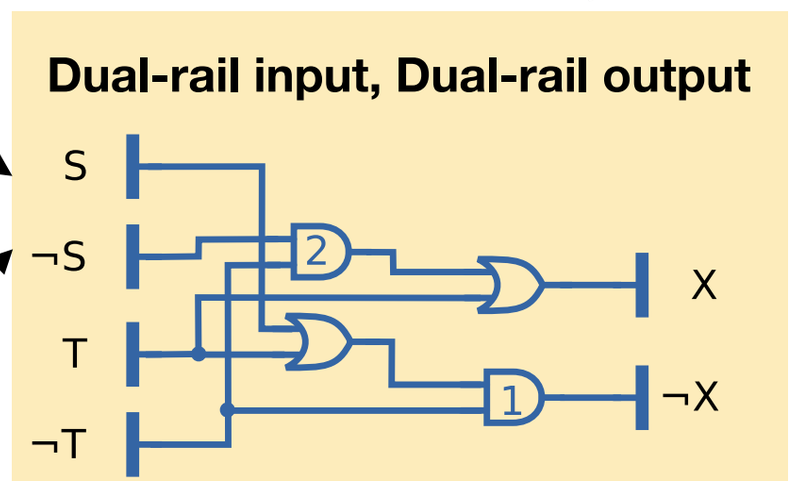
5′-CCCCCCC-3′

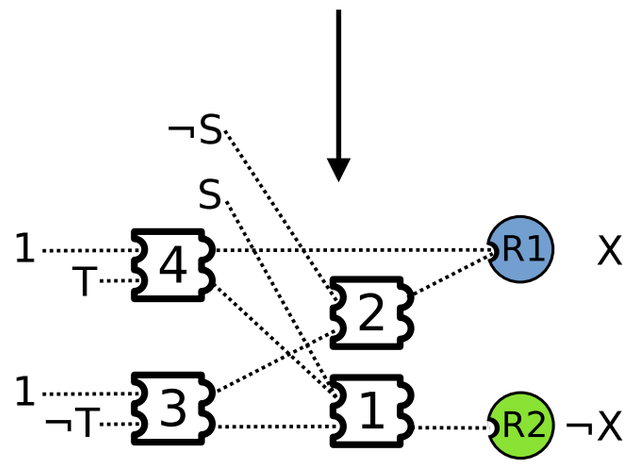
S

5′-TTTTTTT-3′

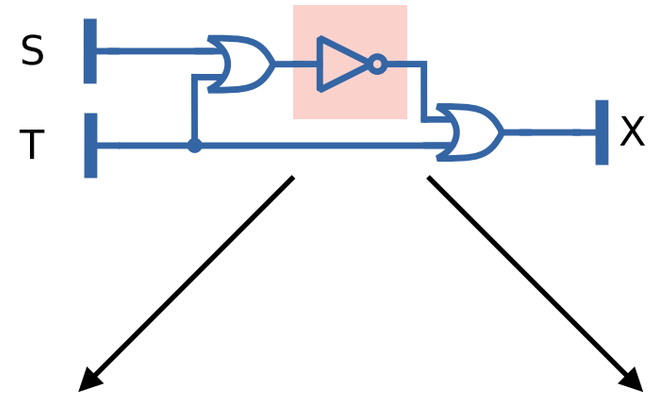
¬S

Dual-rail input, Dual-rail output





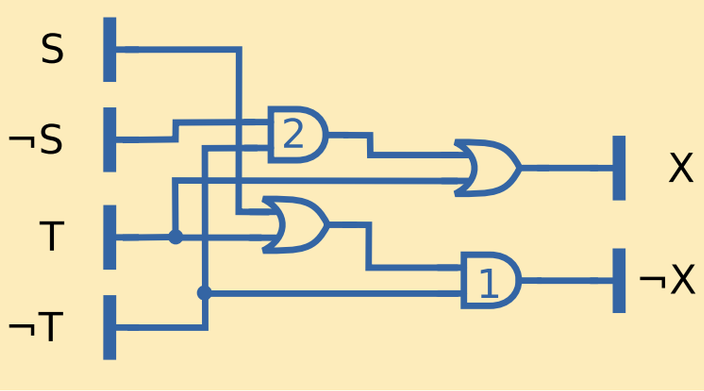
Handling NOT gates

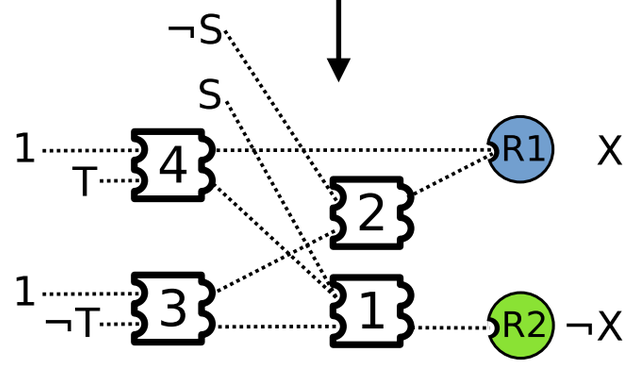


Dual-rail input, Dual-rail output

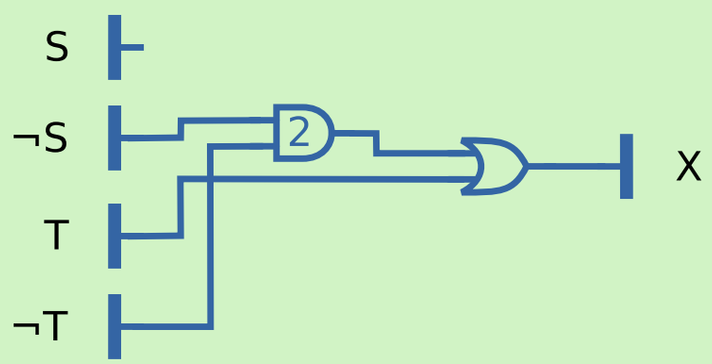
5′-CCCCCCC-3′
S

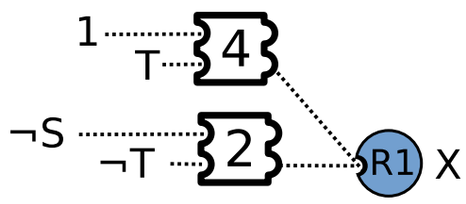
5′-TTTTTTT-3′
¬S



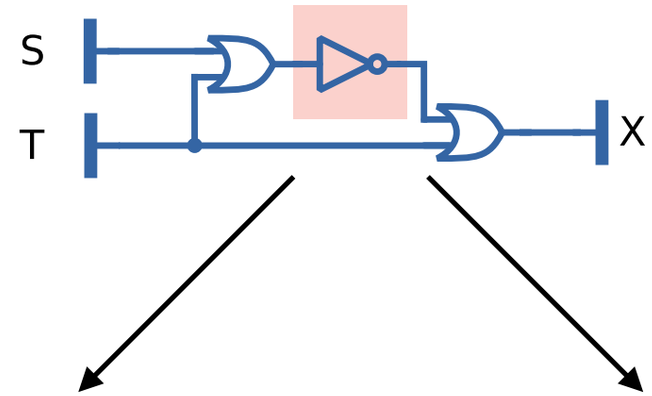


Dual-rail input





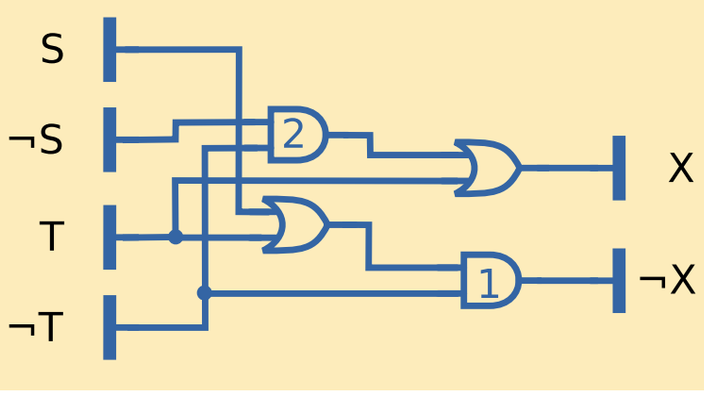
Handling NOT gates

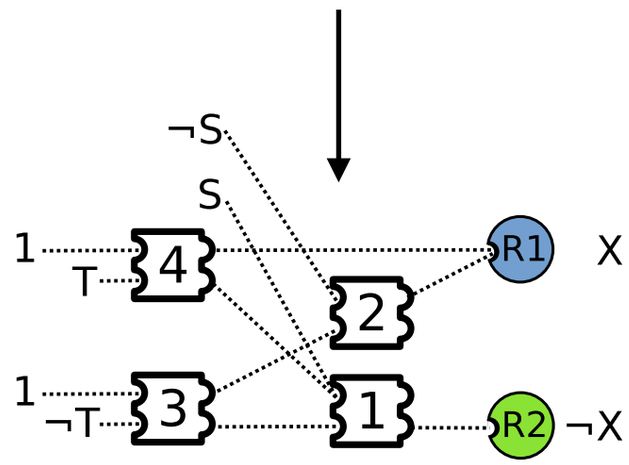


Dual-rail input, Dual-rail output

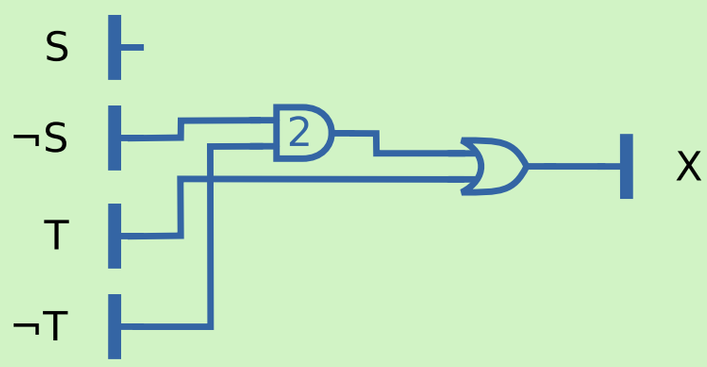
5′-CCCCCCC-3′
S

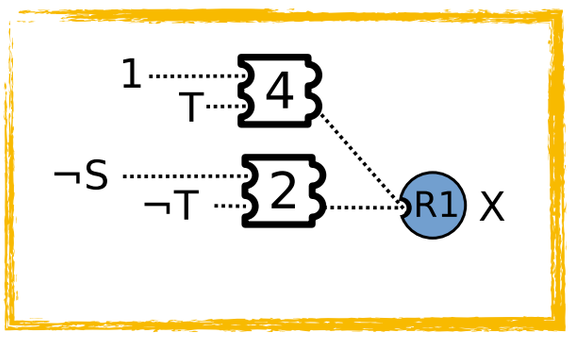
5′-TTTTTTT-3′
¬S





Dual-rail input





With reaction gates, wires, and dual-rail encoding, we can build any combinatorial circuit

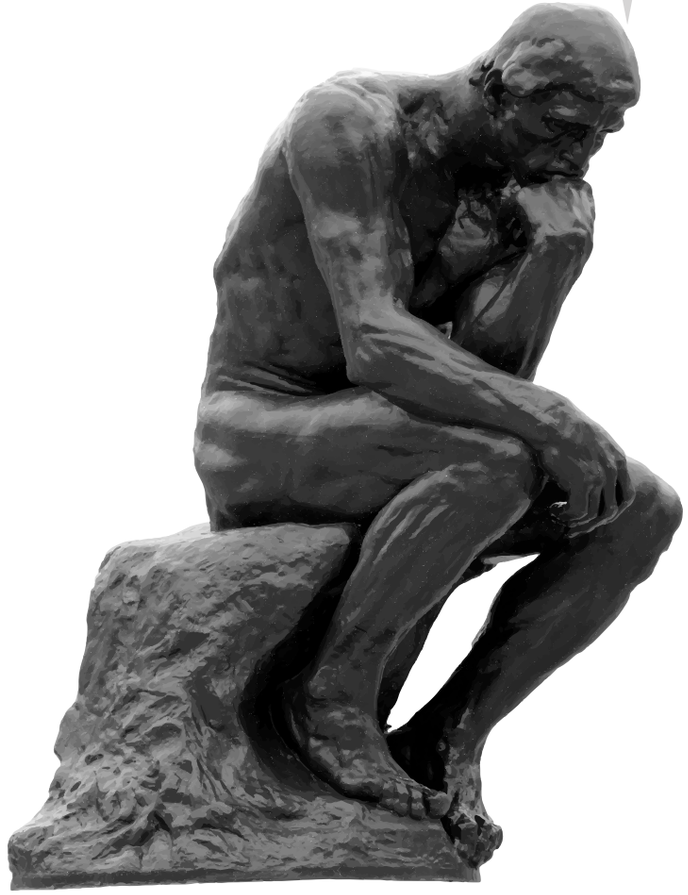
Tutorial Outline

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

How do you design the circuit?

2-input MUX			
A	B	s	out
0	0	0	0
0	1	0	1
1	0	0	0
1	1	0	1
0	0	1	0
0	1	1	0
1	0	1	1
1	1	1	1

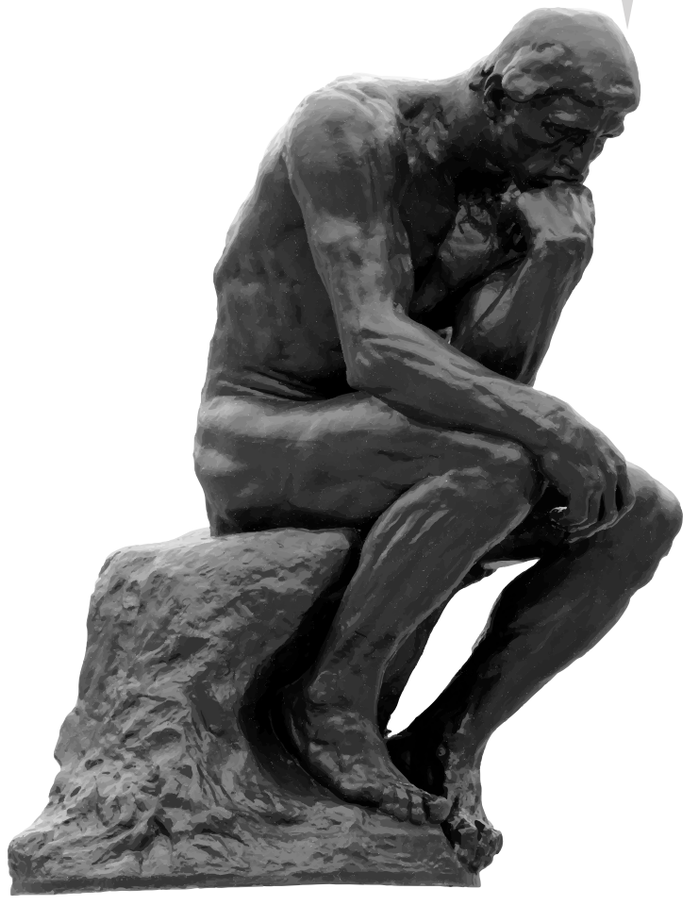
2-output DEMUX			
in	r	X	Y
0	0	0	0
1	0	0	1
0	1	0	0
1	1	1	0



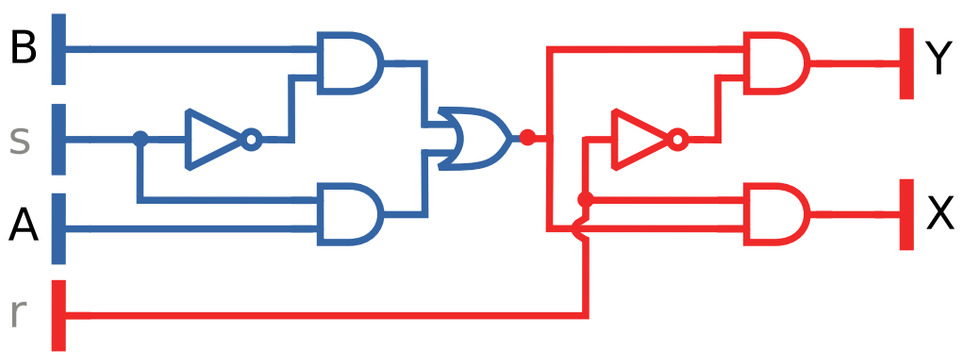
How do you design the circuit?

2-input MUX			
A	B	s	out
0	0	0	0
0	1	0	1
1	0	0	0
1	1	0	1
0	0	1	0
0	1	1	0
1	0	1	1
1	1	1	1

2-output DEMUX			
in	r	X	Y
0	0	0	0
1	0	0	1
0	1	0	0
1	1	1	0



Compile from Verilog, or truth table, into AND-OR-NOT circuit

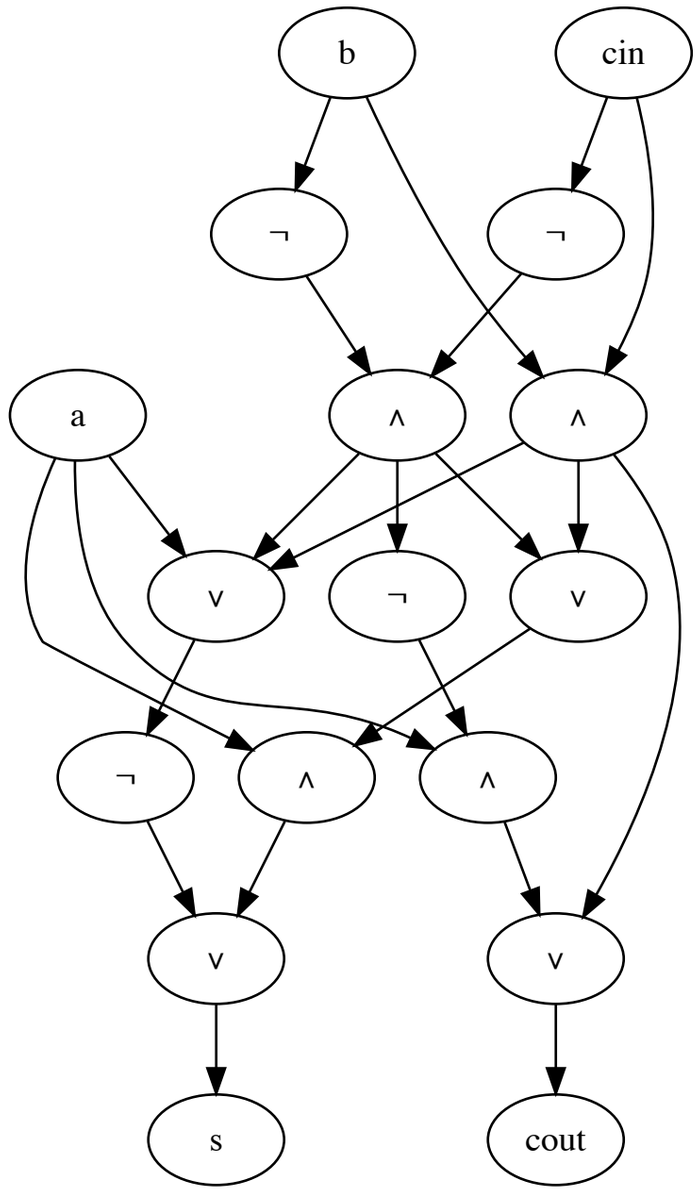


How do you design the circuit?

Inputs			Outputs	
A	***B***	C_{in}	C_{out}	***S***
0	0	0	0	0
0	0	1	0	1
0	1	0	0	1
0	1	1	1	0
1	0	0	0	1
1	0	1	1	0
1	1	0	1	0
1	1	1	1	1

How do you design the circuit?

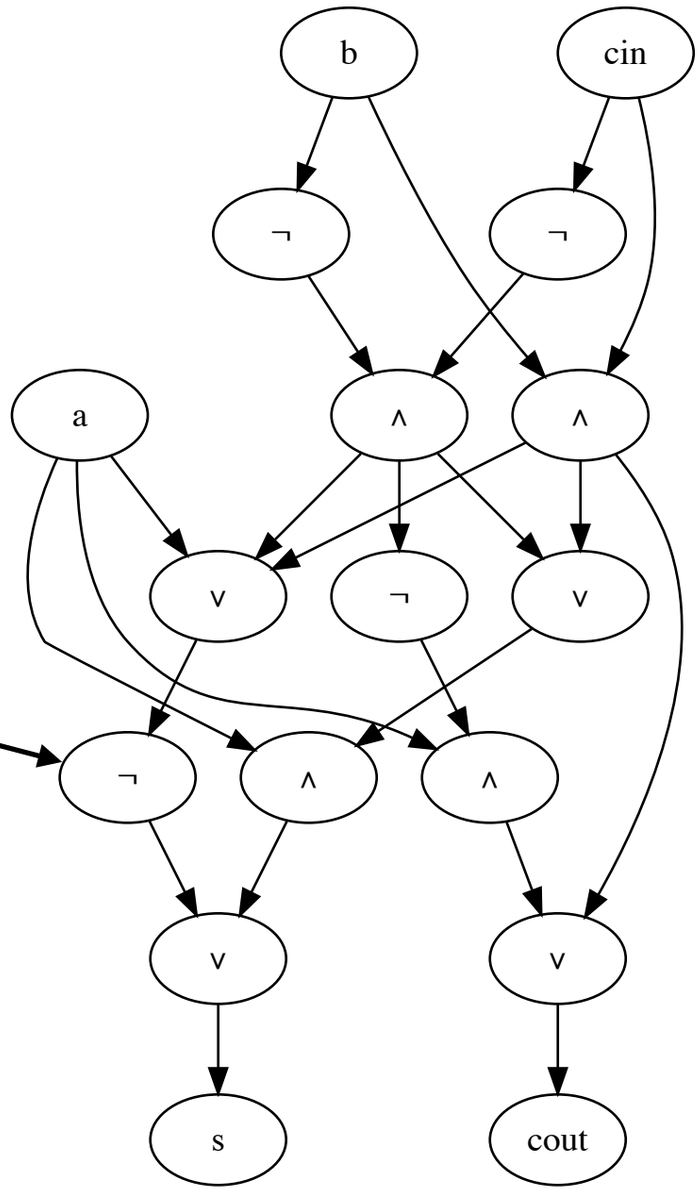
- ☑ Find minimized AND-OR-NOT circuit using **ABC**



Brayton, R., & Mishchenko, A. (2010, July). **ABC**: An academic industrial-strength verification tool. In *International Conference on Computer Aided Verification* (pp. 24-40). Springer, Berlin, Heidelberg.

How do you design the circuit?

☑ Find minimized AND-OR-NOT circuit using **ABC**

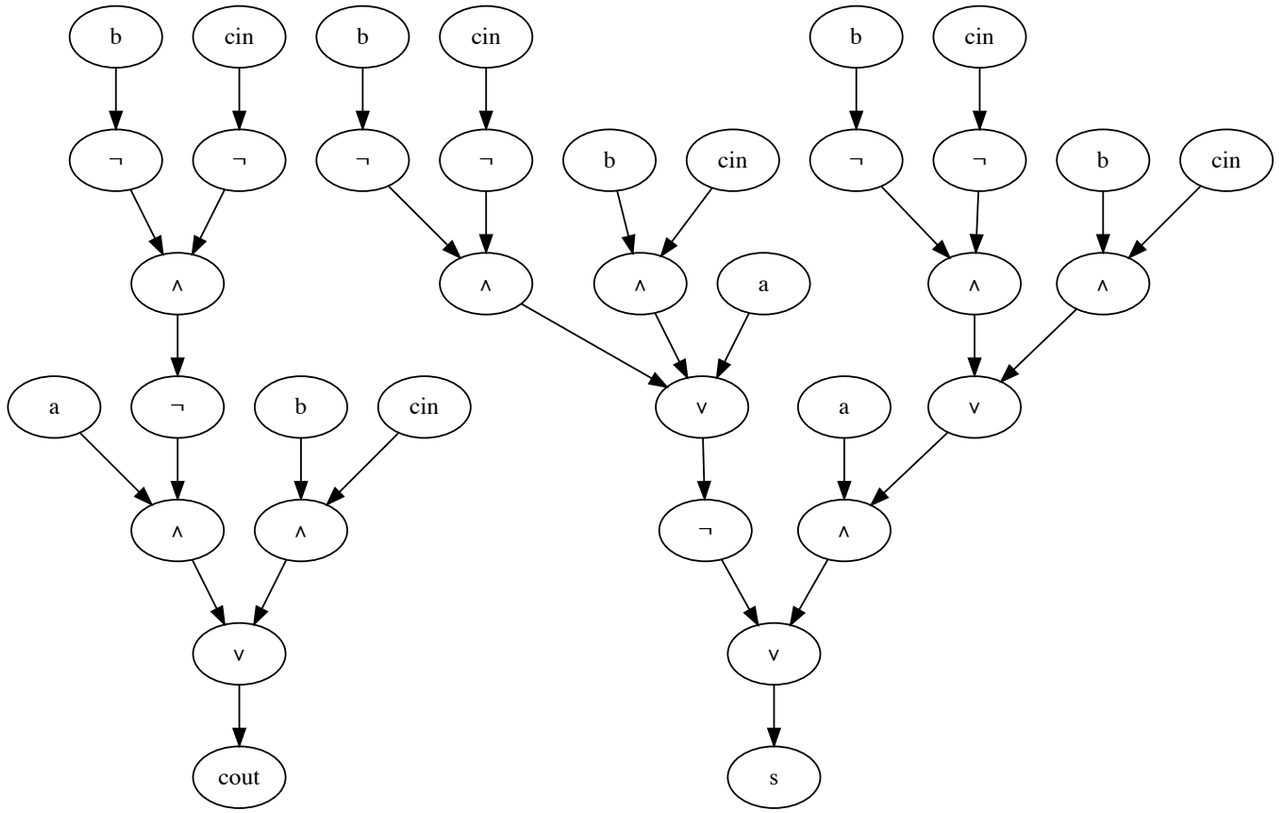


Need to remove explicit NOT gates

Brayton, R., & Mishchenko, A. (2010, July). **ABC**: An academic industrial-strength verification tool. In *International Conference on Computer Aided Verification* (pp. 24-40). Springer, Berlin, Heidelberg.

How do you design the circuit?

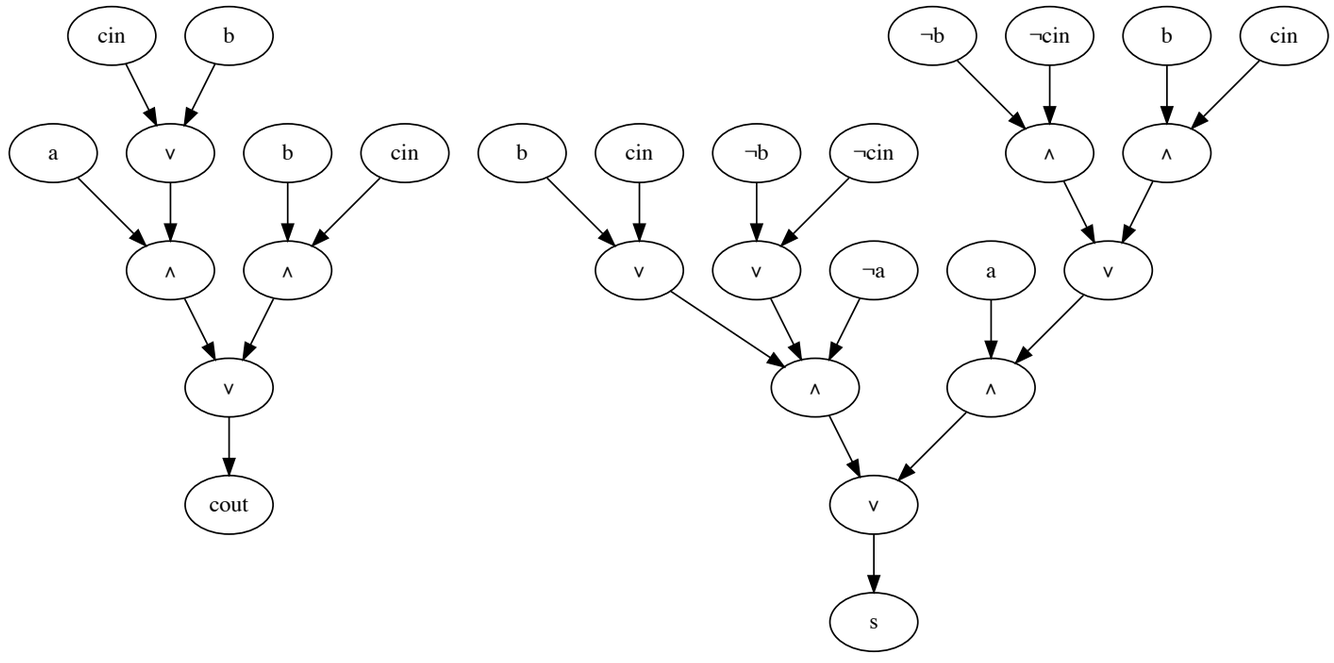
- ☑ Find minimized AND-OR-NOT circuit using ABC
- ☑ “Tree-ify” circuit



Brayton, R., & Mishchenko, A. (2010, July). ABC: An academic industrial-strength verification tool. In *International Conference on Computer Aided Verification* (pp. 24-40). Springer, Berlin, Heidelberg.

How do you design the circuit?

- ☑ Find minimized AND-OR-NOT circuit using ABC
- ☑ “Tree-ify” circuit
- ☑ Push negations to literal level (dual-rail inputs)



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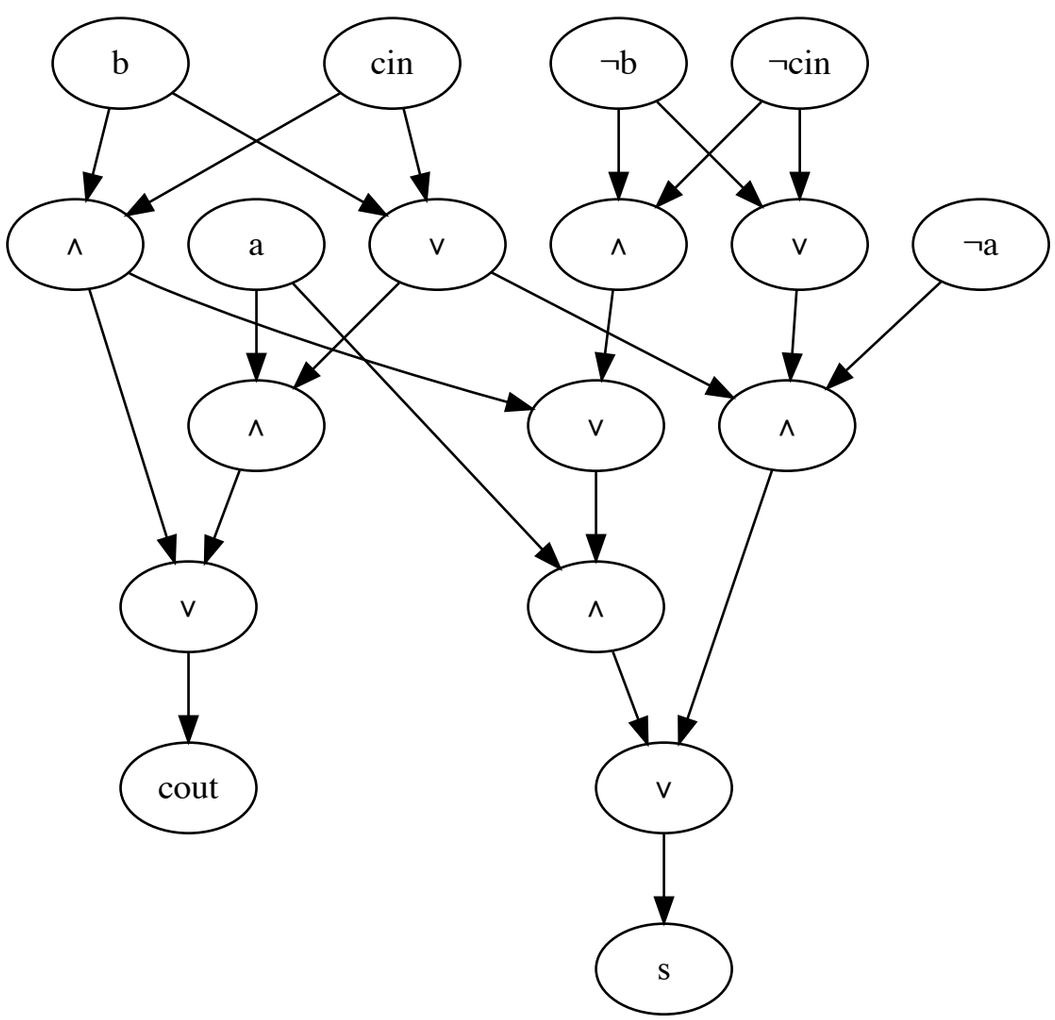
How do you design the circuit?

- ☑ Find minimized AND-OR-NOT circuit using ABC
- ☑ “Tree-ify” circuit
- ☑ Push negations to literal level (dual-rail inputs)
- ☑ Compress circuit

Circuit now using dual-rail input



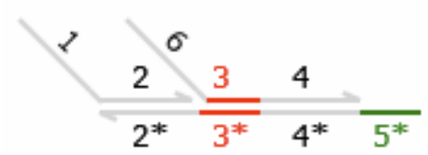
Brayton, R., & Mishchenko, A. (2010, July). ABC: An academic industrial-strength verification tool. In *International Conference on Computer Aided Verification* (pp. 24-40). Springer, Berlin, Heidelberg.

From circuit to DSD system

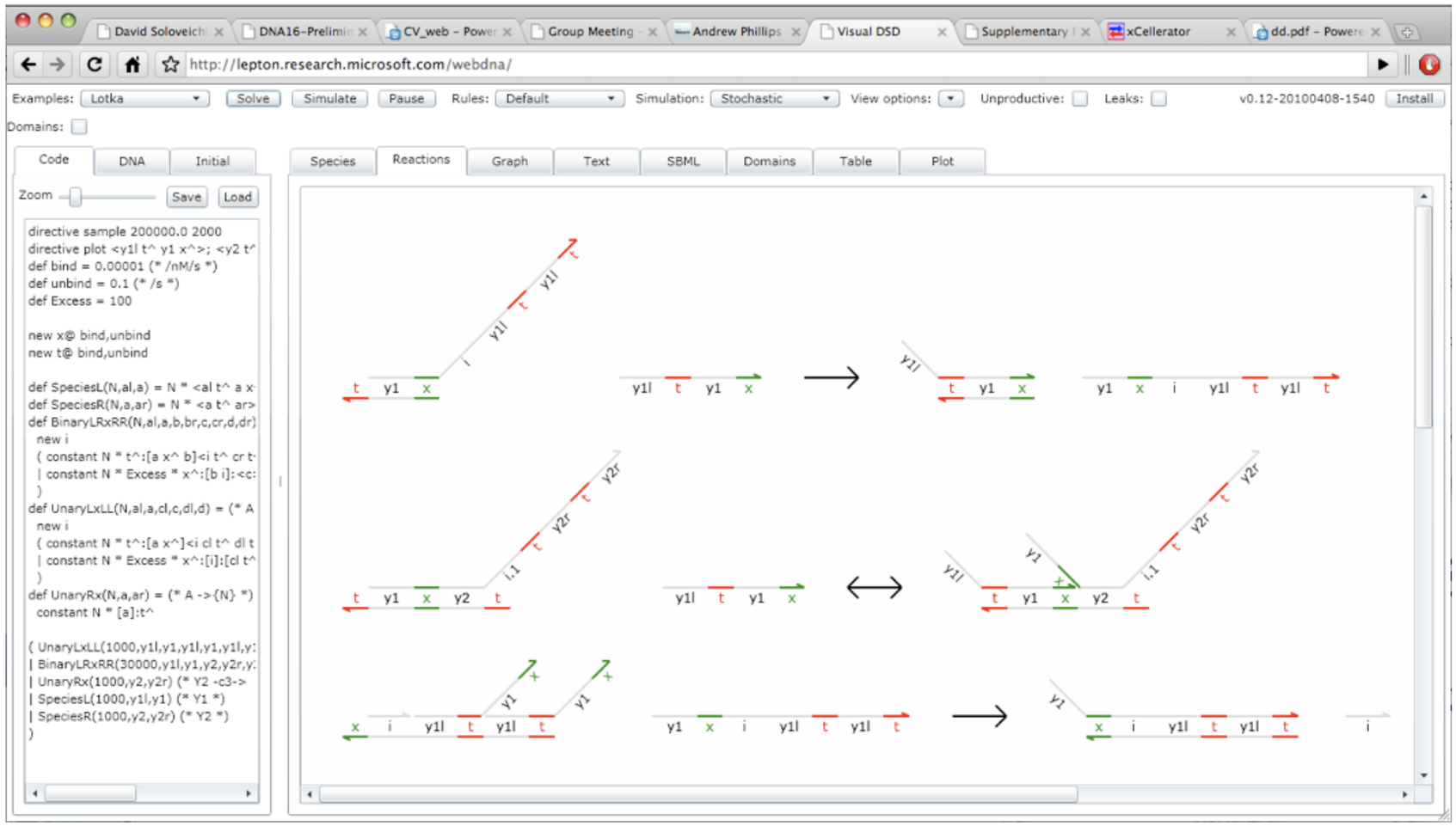
DSD: formal language for describing and modeling strand displacement cascades

<1>[2]:<6>[3^ 4]:5^*

=



http://lepton.research.microsoft.com/webdna/



Phillips, Cardelli, *Journal of Royal Society Interface*, 2009

From circuit to DSD system

DSD: formal language for describing and modeling strand displacement cascades

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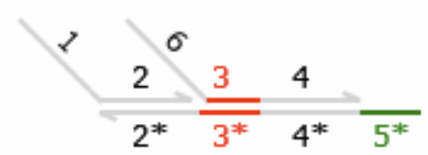
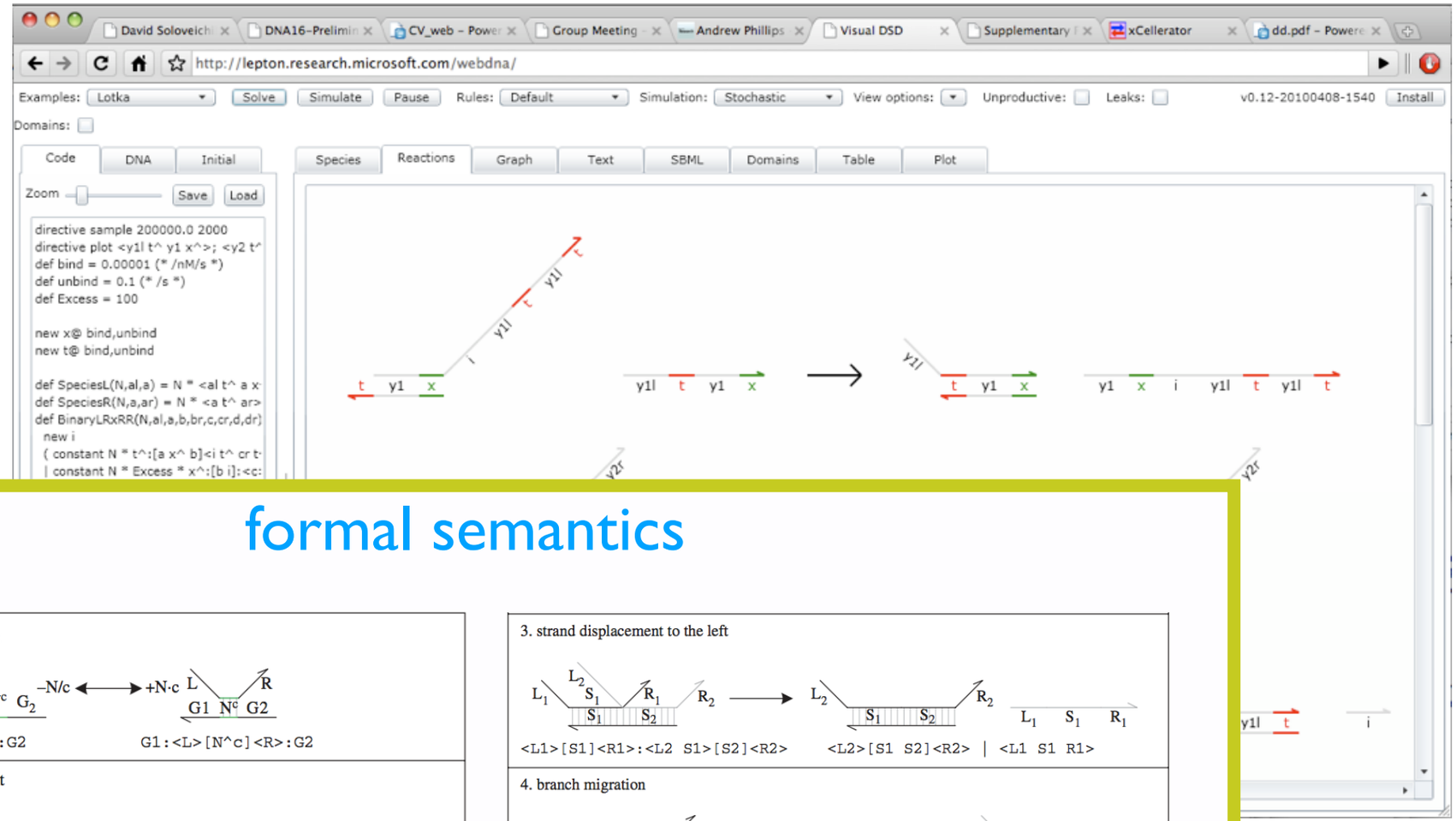




formal semantics

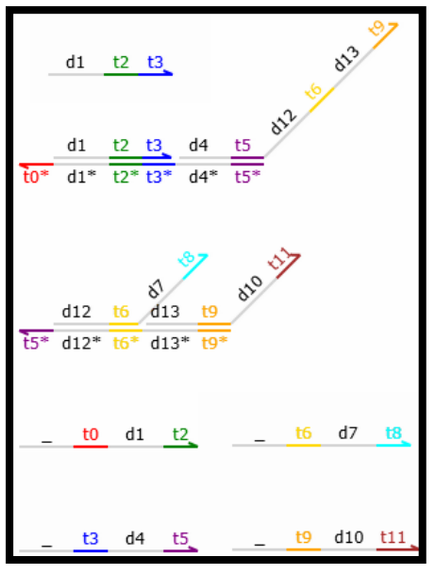
Figure 2. Reduction and branch migration rules of the strand displacement language. For each rule, the graphical representation at the top is equivalent to the program code at the bottom.

Phillips, Cardelli, *Journal of Royal Society Interface*, 2009

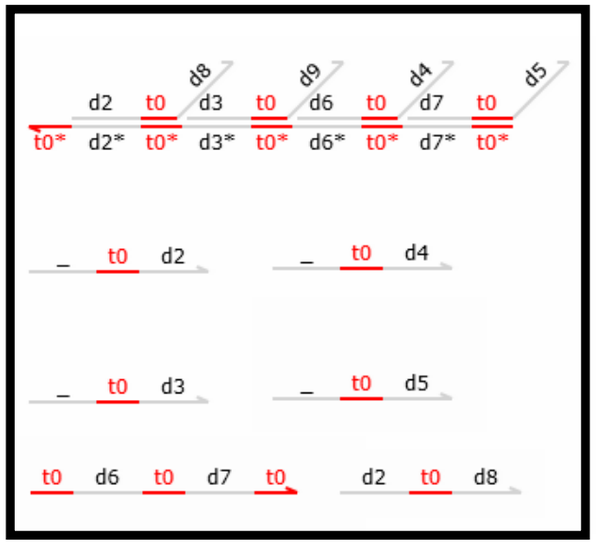
From circuit to DSD system

$$A + B \rightarrow C + D$$

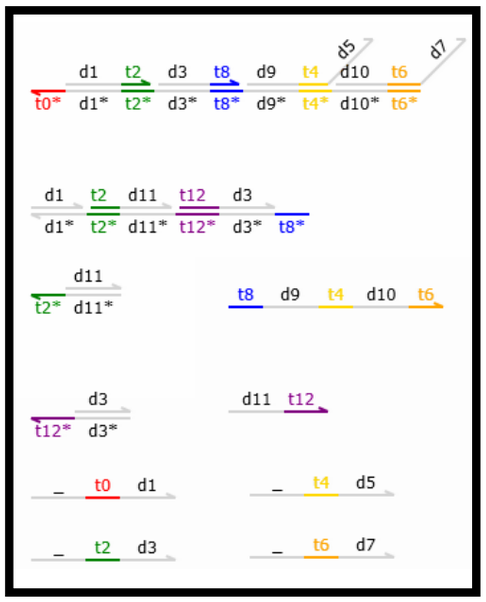
Soloveichik et al. (2010)



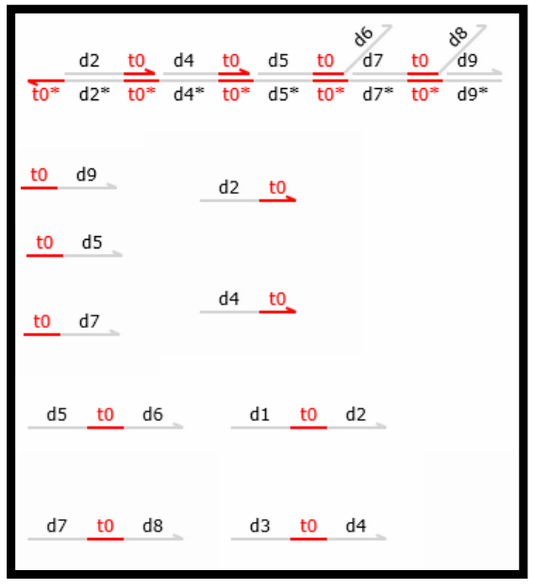
Lakin et al. (2012)



Cardelli (2011)



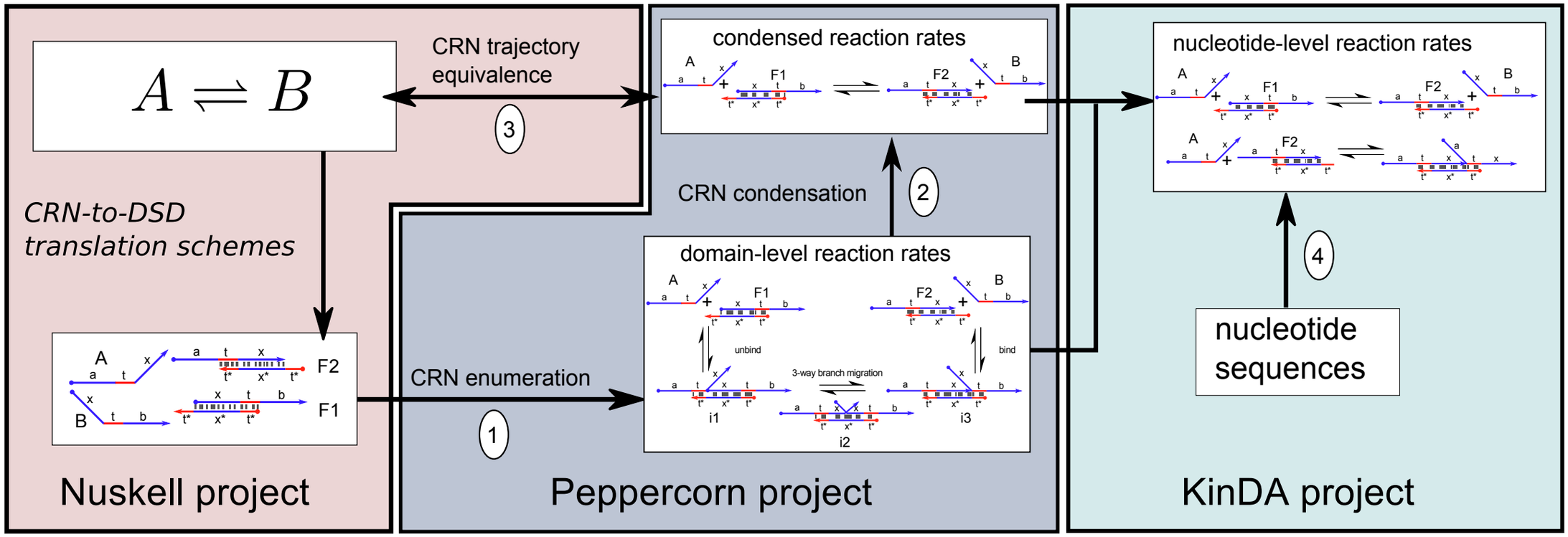
Qian et al. (2011)



Chen et al. (2012), Cardelli (2013), Srinivas (2015), Lakin et al. (2016), ...

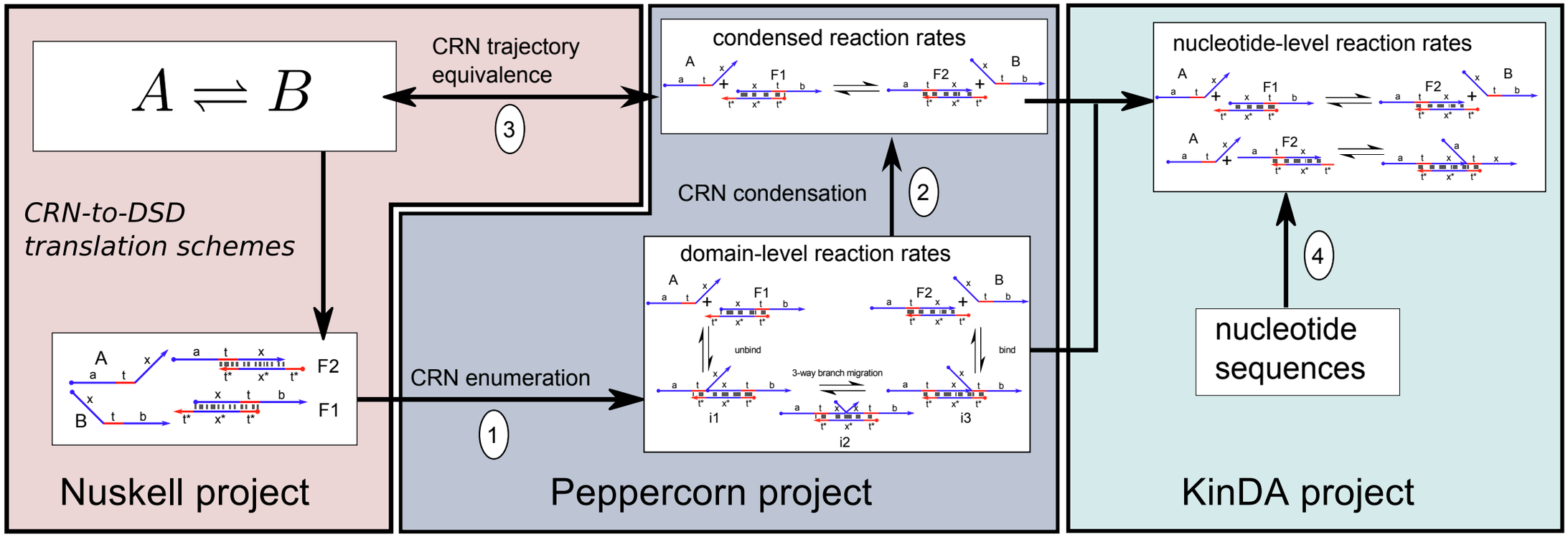
Images drawn using VisualDSD, Lakin et al. (2012)

The Nuskell compiler framework



Badelt et al. (2017) - Nuskell
Grun et al. (2014) - Peppercorn
Shin et al. (2017) - CRN pathway decomposition equivalence
Johnson et al. (2018) - CRN bisimulation equivalence
Berleant et al. (submitted) - KinDA

The Nuskell compiler framework



Badelt et al. (2017) - Nuskell
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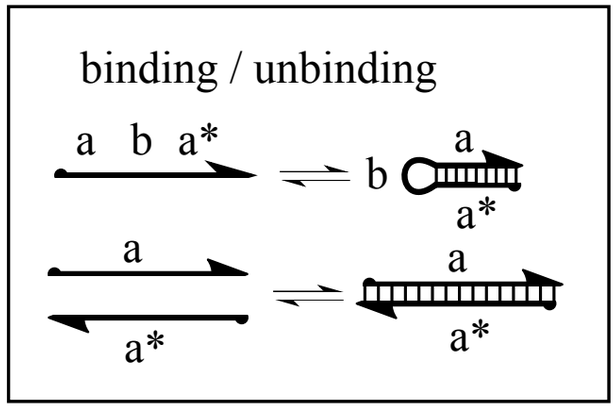
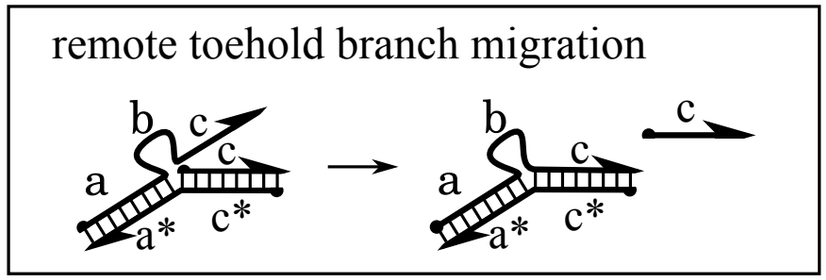
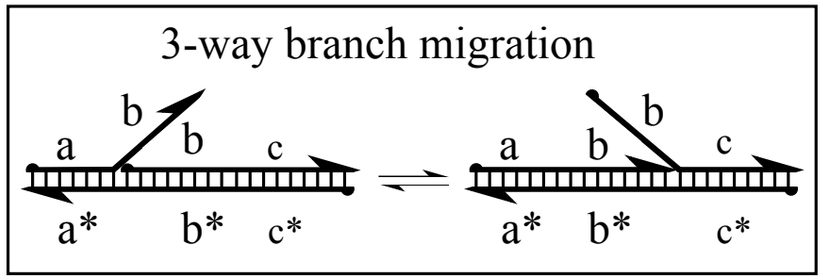
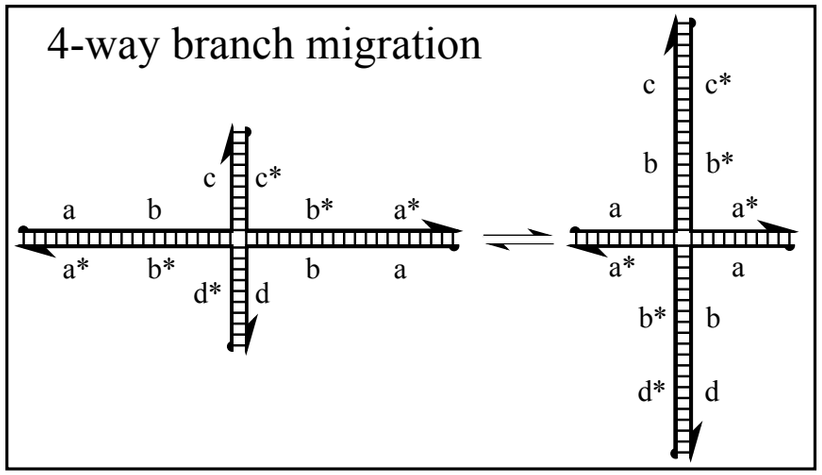
Berleant et al. (submitted) - KinDA

Reaction Enumeration









http://www.github.com/DNA-and-Natural-Algorithms-Group/peppercornenumerator

Grun et al. 2014

CRN equivalence verification

formal input CRN

3 species

7 reactions

```
A     -> A + A
A + A -> A
A + B -> B + B
B     ->
A + C ->
C     -> C + C
C + C -> C
```

enumerated CRN

360 species

668 reactions

translation scheme: qian2011_3D_var1.ts

CRN equivalence verification

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```

Are these networks equivalent?

condensed CRN

42 species

32 reactions

```
f14 + C -> e1428 + f15
e853 + f12 -> C + f13
A + f4 -> f3 + e71
f2 + e25 -> A + f1
A + e25 -> f2 + e7
e996 + f3 -> A + f10
e1428 + f15 -> f14 + C
f3 + e71 -> A + f4
e465 + B -> e418 + f6
e614 + f9 -> e611 + e730
e996 + C -> e1040 + f12
e465 + f5 -> e514 + e368
e308 + f7 -> f8 + B
e418 + B -> e371 + f6
C + f13 -> e853 + f12
A + f1 -> f2 + e25
B + e71 -> e319 + f7
f2 + e7 -> A + e25
e1040 + f11 -> e1162 + e1163 + e1158
e319 + f7 -> B + e71
e308 + f9 -> e614 + e615 + e611
e371 + f6 -> e418 + B
e1040 + f12 -> e996 + C
f8 + B -> e308 + f7
e319 + f5 -> e372 + e371 + e368
f3 + e7 -> A + f0
e853 + f15 -> e1428 + C
e1428 + C -> e853 + f15
A + f10 -> e996 + f3
e1163 + f11 -> e1158 + e1246
e418 + f6 -> e465 + B
A + f0 -> f3 + e7
```

enumerated CRN

360 species

668 reactions

translation scheme: qian2011_3D_var1.ts

CRN equivalence verification

formal input CRN
3 species
7 reactions

```
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A + A -> A
A + B -> B + B
B     ->
A + C ->
C     -> C + C
C + C -> C
```

interpreted CRN
3 species
7 non-trivial reactions

```
C     -> C
C + C -> C
A     -> A
A     -> A
A + A -> A + A
A     -> A
C     -> C
A     -> A
B     -> B
      ->
A + C -> A + C
      ->
B     -> B
B + B -> B + B
C     -> C + C
A     -> A
B + A -> A + B
A + A -> A + A
A + C ->
A + B -> B + A
B     ->
B + B -> B + B
A + C -> A + C
B     -> B
A + B -> B + B
A + A -> A
C + C -> C + C
C + C -> C + C
A     -> A
      ->
B     -> B
A     -> A + A
```

condensed CRN
42 species
32 reactions

```
f14 + C -> e1428 + f15
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enumerated CRN
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Johnson et al. (2016) - CRN bisimulation equivalence
translation scheme: qian2011_3D_var1.ts

CRN equivalence verification

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3 species
7 reactions

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A + A -> A
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A + C ->
C     -> C + C
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interpreted CRN
3 species
7 non-trivial reactions

```
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A     -> A
A     -> A
A + A -> A + A
A     -> A
C     -> C
A     -> A
B     -> B
      ->
A + C -> A + C
      ->
B     -> B
B + B -> B + B
C     -> C + C
A     -> A
B + A -> A + B
A + A -> A + A
A + C ->
A + B -> B + A
B     ->
B + B -> B + B
A + C -> A + C
B     -> B
A + B -> B + B
A + A -> A
C + C -> C + C
C + C -> C + C
A     -> A
      ->
B     -> B
A     -> A + A
```

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32 reactions

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3 species
7 non-trivial reactions

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C + C -> C
A     -> A
A     -> A
A + A -> A + A
A     -> A
C     -> C
A     -> A
B     -> B
      ->
A + C -> A + C
      ->
B     -> B
B + B -> B + B
C     -> C + C
A     -> A
B + A -> A + B
A + A -> A + A
A + C ->
A + B -> B + A
B     ->
B + B -> B + B
A + C -> A + C
B     -> B
A + B -> B + B
A + A -> A
C + C -> C + C
C + C -> C + C
A     -> A
      ->
B     -> B
A     -> A + A
```

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32 reactions

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enumerated CRN
360 species
668 reactions

Johnson et al. (2016) - CRN bisimulation equivalence
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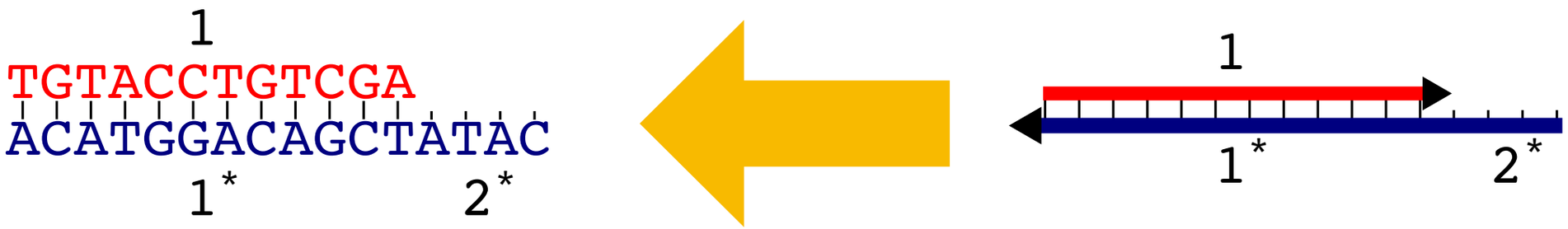
Designing Sequences



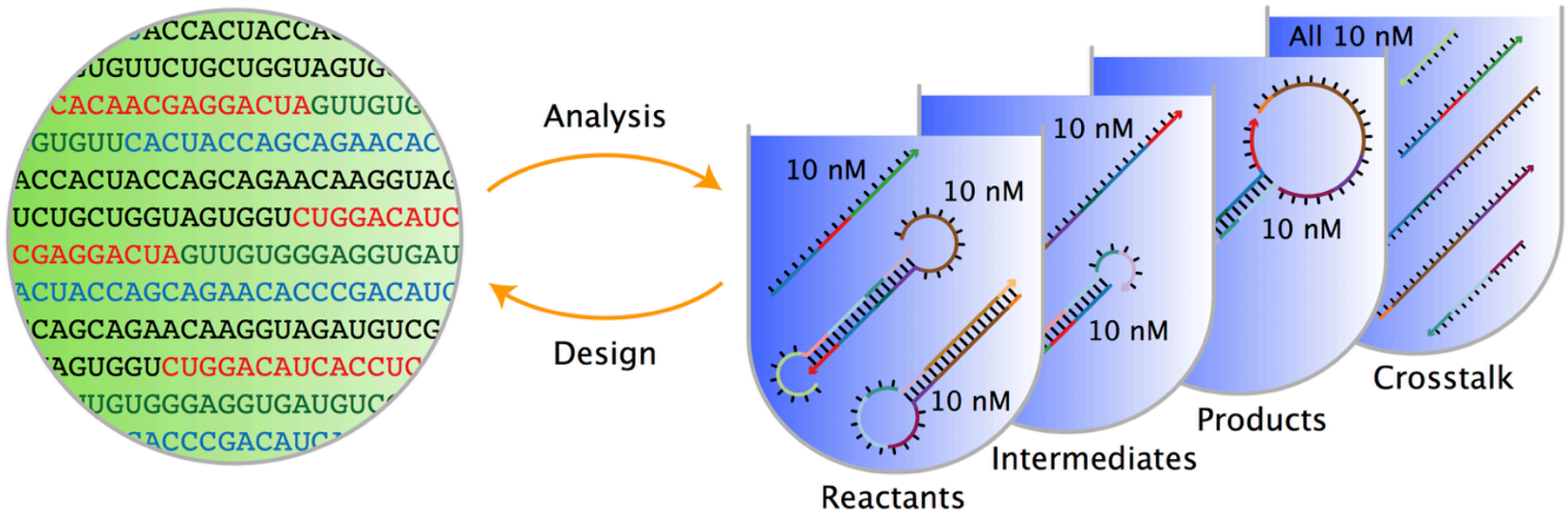
Designing Sequences

NUPACK

Analysis Design Utilities Downloads

NUPACK is a growing software suite for the analysis and design of nucleic acid structures, devices, and systems.

The NUPACK web application enables analysis and design of the equilibrium base-pairing properties of one or more test tubes of interacting nucleic acid strands:



Please cite the web application and algorithms appropriately; usage statistics are an important component in helping to secure funding for NUPACK development. We are happy to provide advice and technical support.

— The NUPACK Team

News: Constrained multistate test tube design for reaction pathway engineering is now published! (pdf, supp info, source code, user guide)

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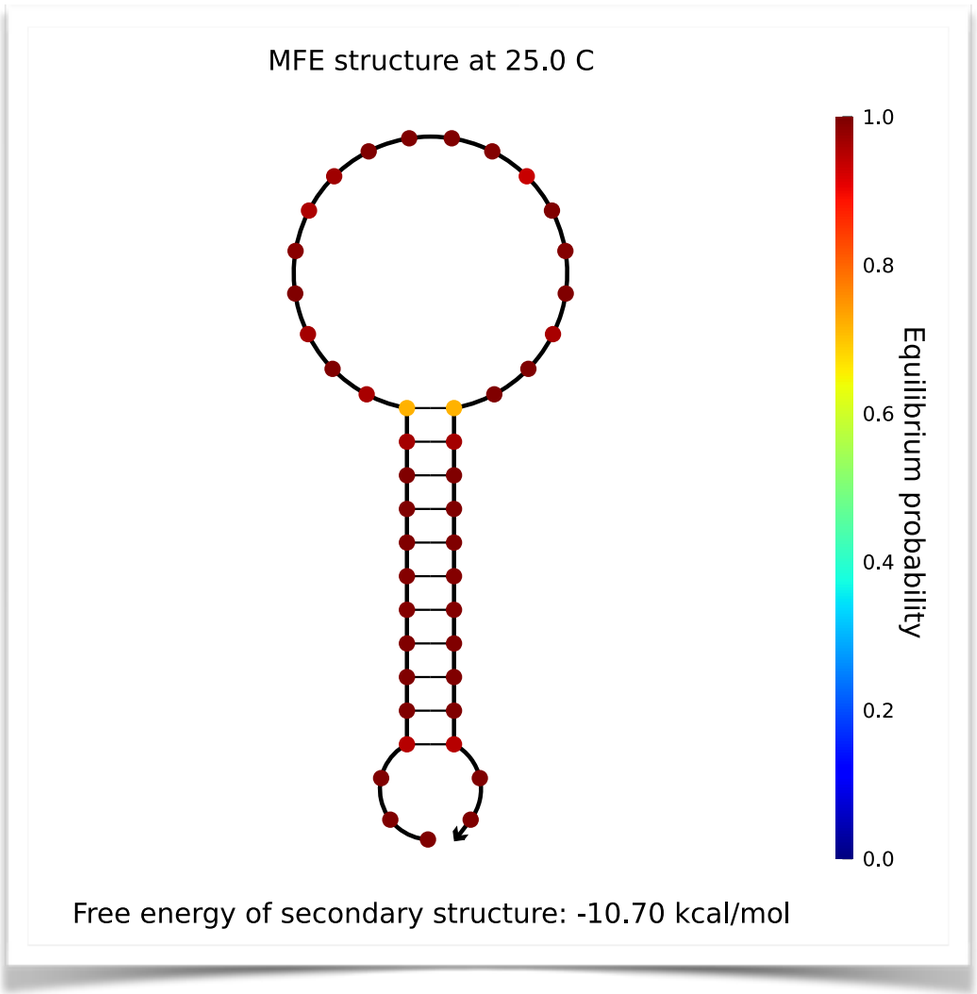
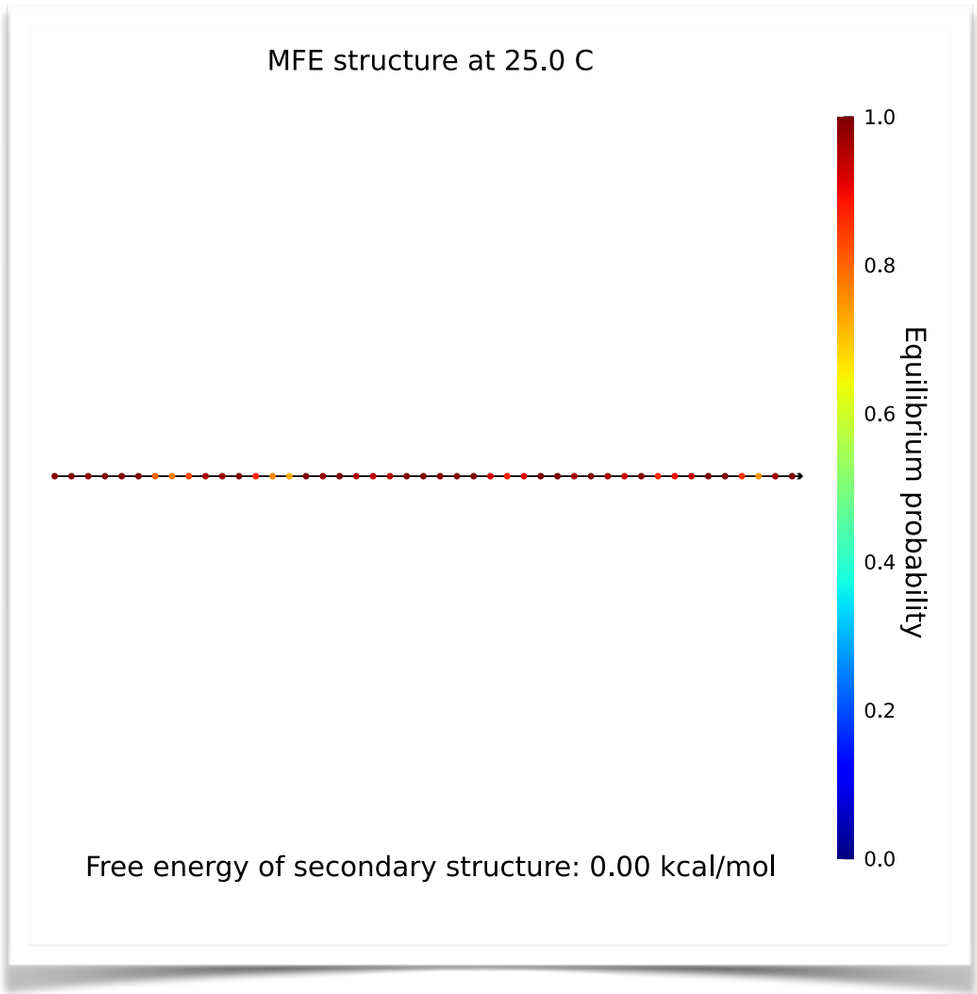
Designing Sequences

input X

poor sequence for a signal strand

good sequence for a signal strand



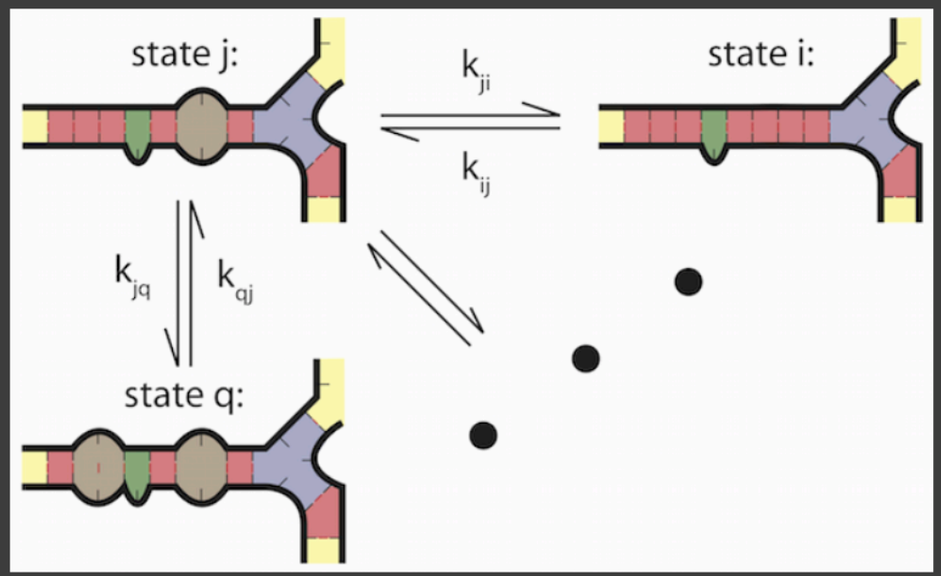


Mulitstrand.org to determine reaction rates

Multistrand is a software package for simulating the kinetics of multiple interacting nucleic acid strands. It is developed at the Winfree lab at the California Institute of Technology.

- DNA and Natural Algorithms Group @ Caltech

Live demo



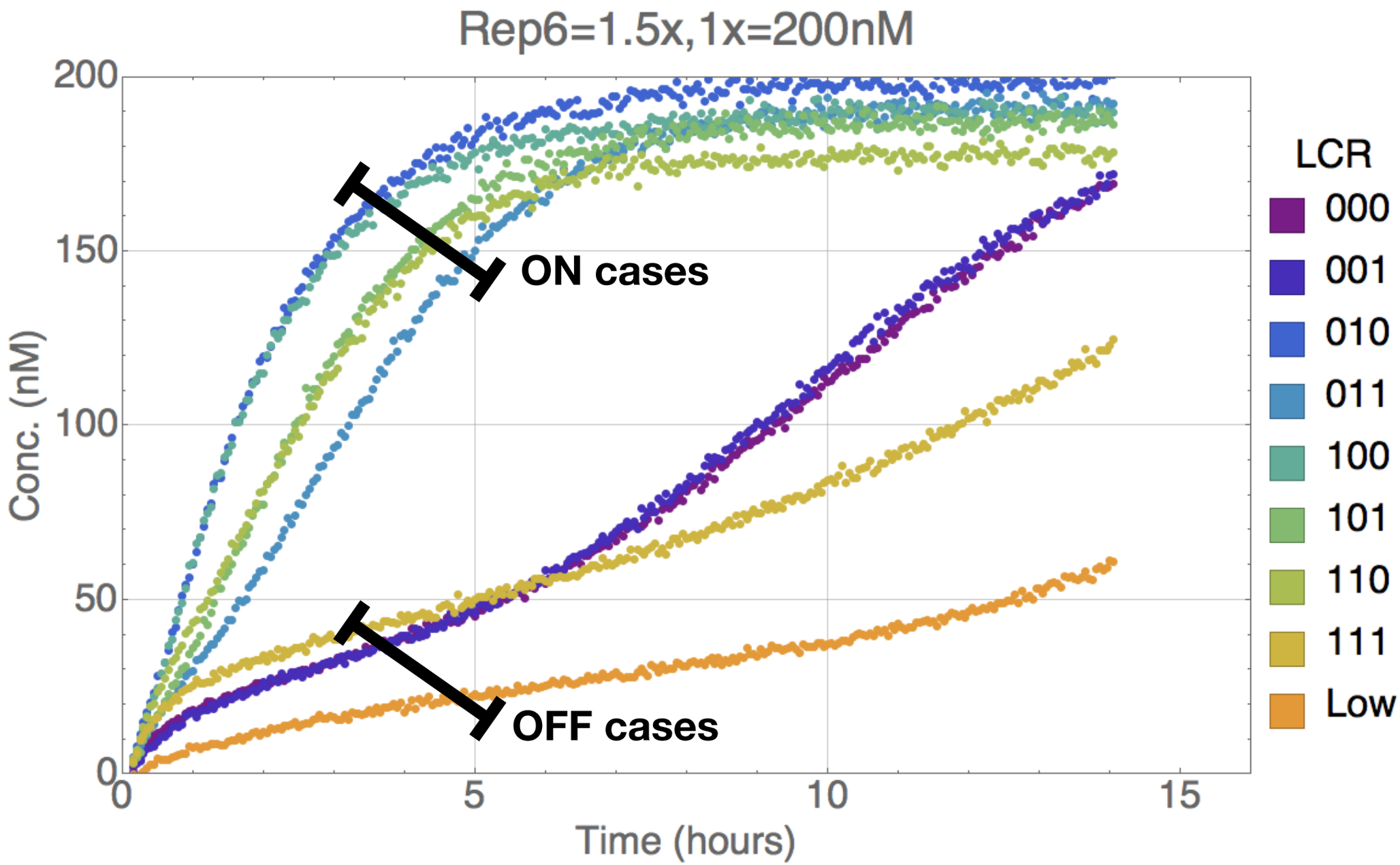
Key Features

- Kinetic simulations of nucleic acids as random walk on thermodynamic energy model
- Supports multiple interacting strands
- Equilibrium consistent with NUPACK
- Various usage modes to study kinetic trajectories
- Distributed as a Python package
- MIT License

Tutorial Outline

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

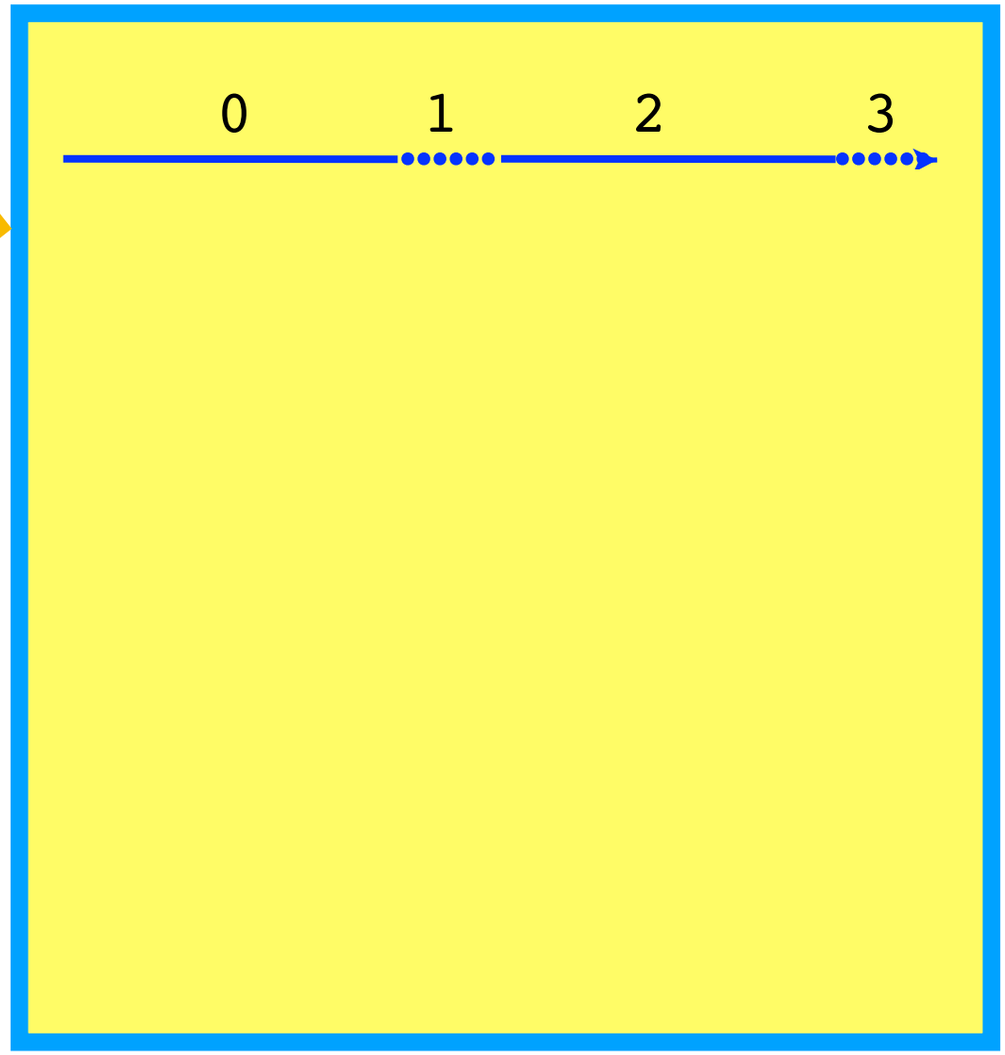
Why is this circuit not *robust*?



What causes signal *leak*?

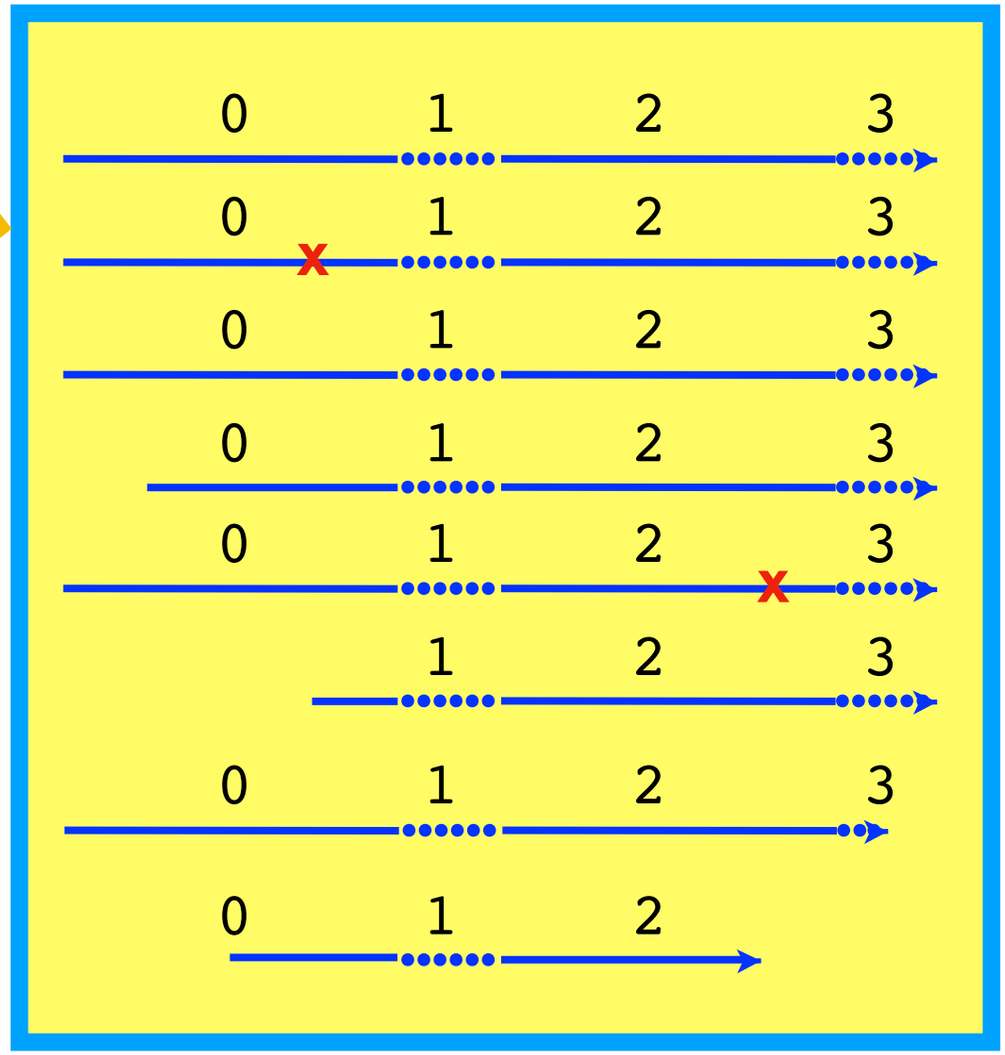
Problem 1: Molecules are not perfect

Imperfect strands from imperfect synthesis



Problem 1: Molecules are not perfect

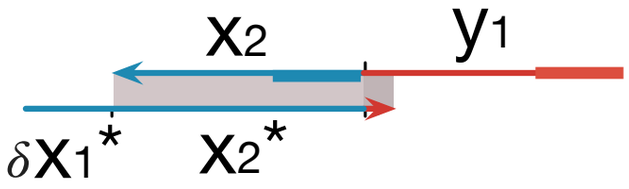
Imperfect strands from imperfect synthesis



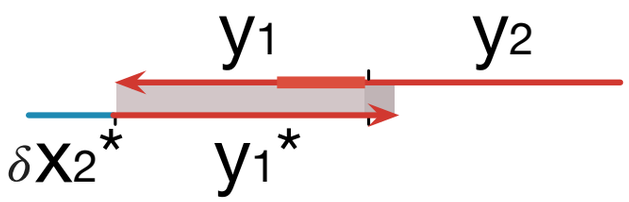
Problem 1: Molecules are not perfect

translator cascade
with perfect molecules

F1

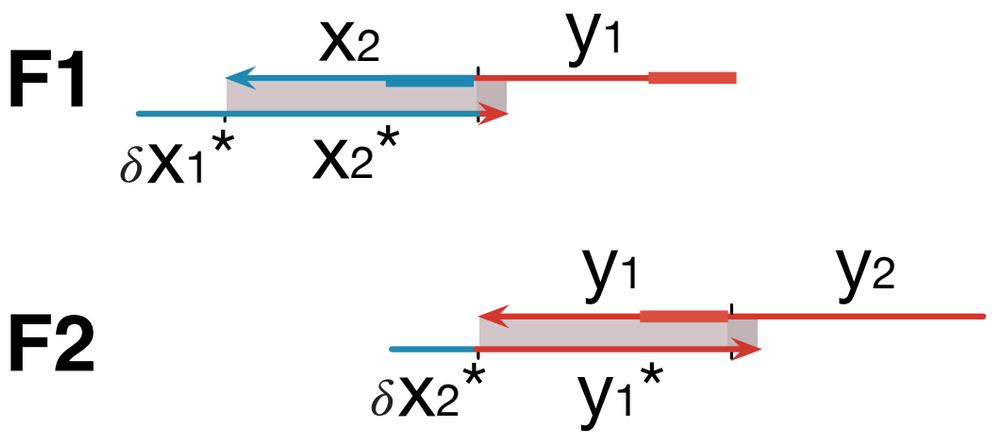


F2

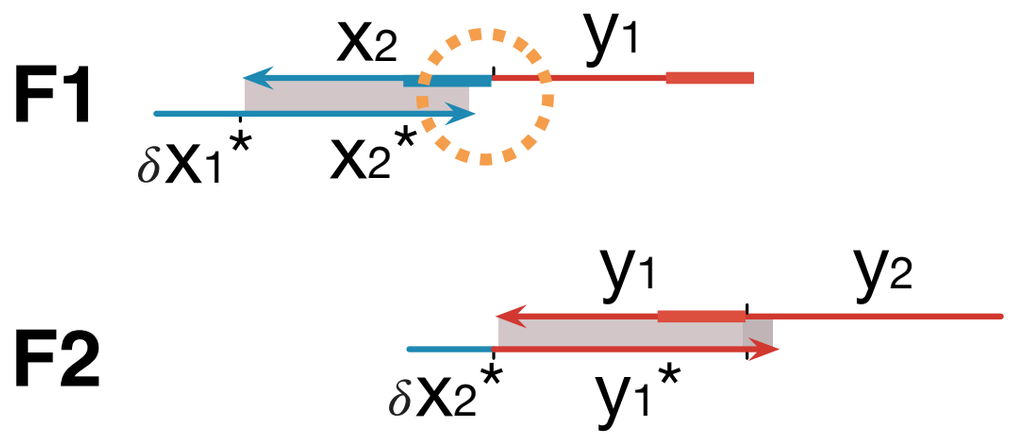


Problem 1: Molecules are not perfect

translator cascade with perfect molecules

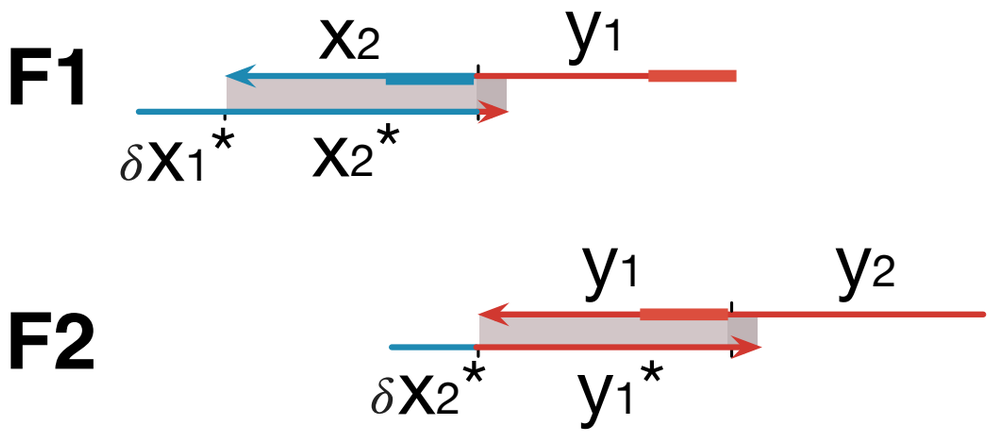


translator cascade with imperfect molecules

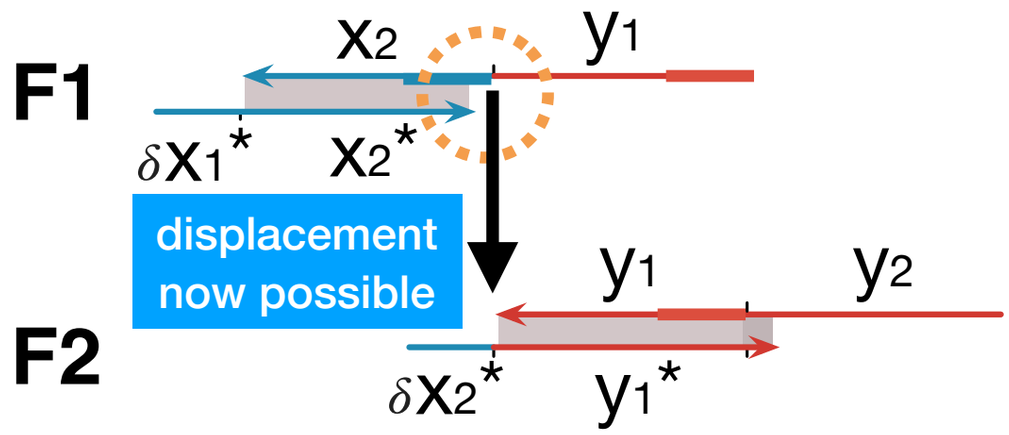


Problem 1: Molecules are not perfect

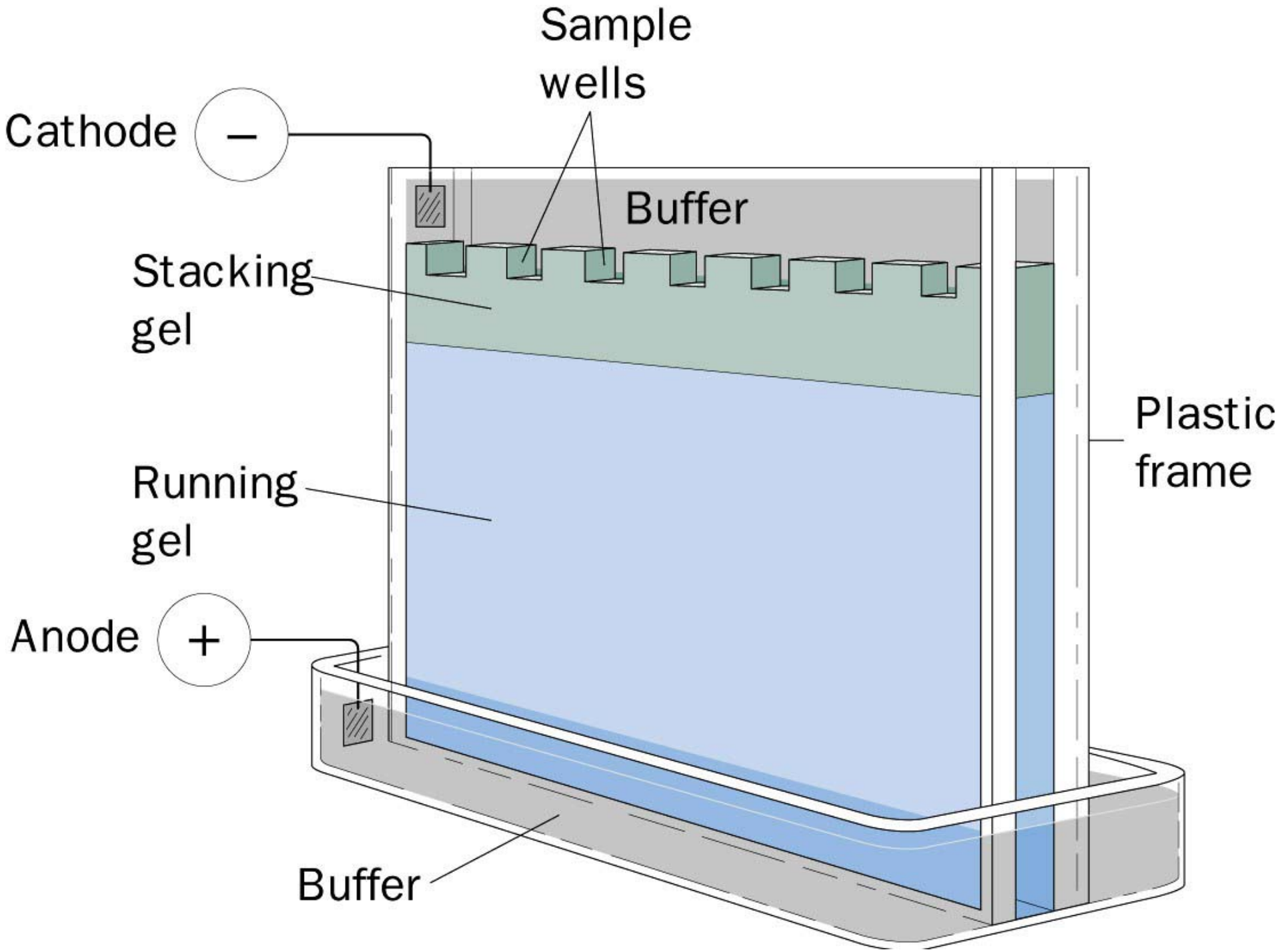
translator cascade with perfect molecules



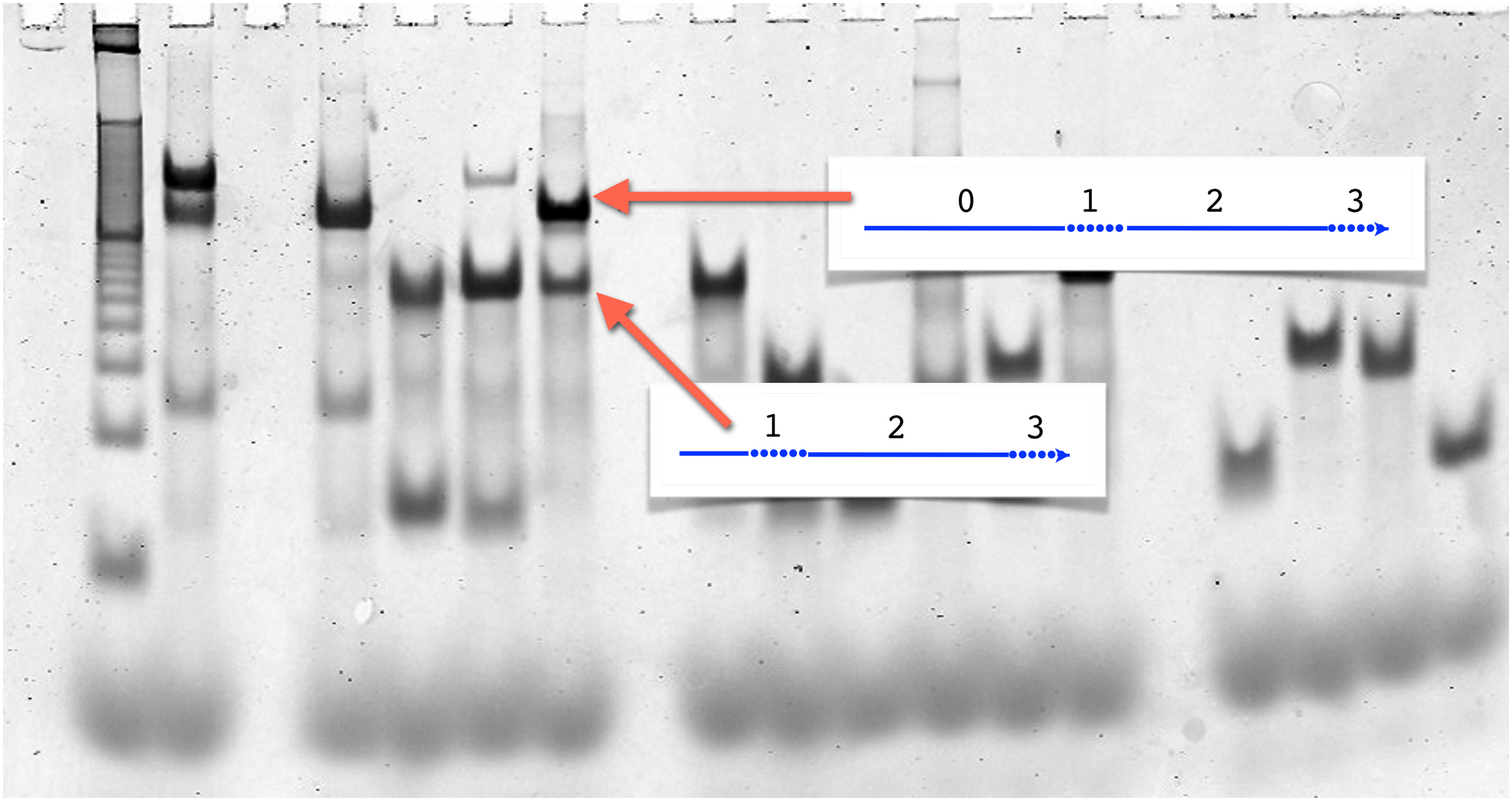
translator cascade with imperfect molecules



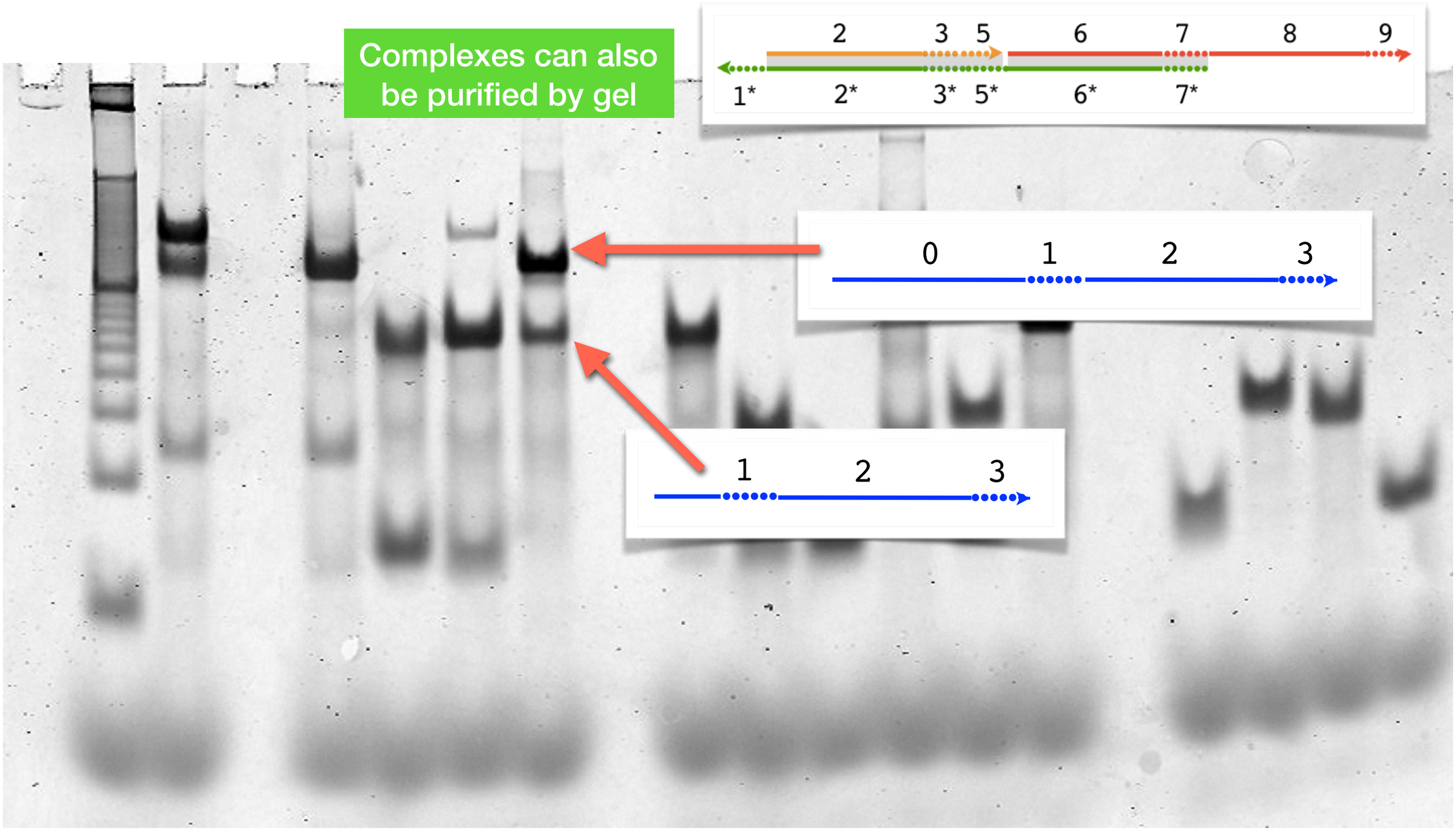
(Partial) solution to Problem 1



(Partial) solution to Problem 1



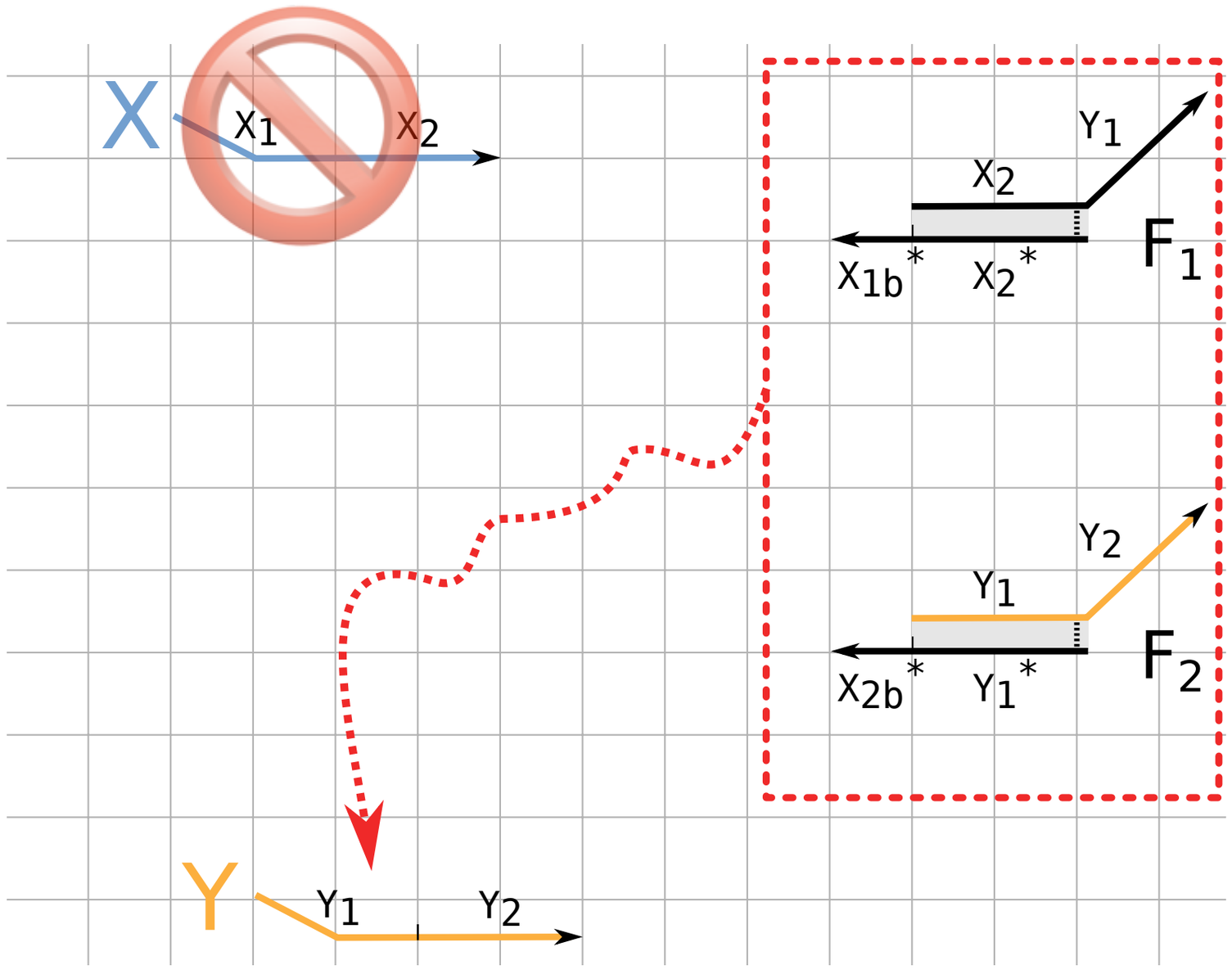
(Partial) solution to Problem 1



Problem 2: Spurious reactions occur

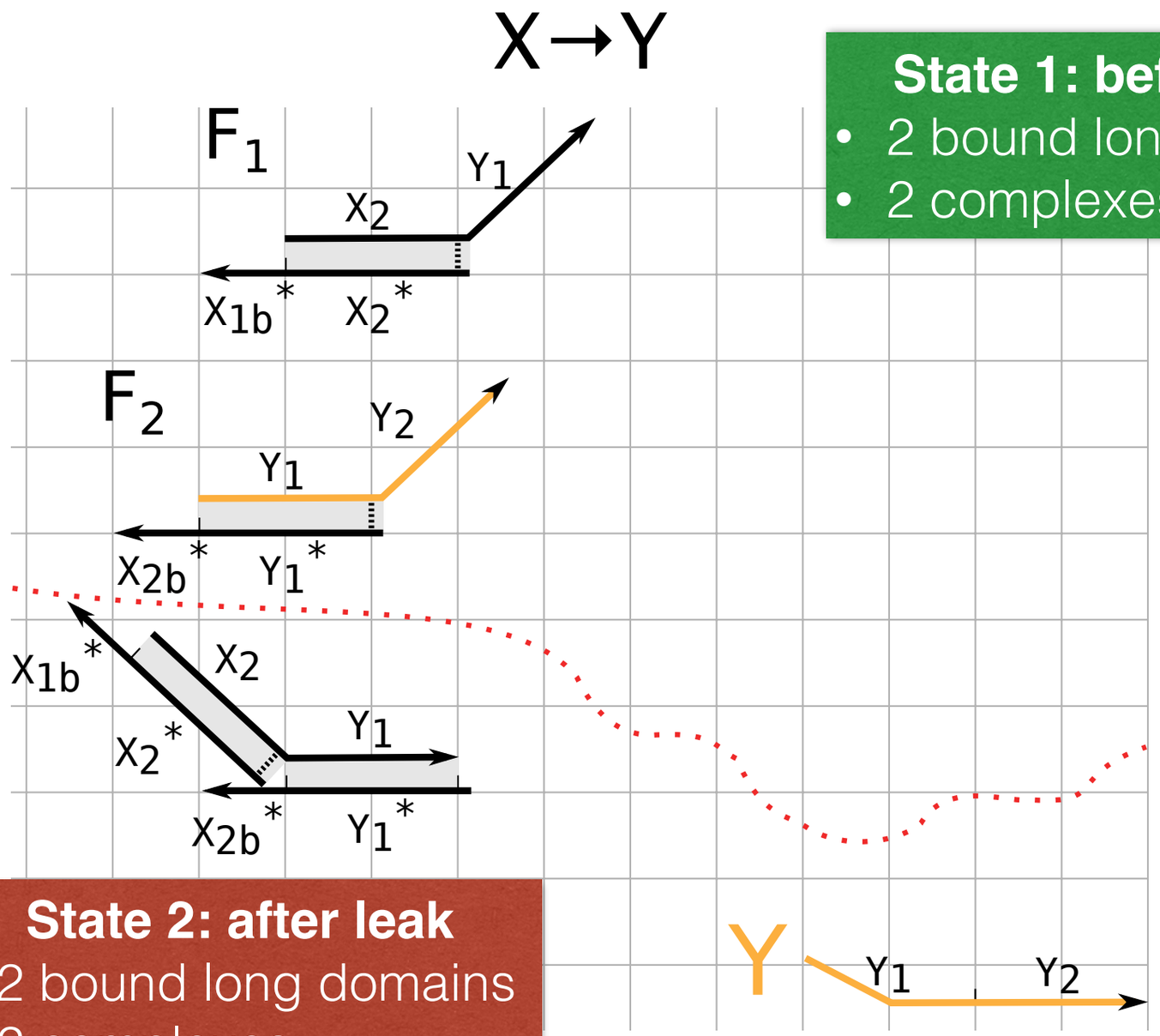
(even with perfect molecules)

X→Y



Y has been spuriously “produced”

Some rough energy accounting



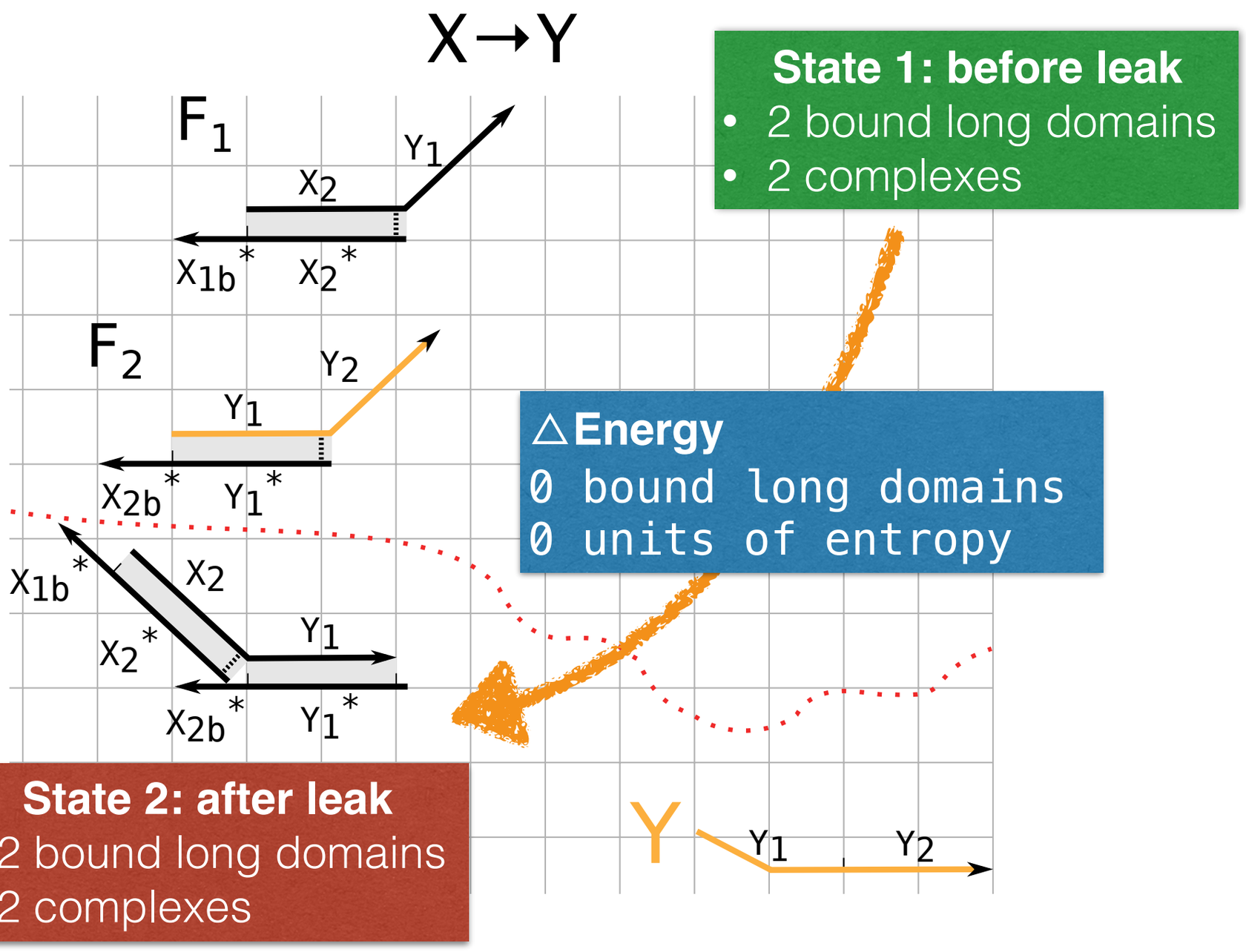
State 1: before leak

- 2 bound long domains
- 2 complexes

State 2: after leak

- 2 bound long domains
- 2 complexes

Some rough energy accounting



State 1: before leak
- 2 bound long domains
- 2 complexes

△Energy
0 bound long domains
0 units of entropy

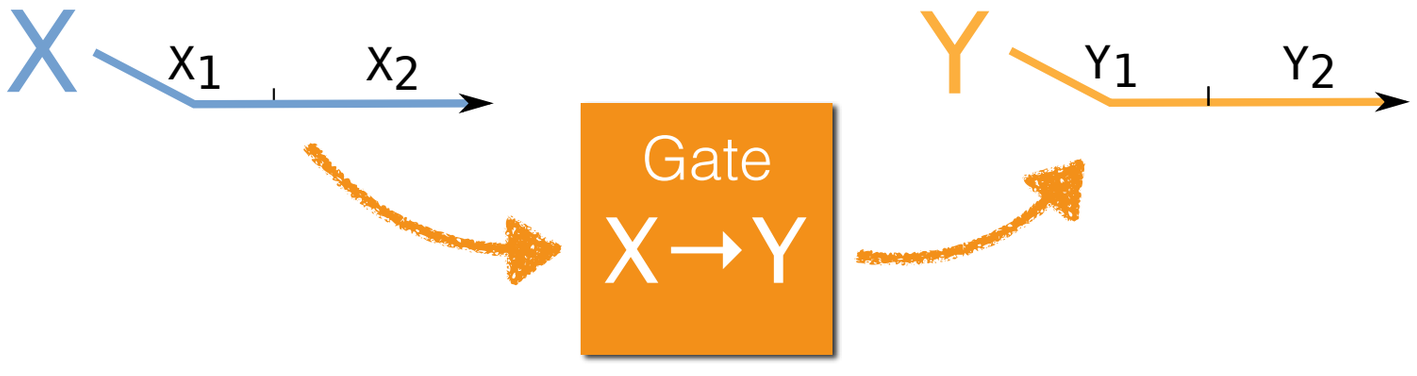
State 2: after leak
- 2 bound long domains
- 2 complexes

A Motivating Question

Can we rationally design *composable*, *leakless* DSD gates?

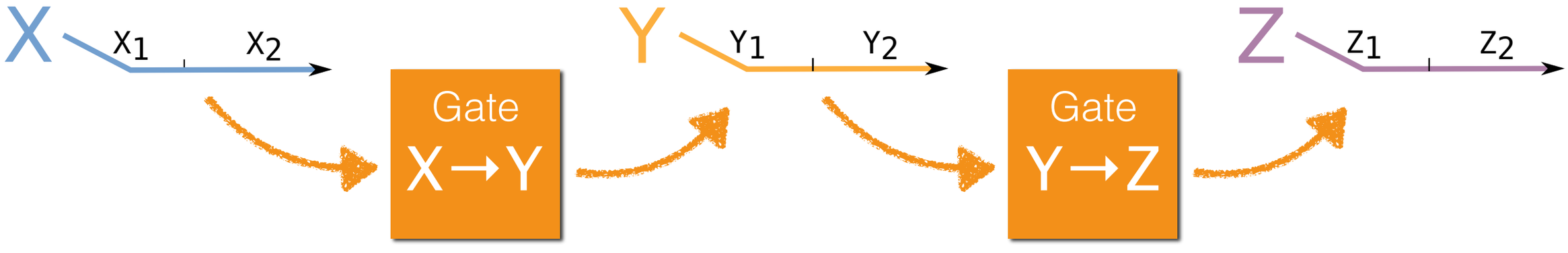
A Motivating Question

Can we rationally design *composable*, *leakless* DSD gates?

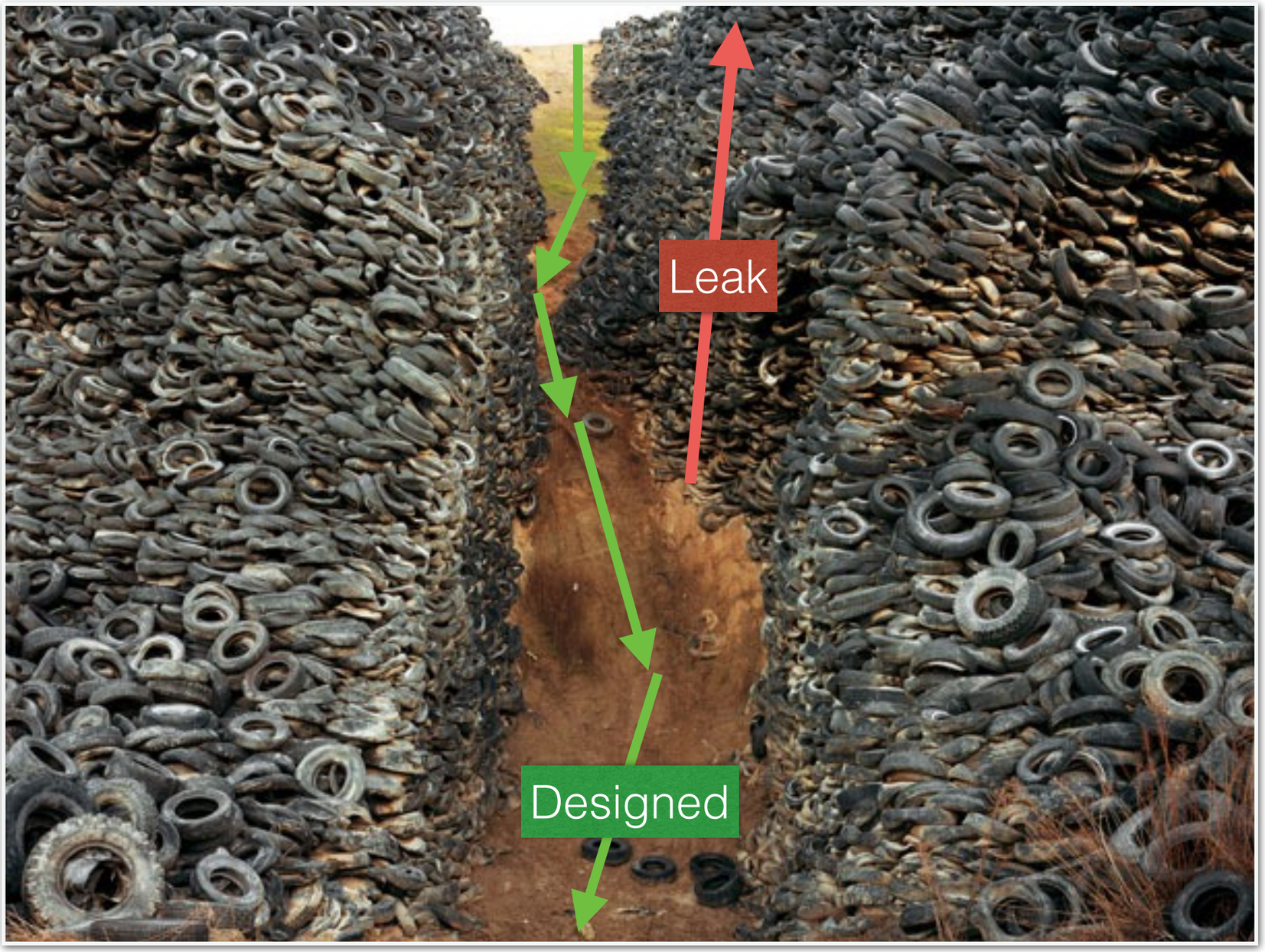


A Motivating Question

Can we rationally design *composable*, *leakless* DSD gates?

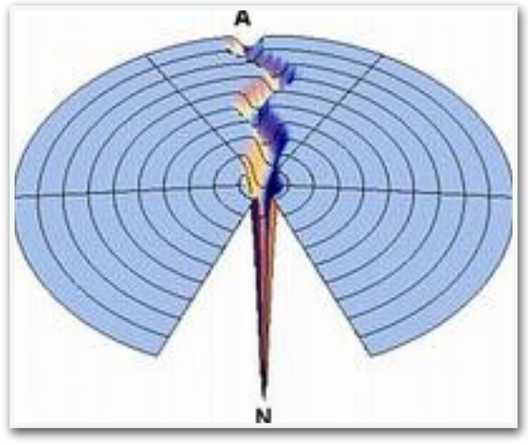


What do we mean by *leakless?*



“Golf funnel with deep groove” pathway

K. Dill & Bromberg (2002). Molecular Driving Forces.



(Partial) solution to Problem 2

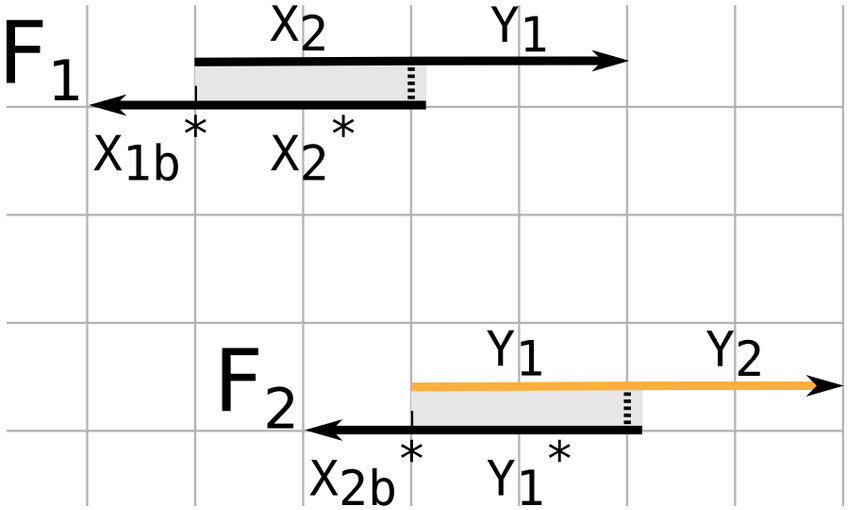
For a redundancy parameter **N**, there exist translator and AND gates using **N** long domains that have the following property:

even at thermodynamic equilibrium,

the net leak decreases exponentially with **N.**

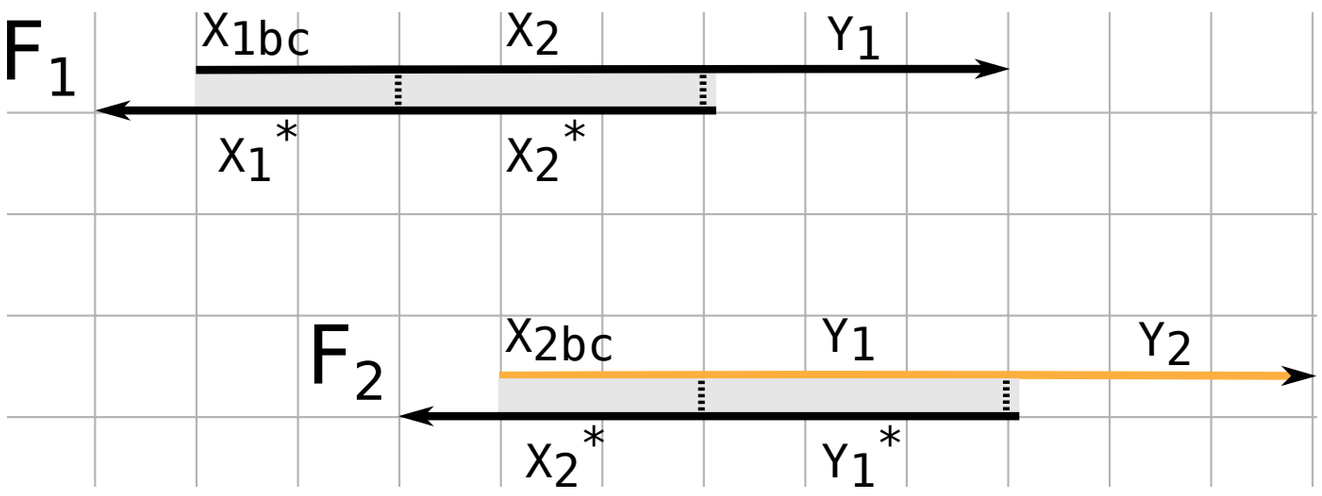
Thachuk, Winfree, David Soloveichik. (2015)
Leakless DNA strand displacement. DNA 21.

Typical translator using “Single Long Domain” (SLD)



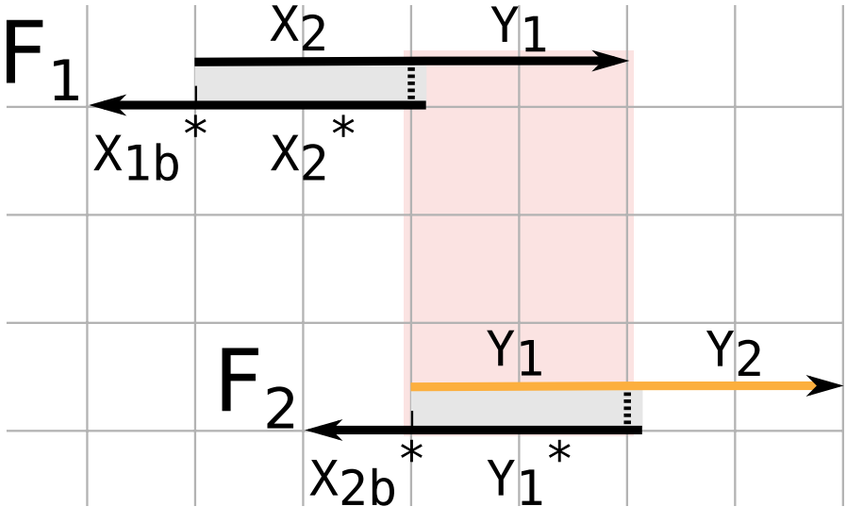
- Designed pathways: bimolecular
- Leak pathways: bimolecular

DLD translator using “Double Long Domain” (DLD)



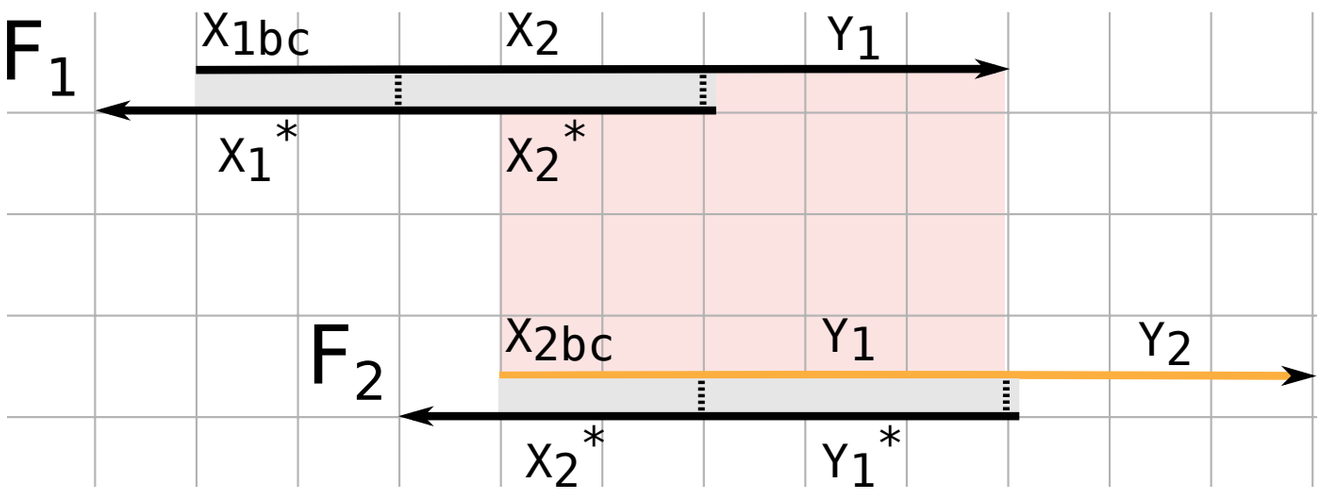
- Designed pathways: bimolecular
- Leak pathways: **trimolecular**

Typical translator using “Single Long Domain” (SLD)



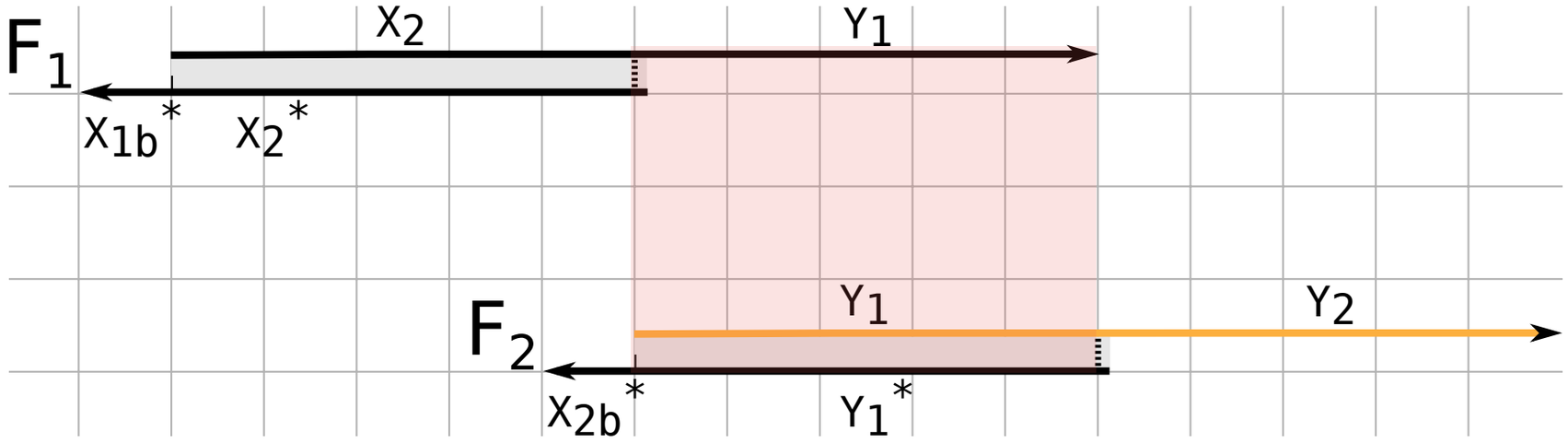
- Designed pathways: bimolecular
- Leak pathways: bimolecular

DLD translator using “Double Long Domain” (DLD)



- Designed pathways: bimolecular
- Leak pathways: **trimolecular**

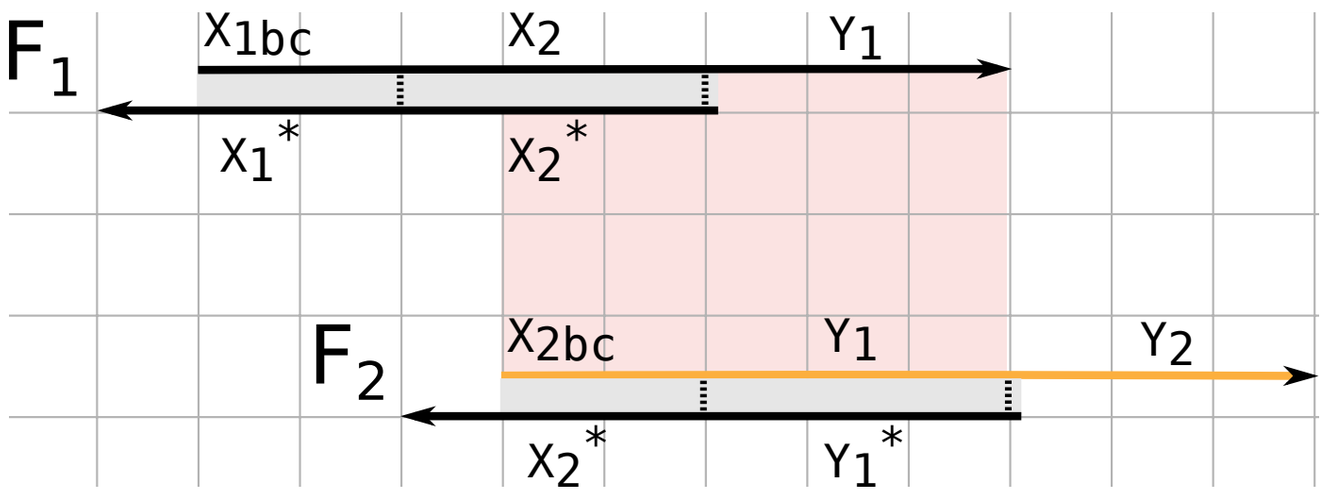
Typical translator using “Single Long Domain” (SLD)



- Designed pathways: bimolecular
- Leak pathways: bimolecular

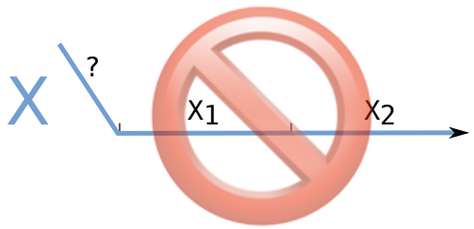
Lengthening recognition domains does not help

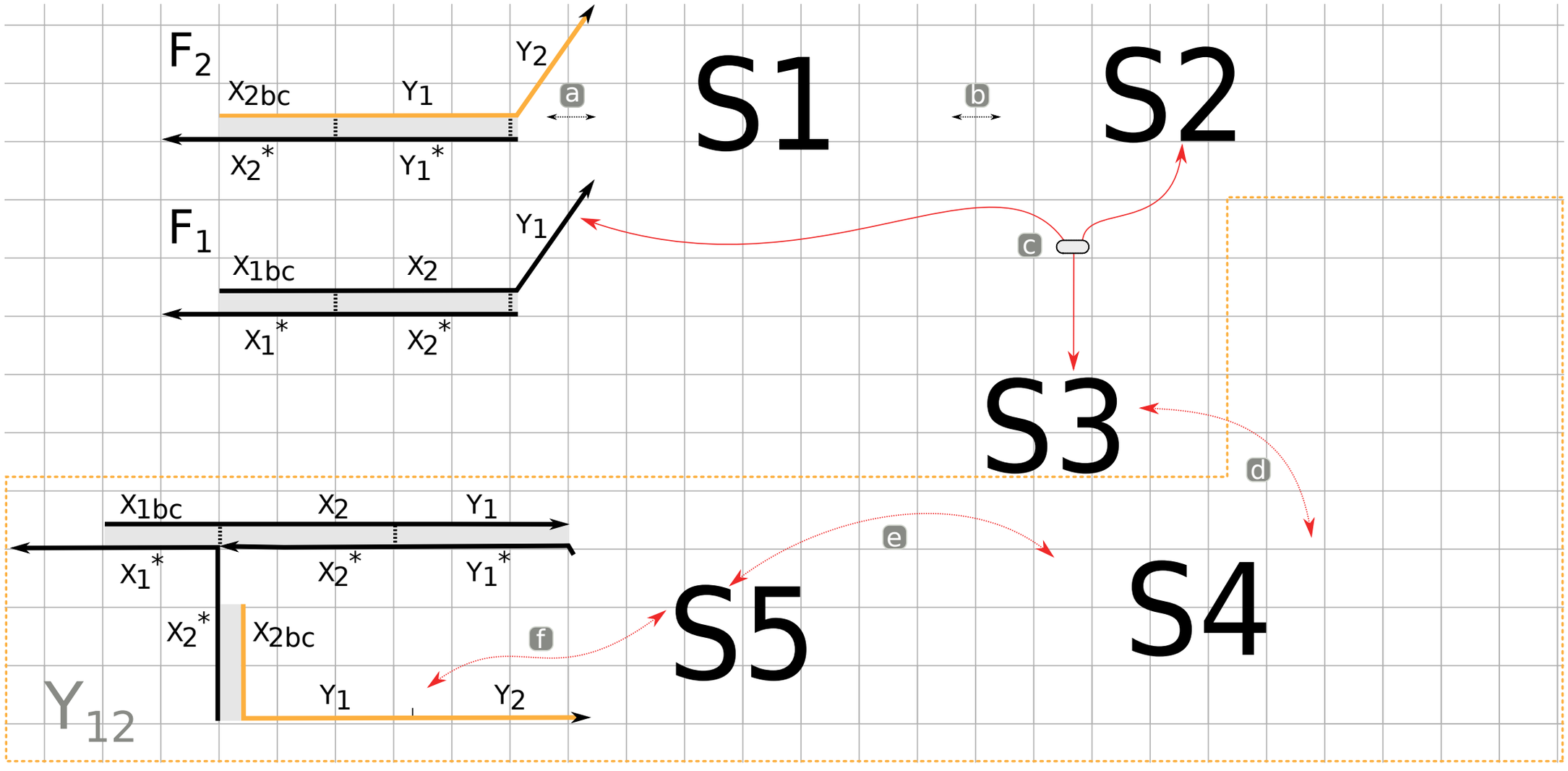
DLD translator using “Double Long Domain” (DLD)



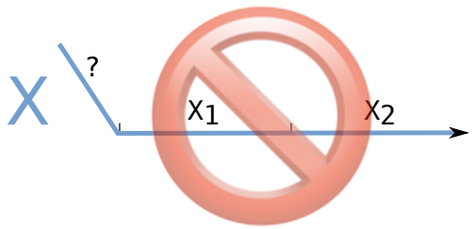
- Designed pathways: bimolecular
- Leak pathways: **trimolecular**

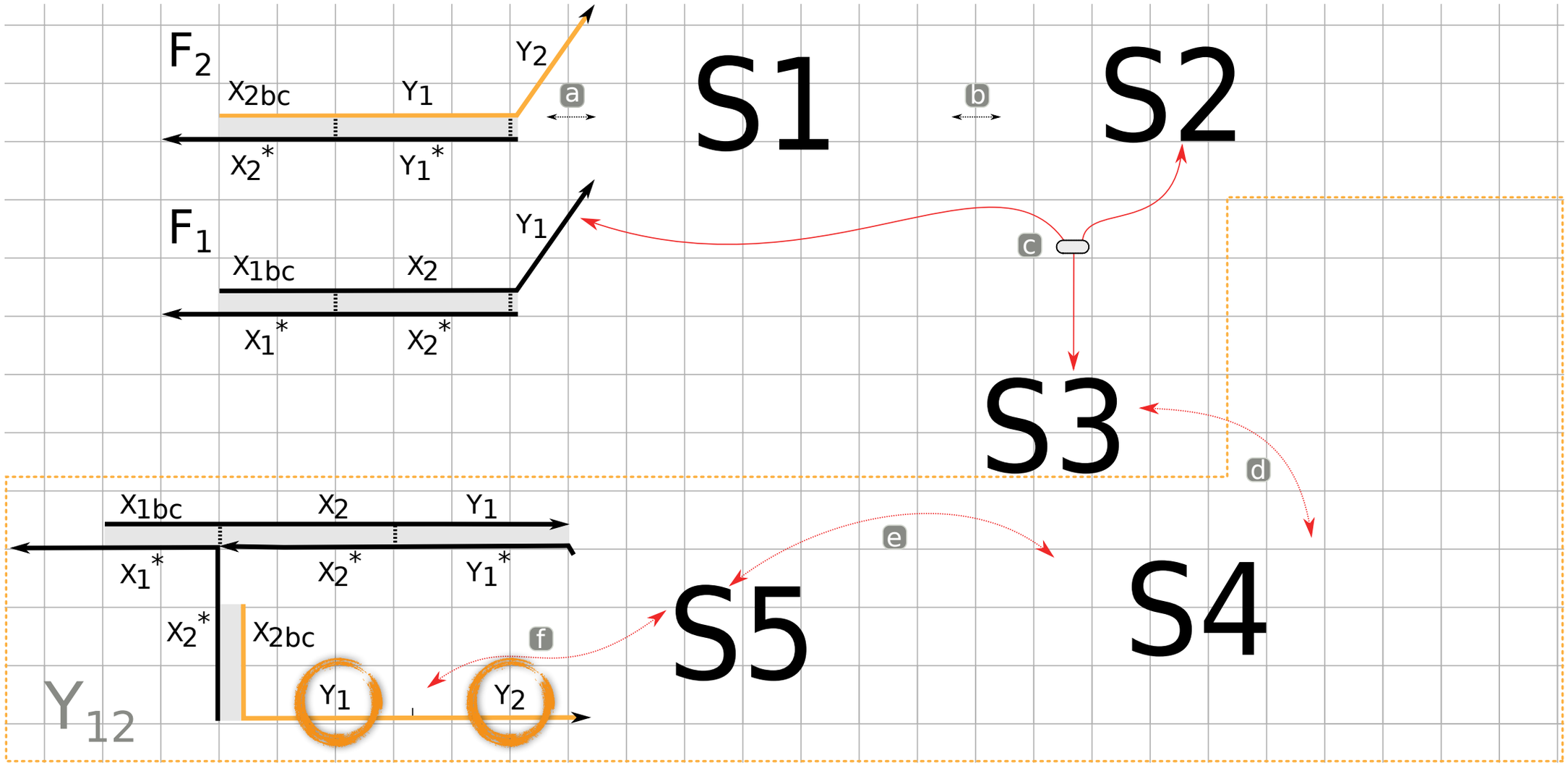
DLD translators are intrinsically less “leaky”



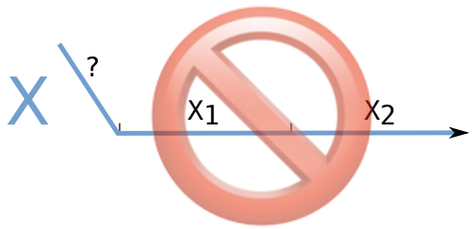


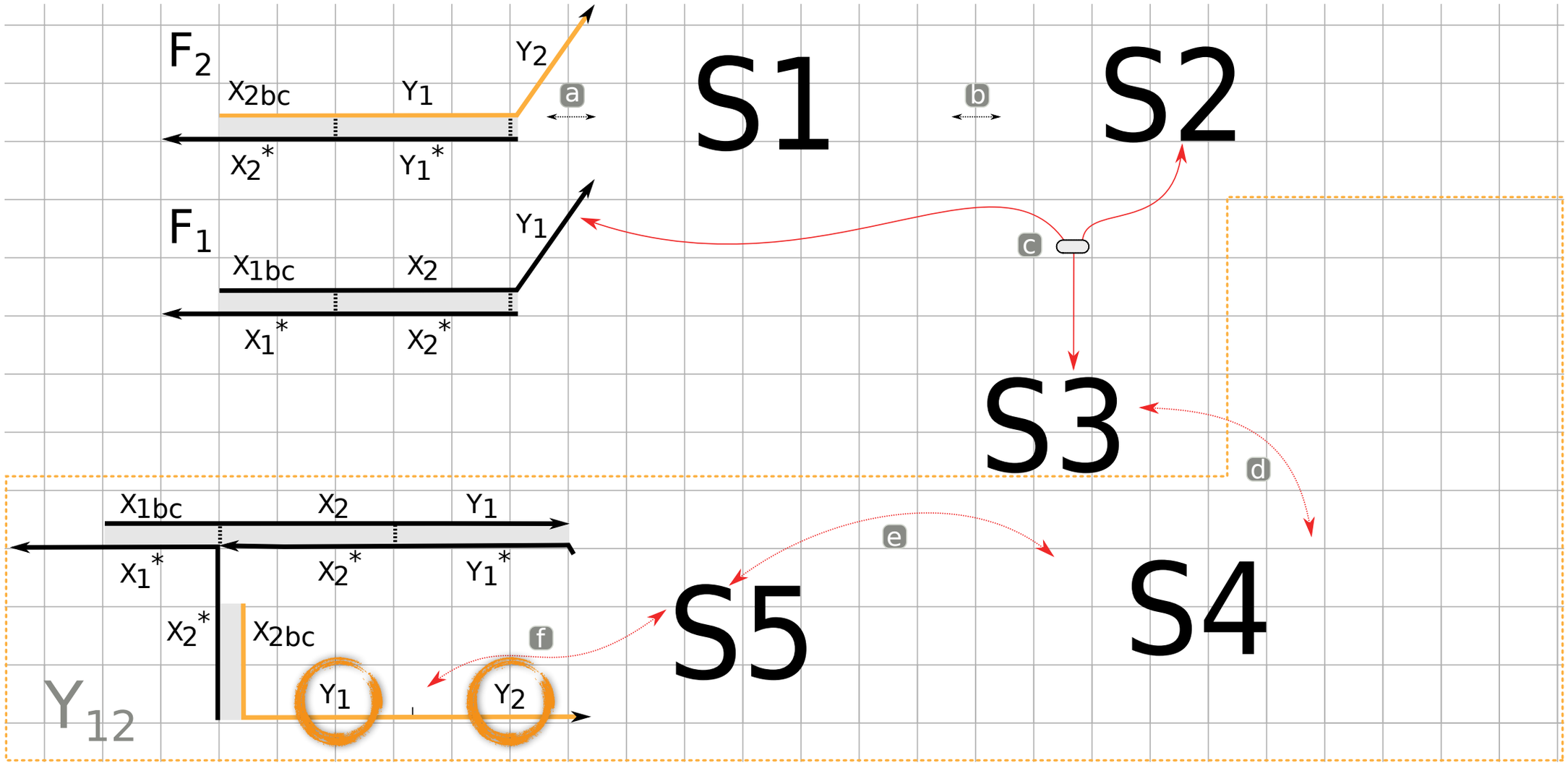
DLD translators are intrinsically less “leaky”

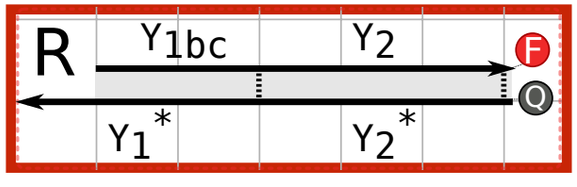




DLD translators are intrinsically less “leaky”

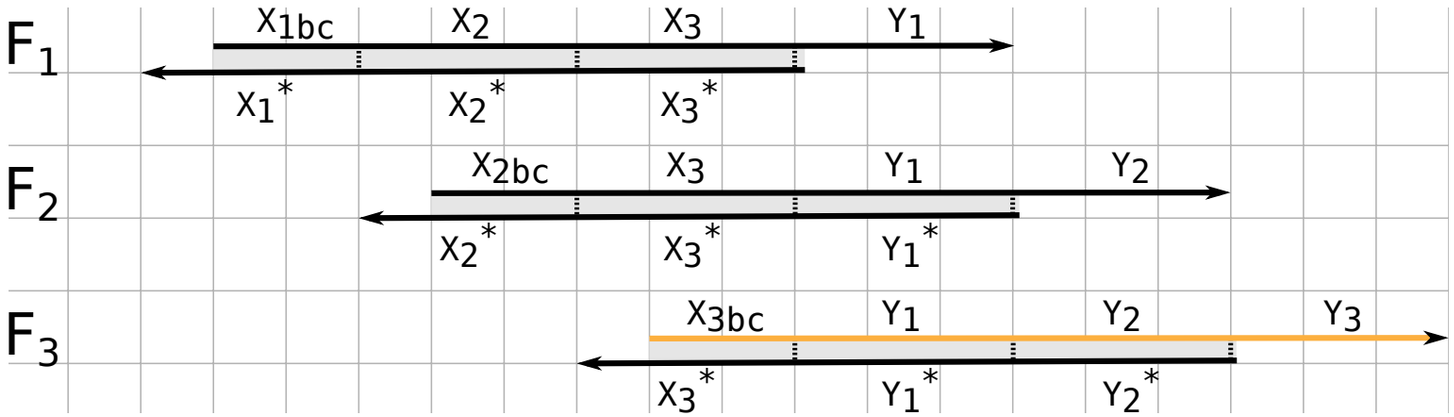






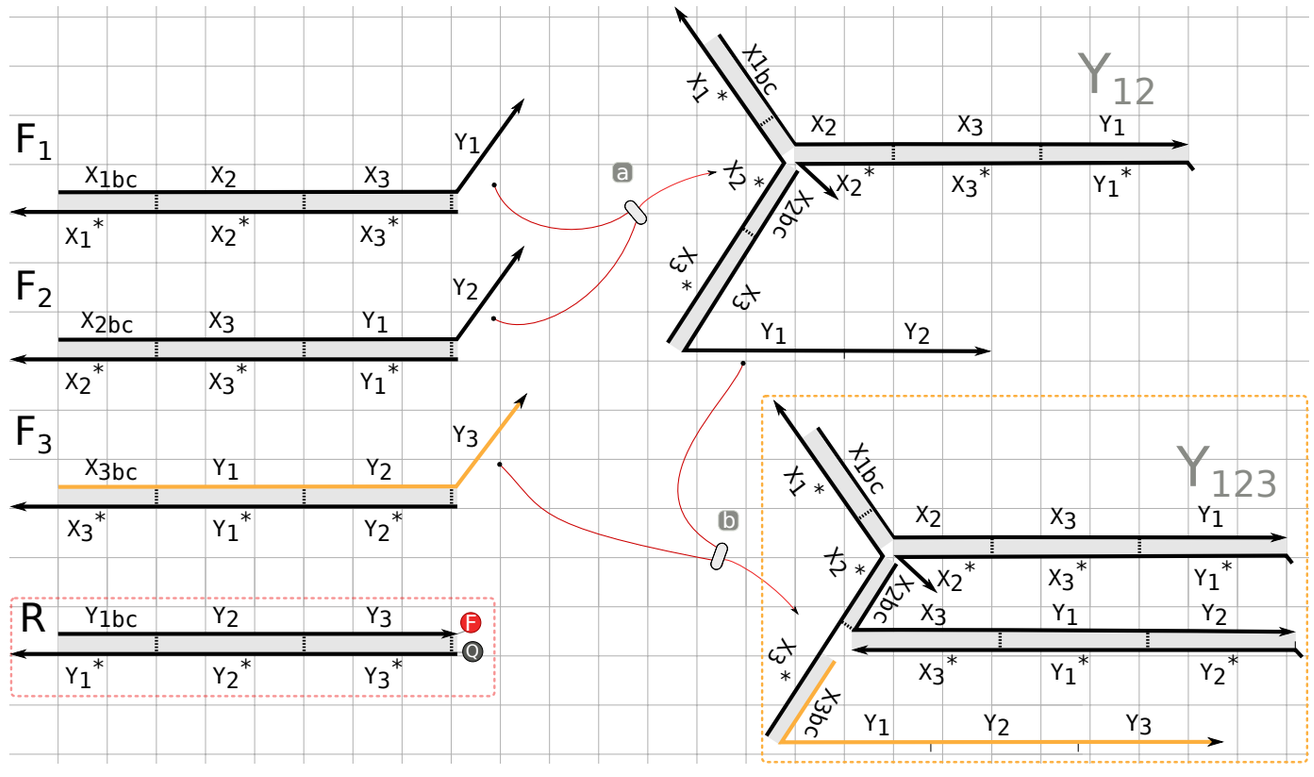
Can we generalize the DLD motif?

Translator using Triple Long Domain (TLD) motif

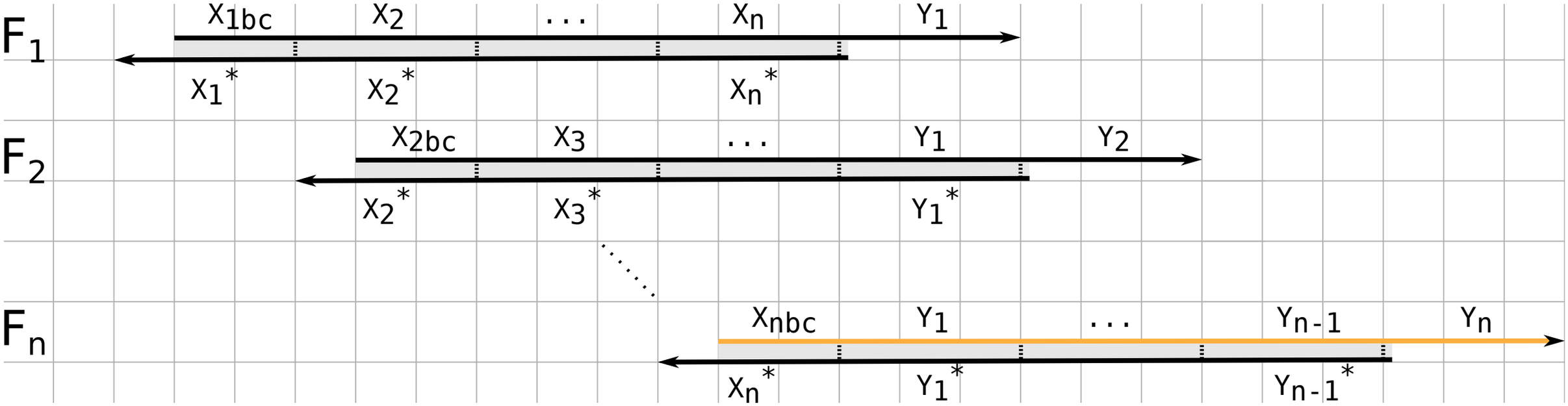


Three fuel complexes must combine to activate output signal.

△Energy
0 bound long domains
−2 units of entropy



Translator using *N* Long Domain (NLD) motif

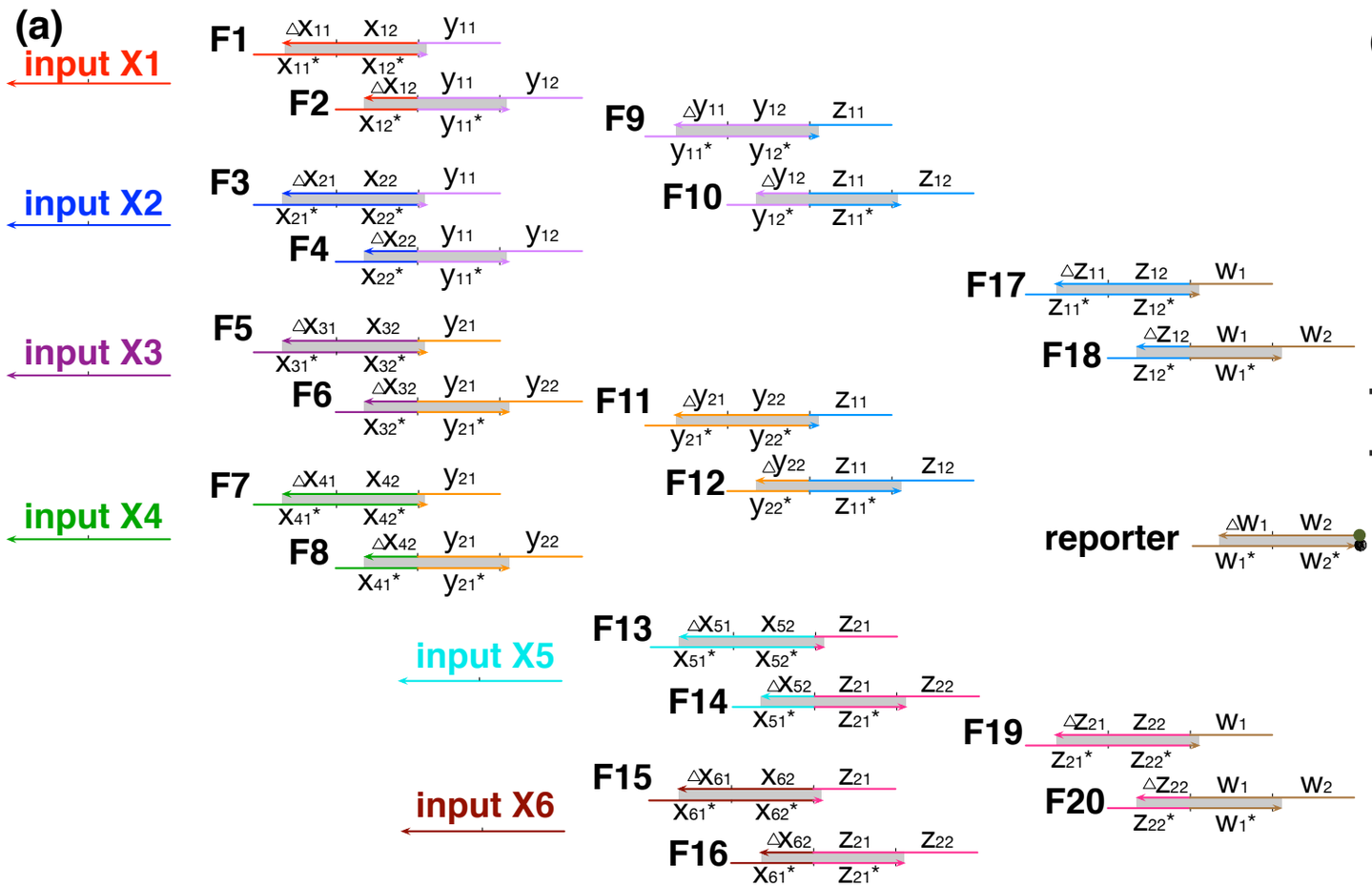


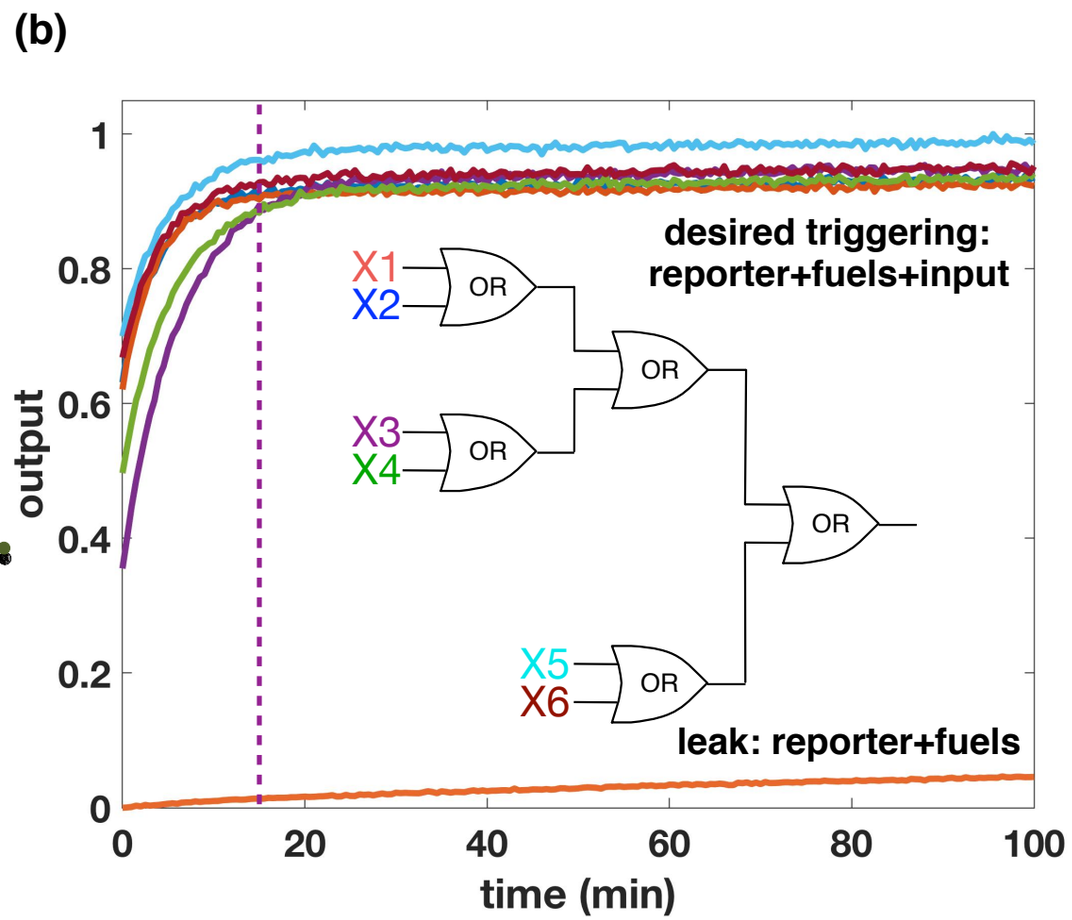
N fuel complexes must combine to activate output signal.

△Energy to leak state

```
 0      bound long domains
-(N-1) units of entropy
```

Building OR circuits from DLD translators

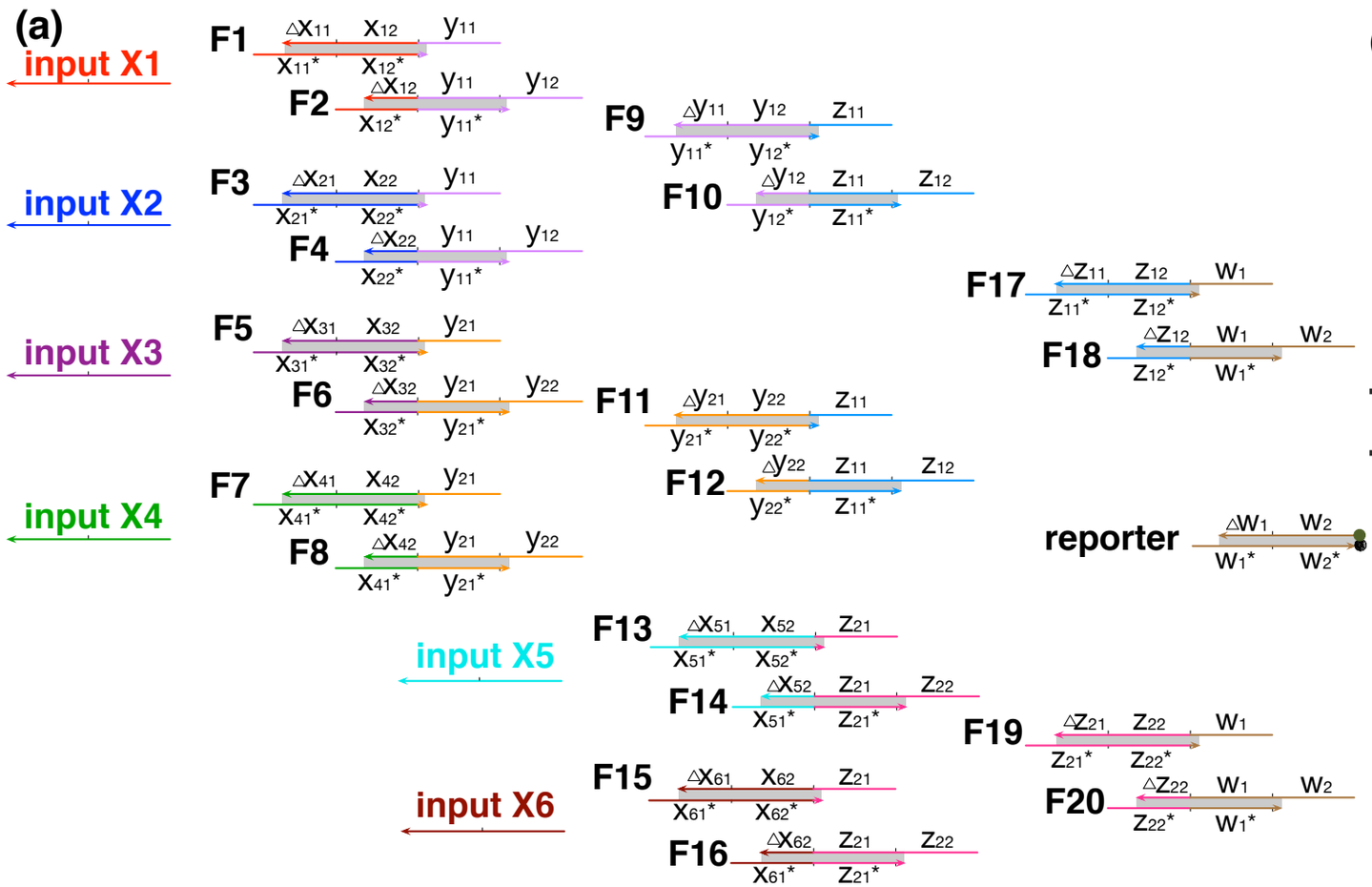


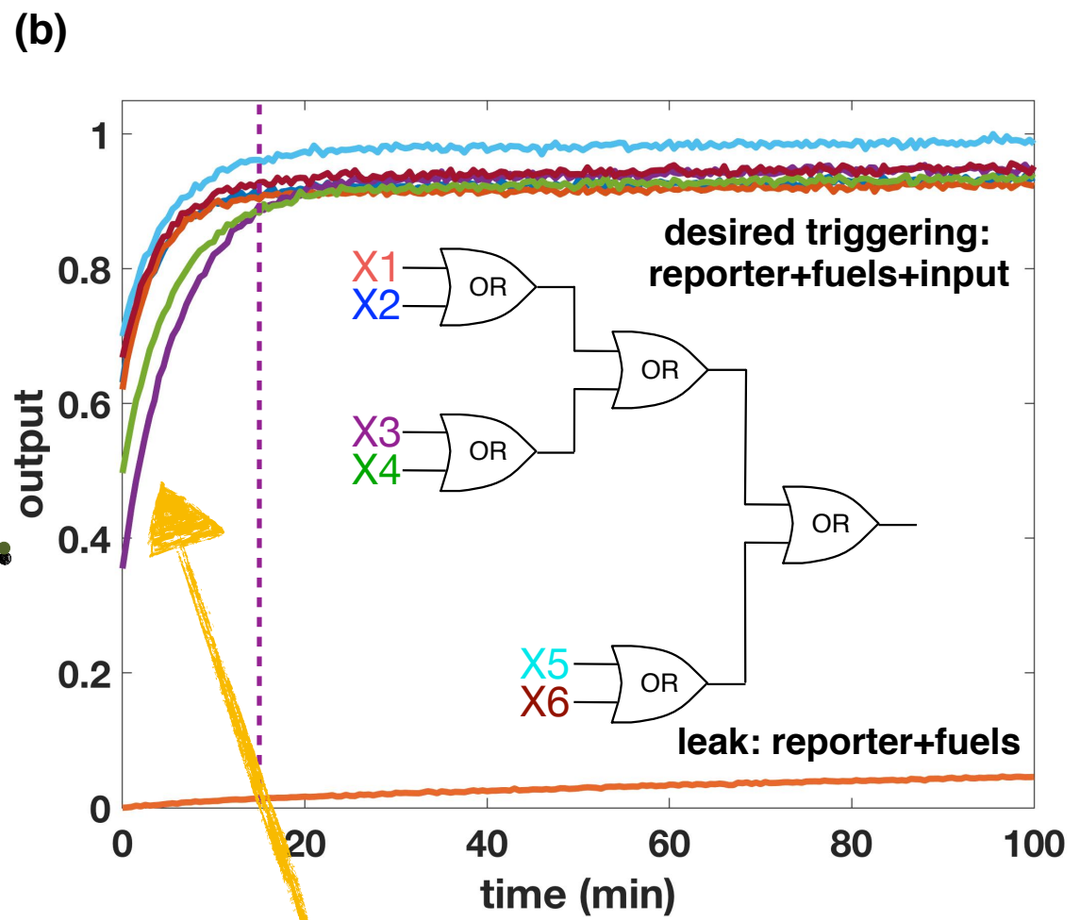


[fuel]=[input]=1000nM
[reporter]=500nM

Boya Wang, Thachuk, Ellington, Winfree, **David Soloveichik**. (In Review)
Effective Design Principles for Leakless Strand Displacement Systems

Building OR circuits from DLD translators





[fuel]=[input]=1000nM
[reporter]=500nM

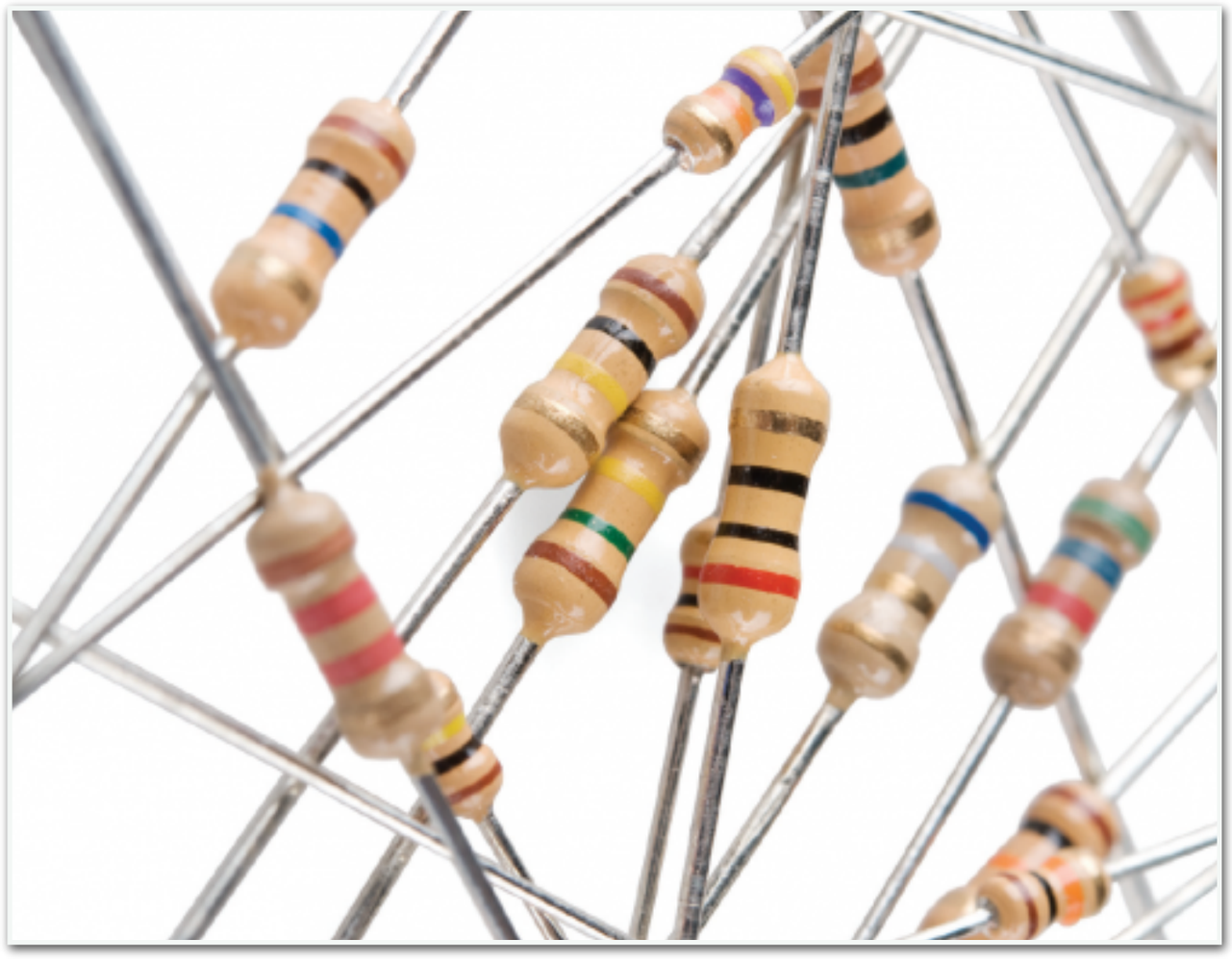
Half-time completion ~6 minutes
No signal restoration used.

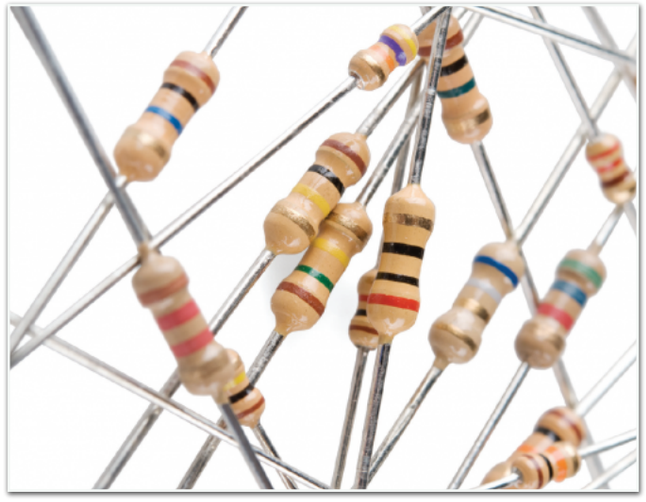
Boya Wang, Thachuk, Ellington, Winfree, **David Soloveichik**. (In Review)
Effective Design Principles for Leakless Strand Displacement Systems

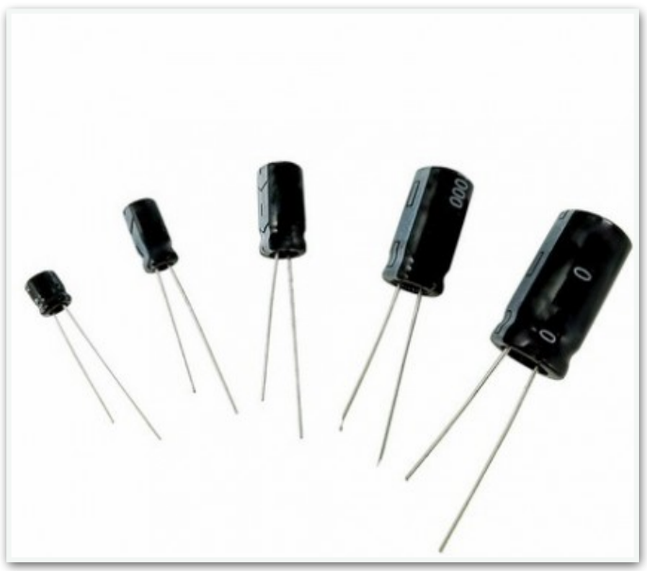
Tutorial Outline

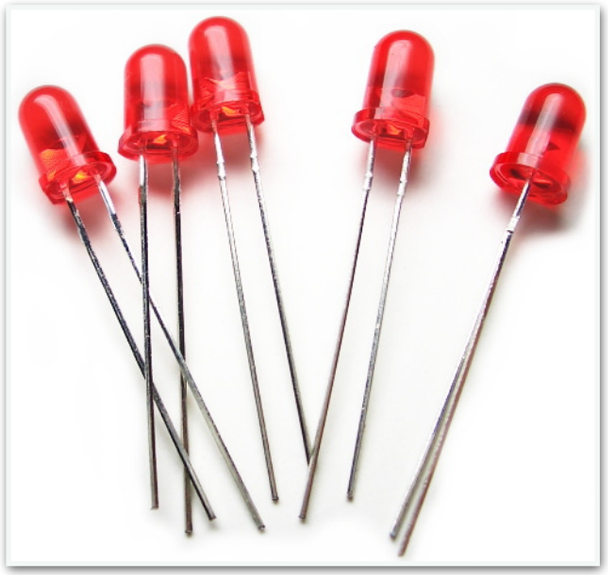
- Review of strand displacement
- Building and composing logic gates
- Tools for designing and verifying circuits
- Robustness of strand displacement
- **(Bonus) DSD circuits the easy way**

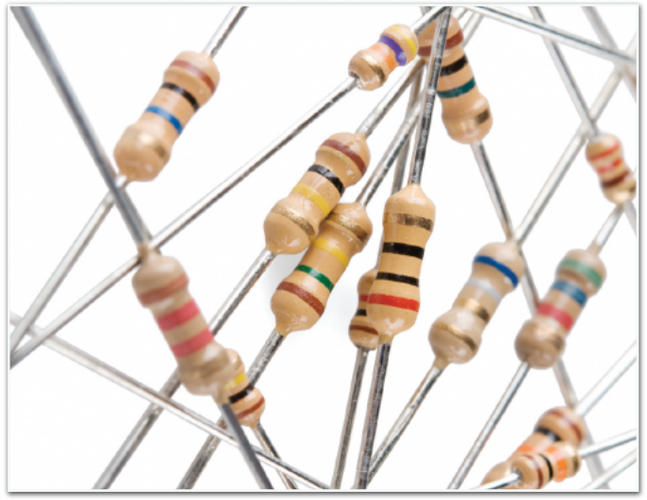
Does it need to be this difficult to build a circuit?

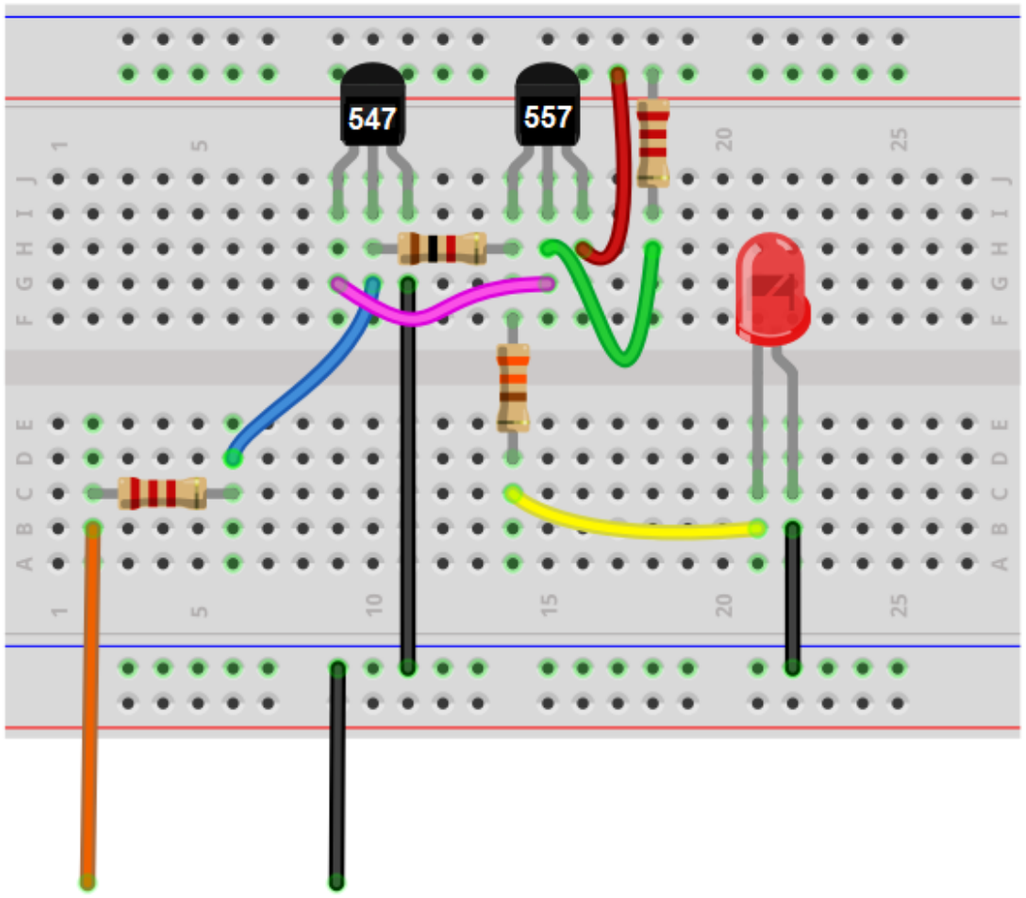


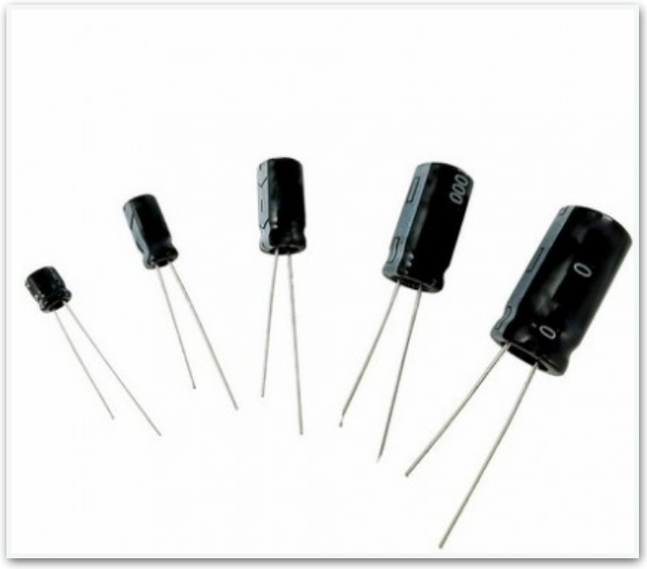


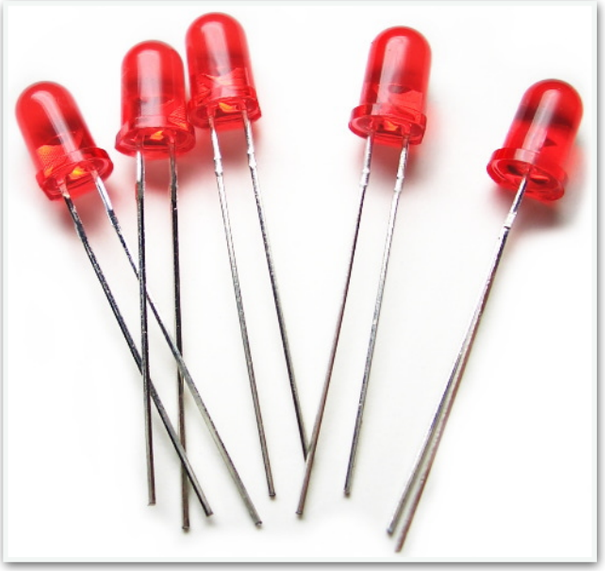







547
557
+ Input Voltage -



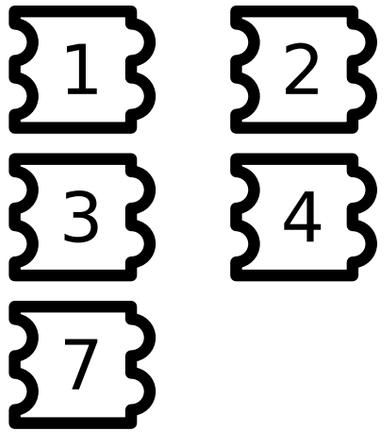


Molecular breadboard 1.0

input signals

A1	B1
A2	B2
A3	B3
A4	B4
A7	B7

reaction gates



wires

X1	R1	Y3	A2
Y1	R2	Y3	B2
X2	B1	X4	B1
X2	R2	X4	B2
Y2	R1	Y4	A2
X3	B1	Y4	R2
X3	B4	X7	A1
X3	R2		

reporters

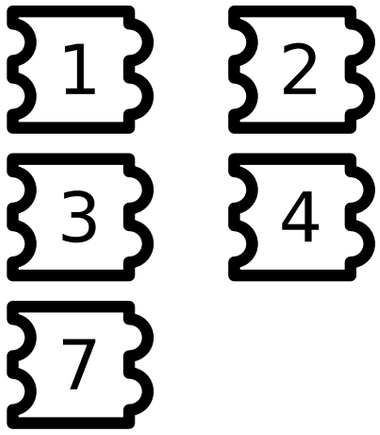
Built using leakless motif

Molecular breadboard 1.0

input signals

A1 B1
A2 B2
A3 B3
A4 B4
A7 B7

reaction gates



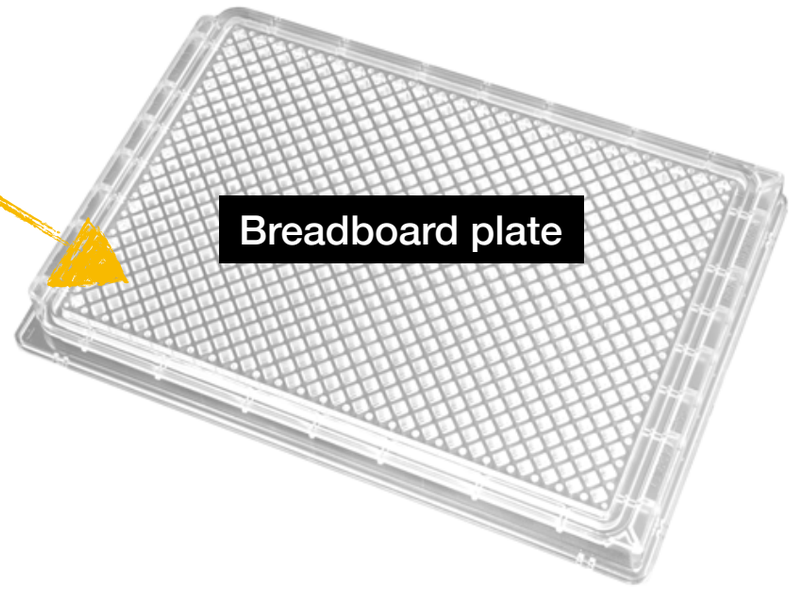
wires

X1 ..... R1
Y1 ..... R2
X2 ..... B1
X2 ..... R2
Y2 ..... R1
X3 ..... B1
X3 ..... B4
X3 ..... R2
Y3 ..... A2
Y3 ..... B2
X4 ..... B1
X4 ..... B2
Y4 ..... A2
Y4 ..... R2
X7 ..... A1

reporters

Built using leakless motif

Load breadboard components onto 384-well plate

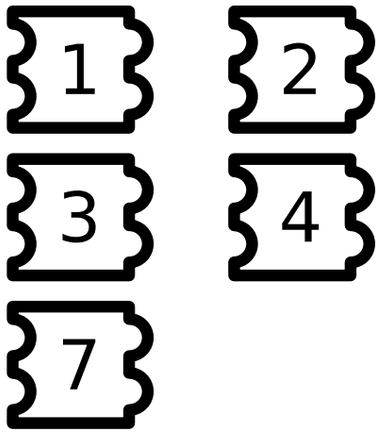


Molecular breadboard 1.0

input signals

A1 B1
A2 B2
A3 B3
A4 B4
A7 B7

reaction gates



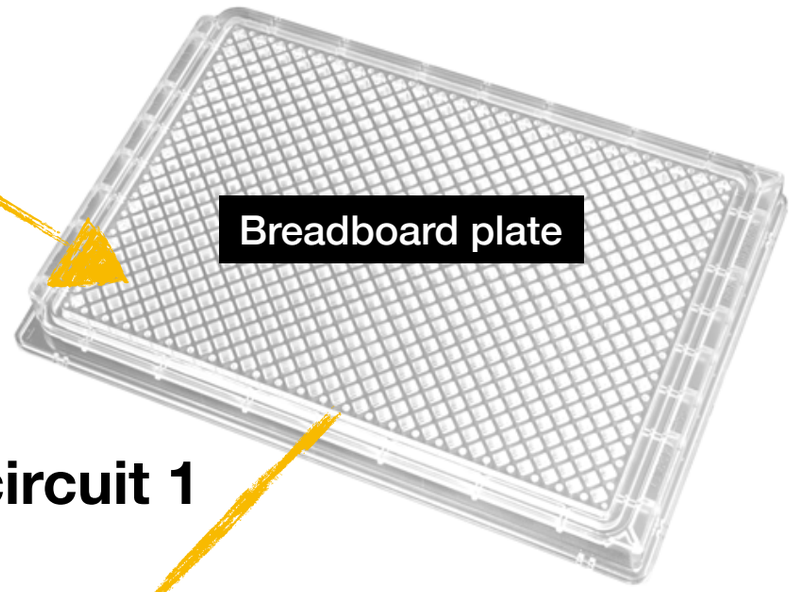
wires

X1 ..... R1
Y1 ..... R2
X2 ..... B1
X2 ..... R2
Y2 ..... R1
X3 ..... B1
X3 ..... B4
X3 ..... R2
Y3 ..... A2
Y3 ..... B2
X4 ..... B1
X4 ..... B2
Y4 ..... A2
Y4 ..... R2
X7 ..... A1

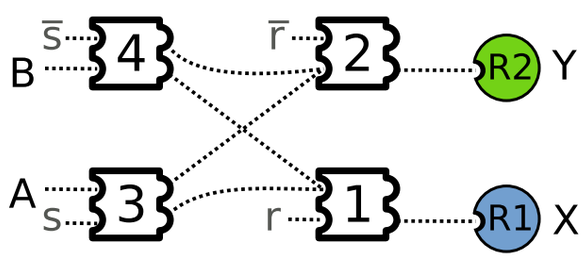
reporters

Built using leakless motif

Load breadboard components onto 384-well plate



circuit 1

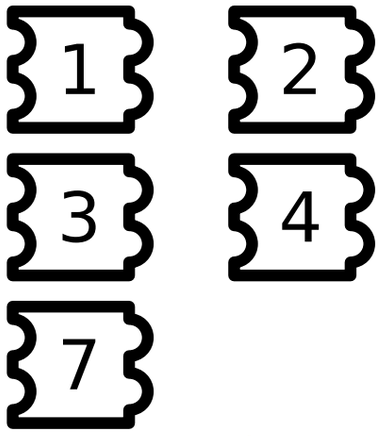


Molecular breadboard 1.0

input signals

A1 B1
A2 B2
A3 B3
A4 B4
A7 B7

reaction gates



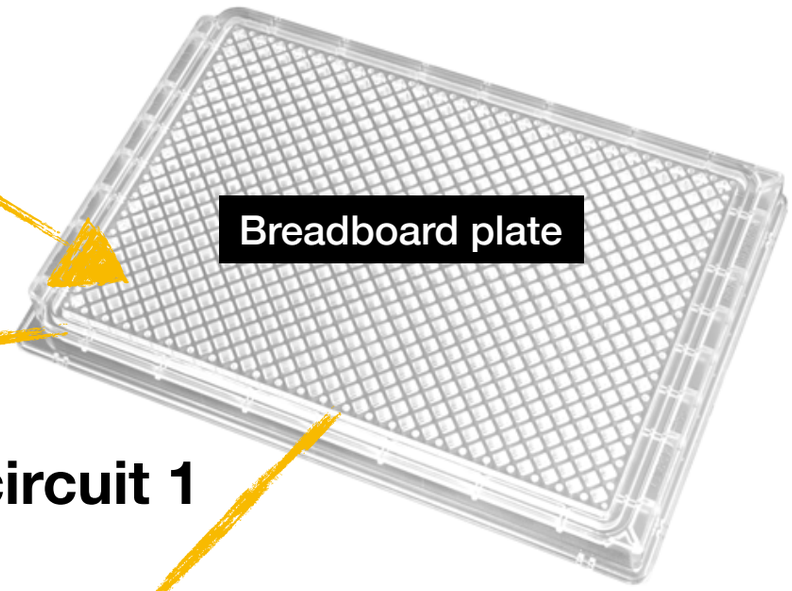
wires

X1 ..... R1
Y1 ..... R2
X2 ..... B1
X2 ..... R2
Y2 ..... R1
X3 ..... B1
X3 ..... B4
X3 ..... R2
Y3 ..... A2
Y3 ..... B2
X4 ..... B1
X4 ..... B2
Y4 ..... A2
Y4 ..... R2
X7 ..... A1

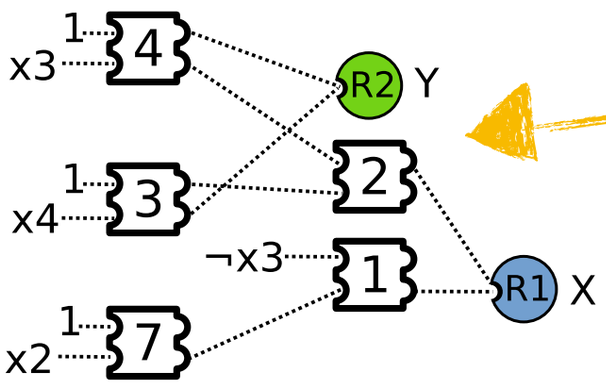
reporters

Built using leakless motif

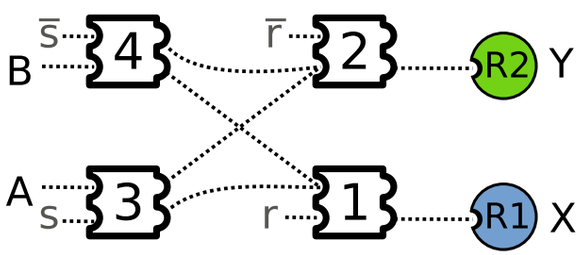
Load breadboard components onto 384-well plate



circuit 2



circuit 1

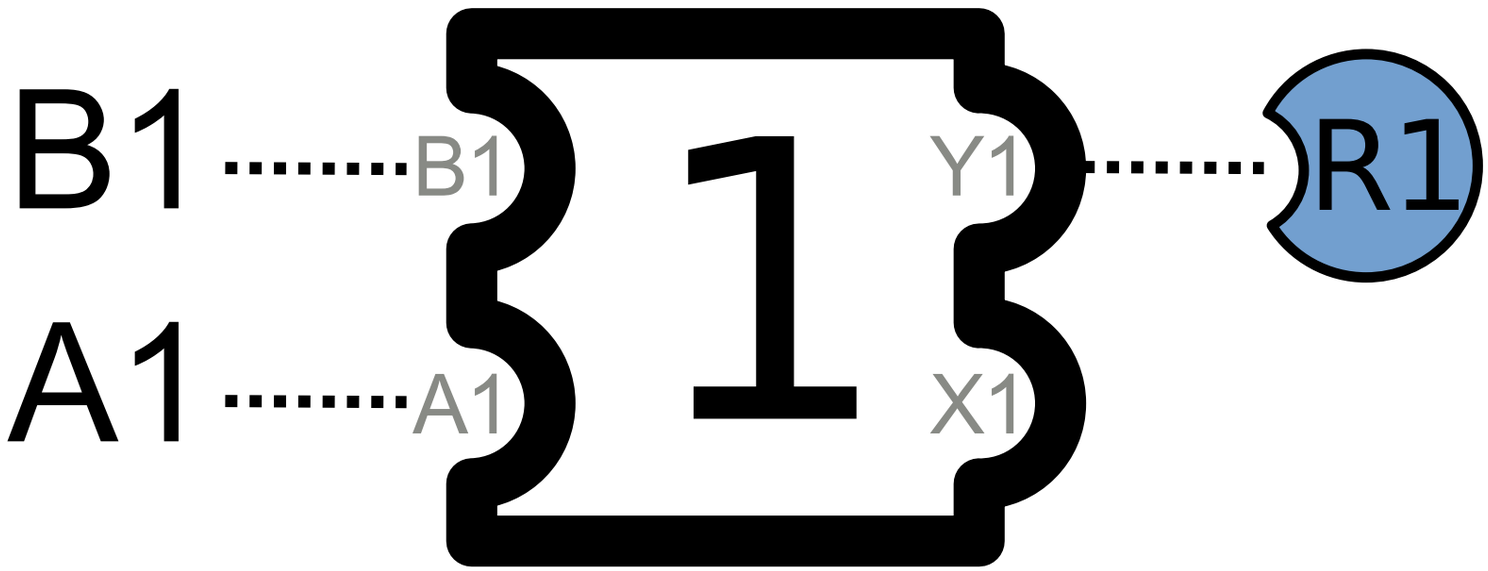


Testing breadboard components

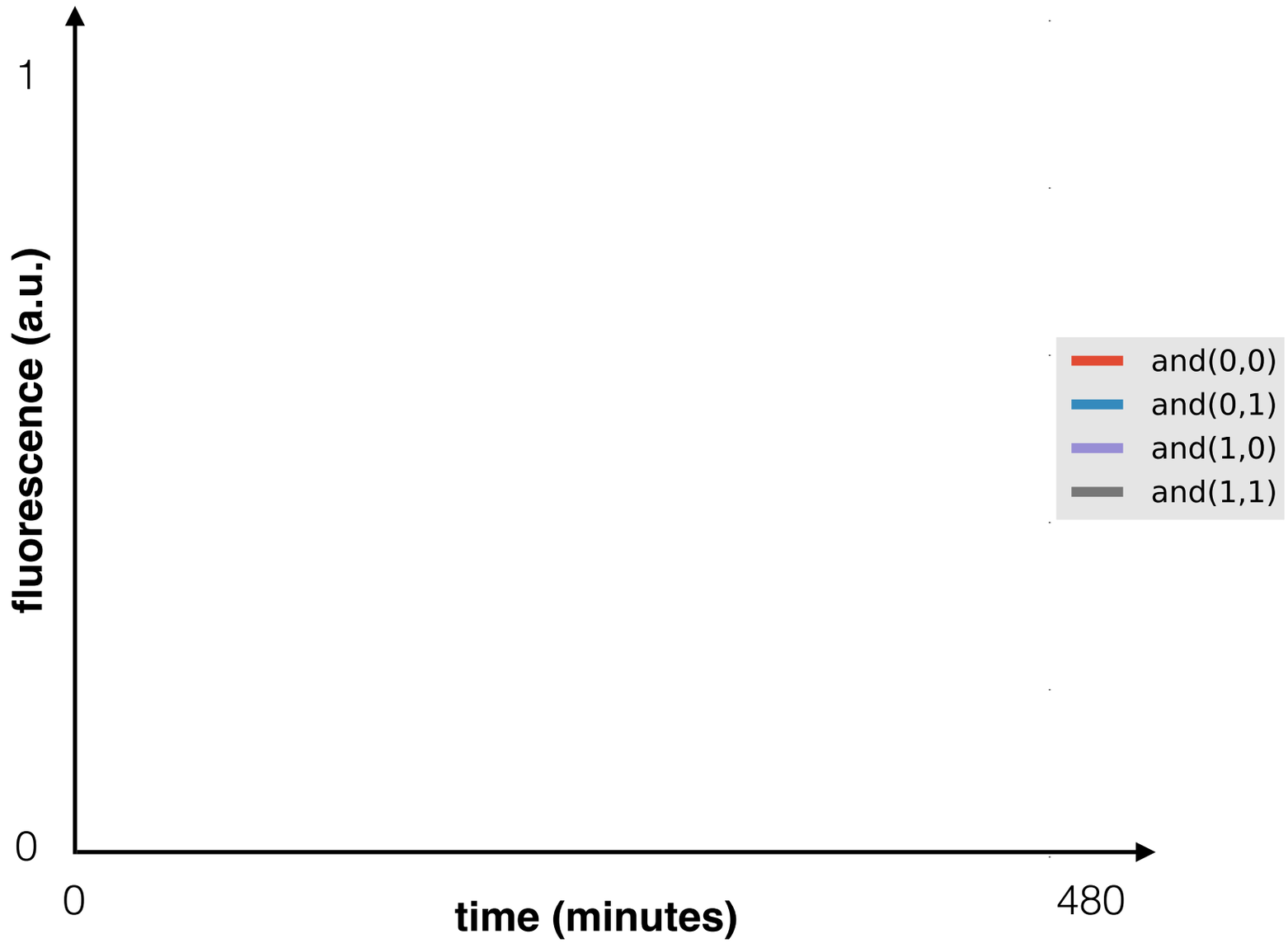
- Typical DSD circuits are 50nM - 200nM concentration
  (our circuits can operate at these concentrations)

- To demonstrate *robustness*, all experiments will be at 2uM
  (~20x higher than typical concentrations)

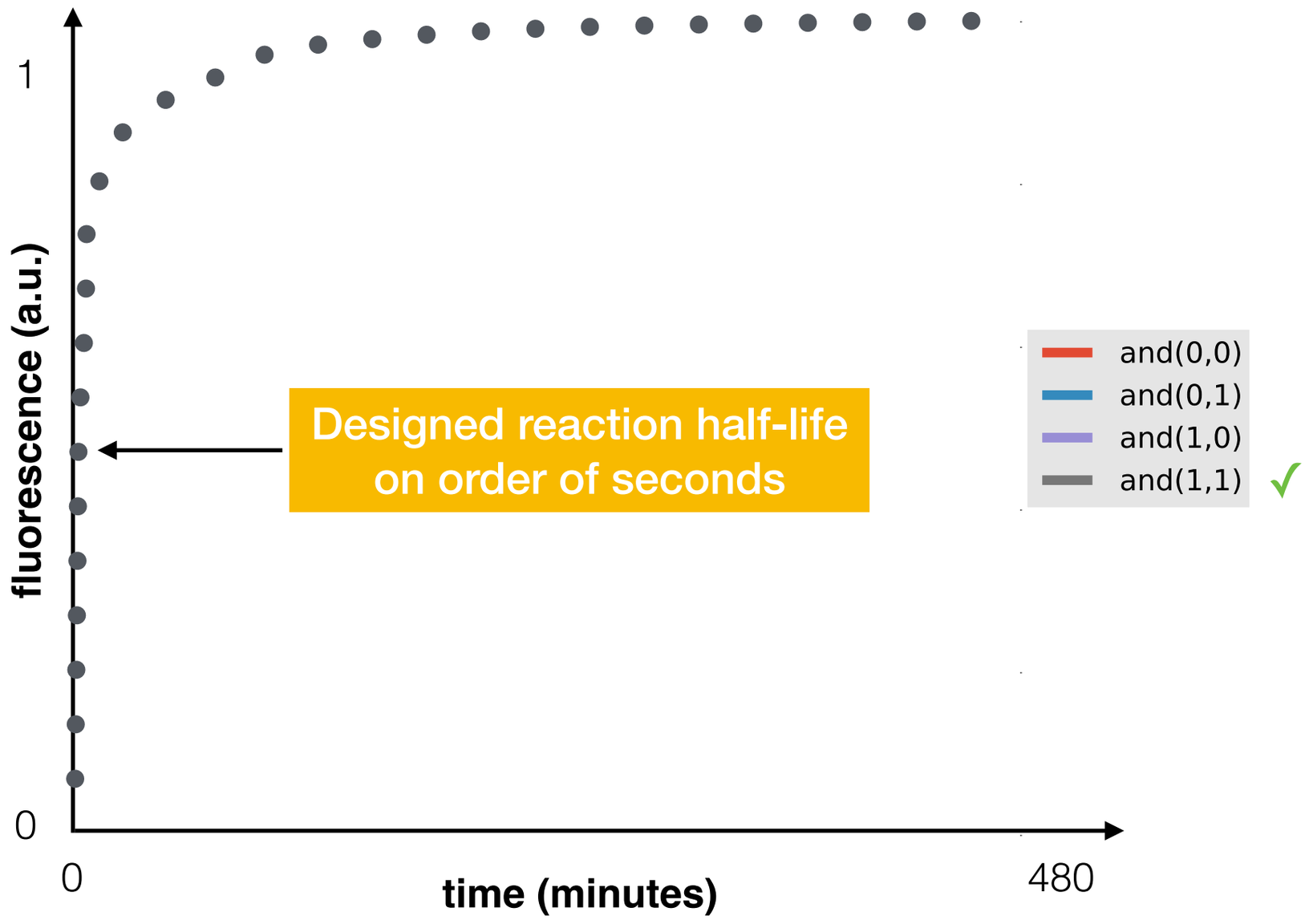
AND gate



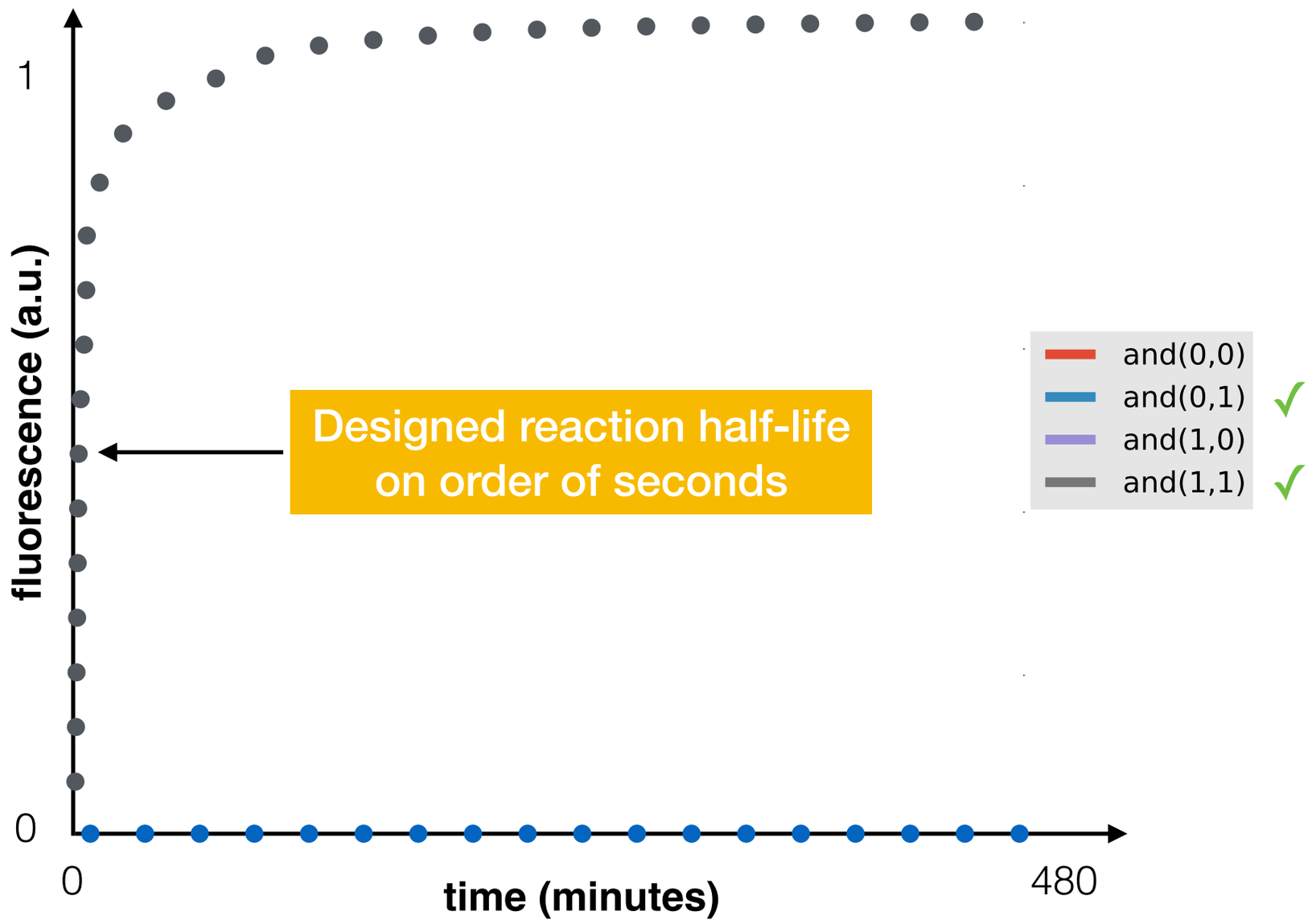
Ideal AND gate simulation @ 2 μM



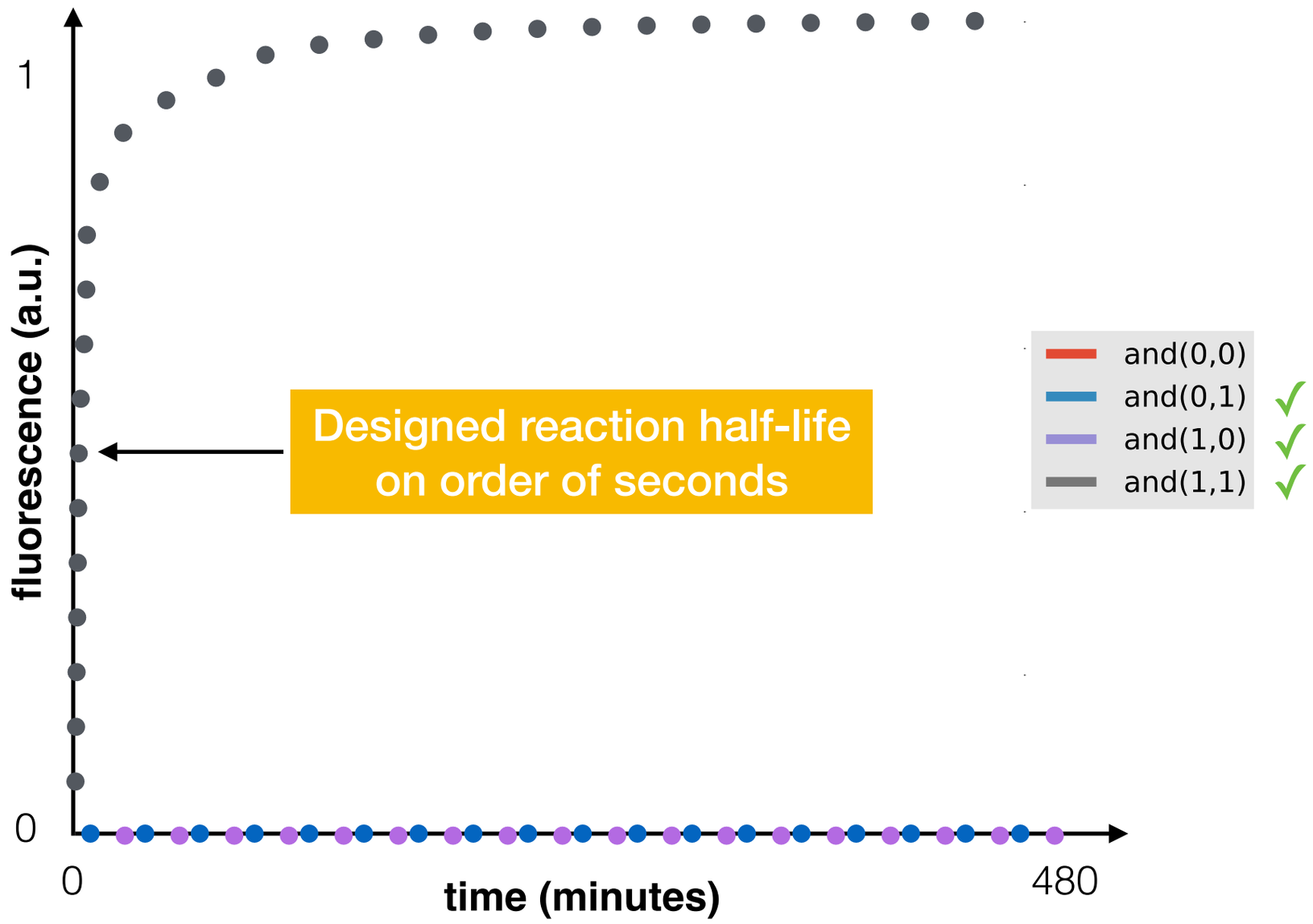
Ideal AND gate simulation @ 2 μM



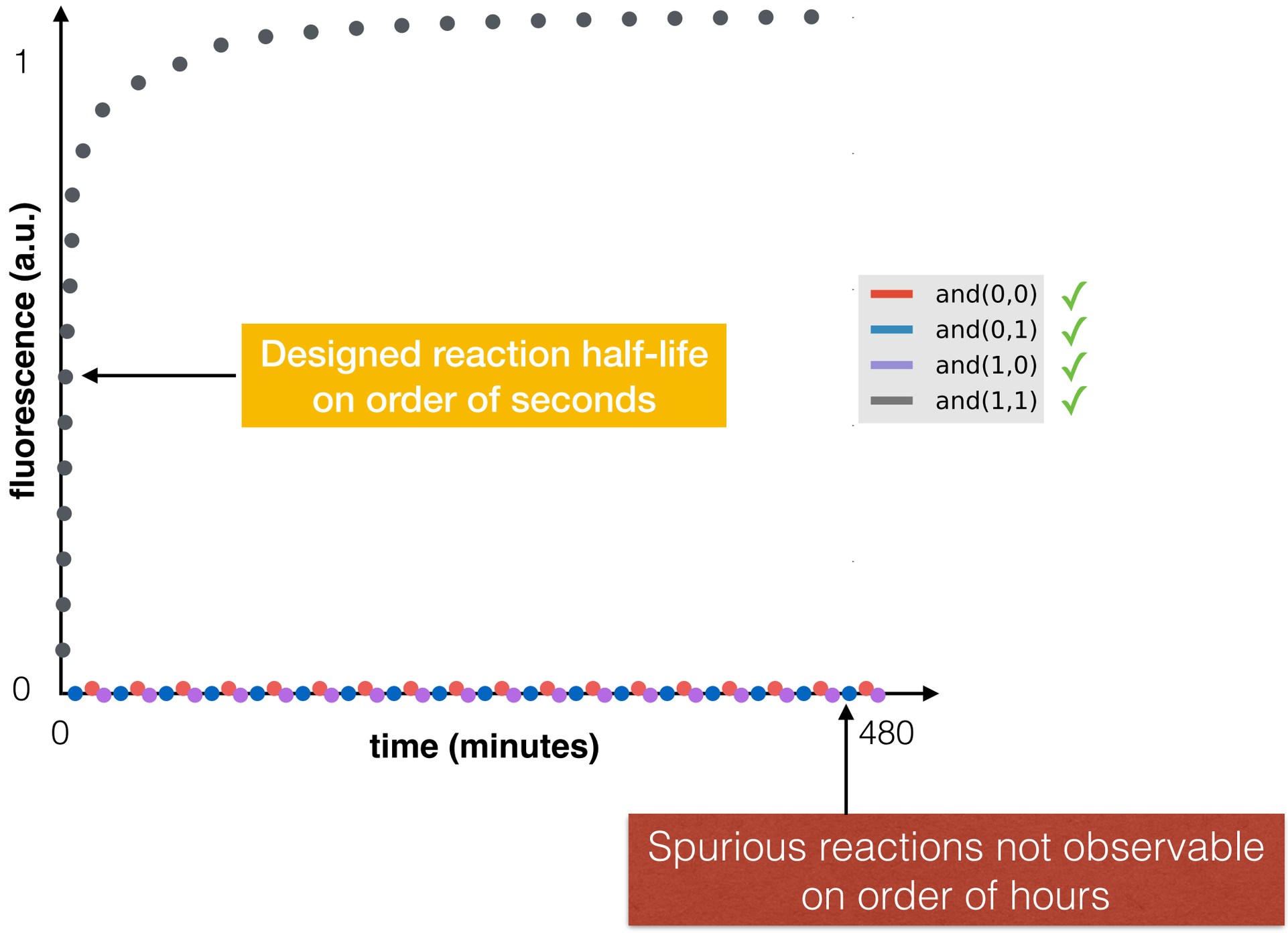
Ideal AND gate simulation @ 2 μM



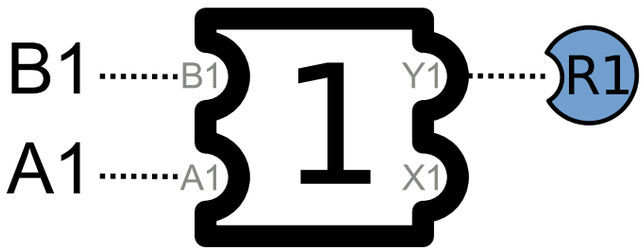
Ideal AND gate simulation @ 2 μM

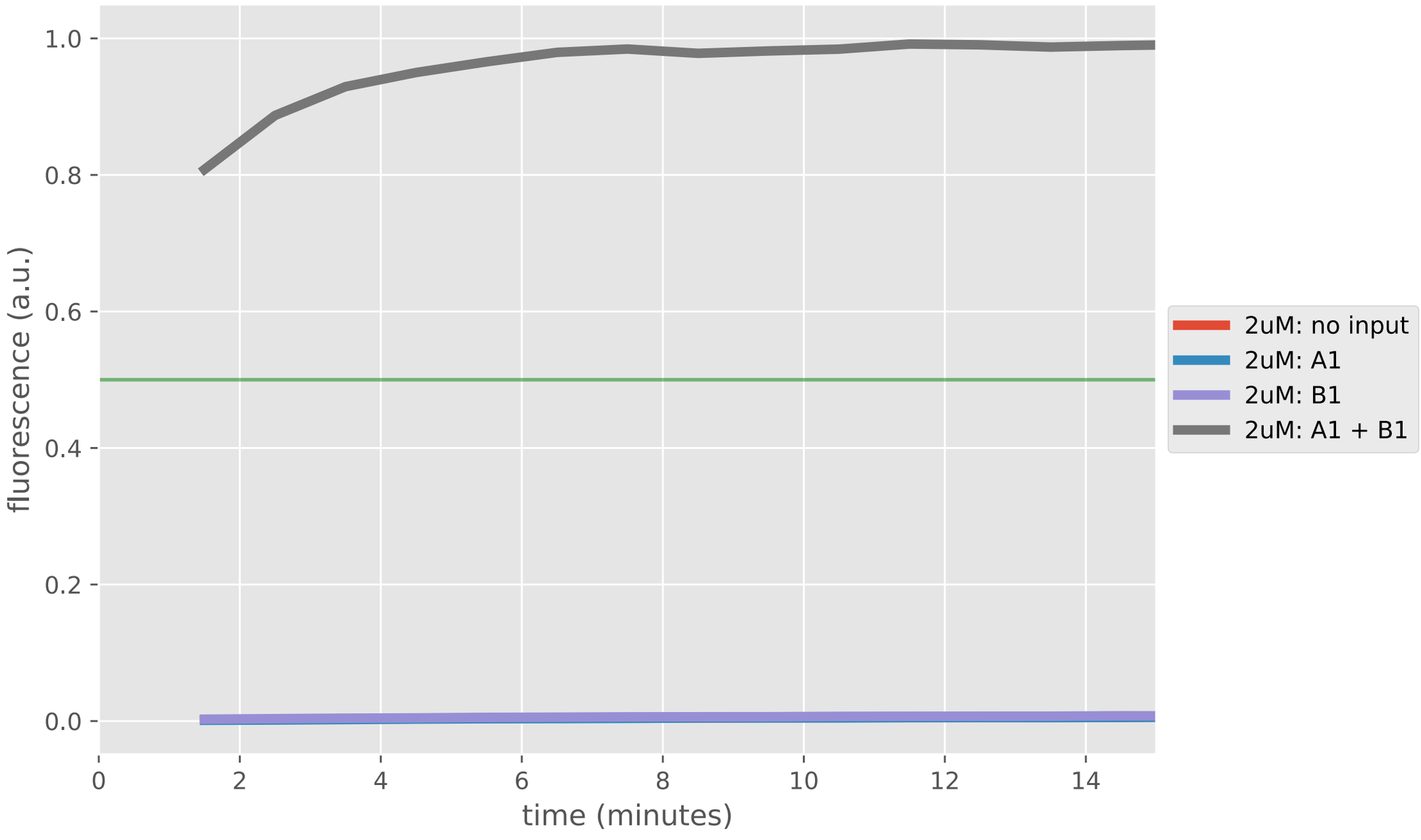


Ideal AND gate simulation @ 2 μM



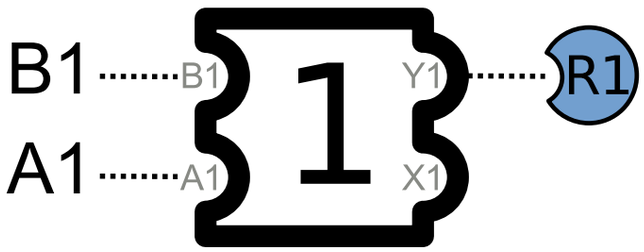
AND gate @ 2 μM

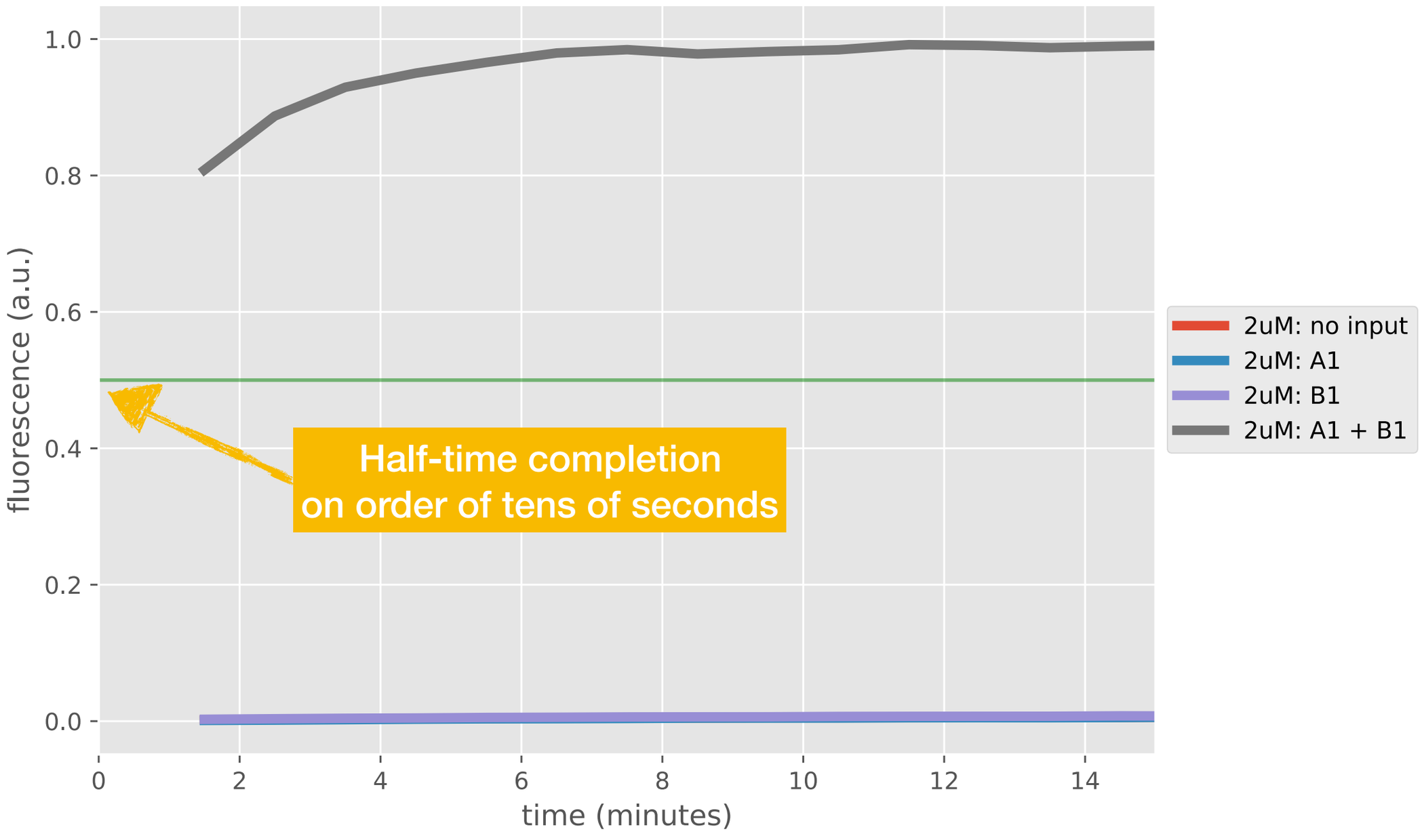




[fuel]=[input]=2uM, [reporter]=1uM

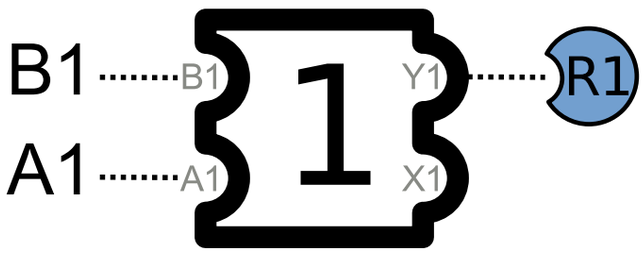
AND gate @ 2 μM

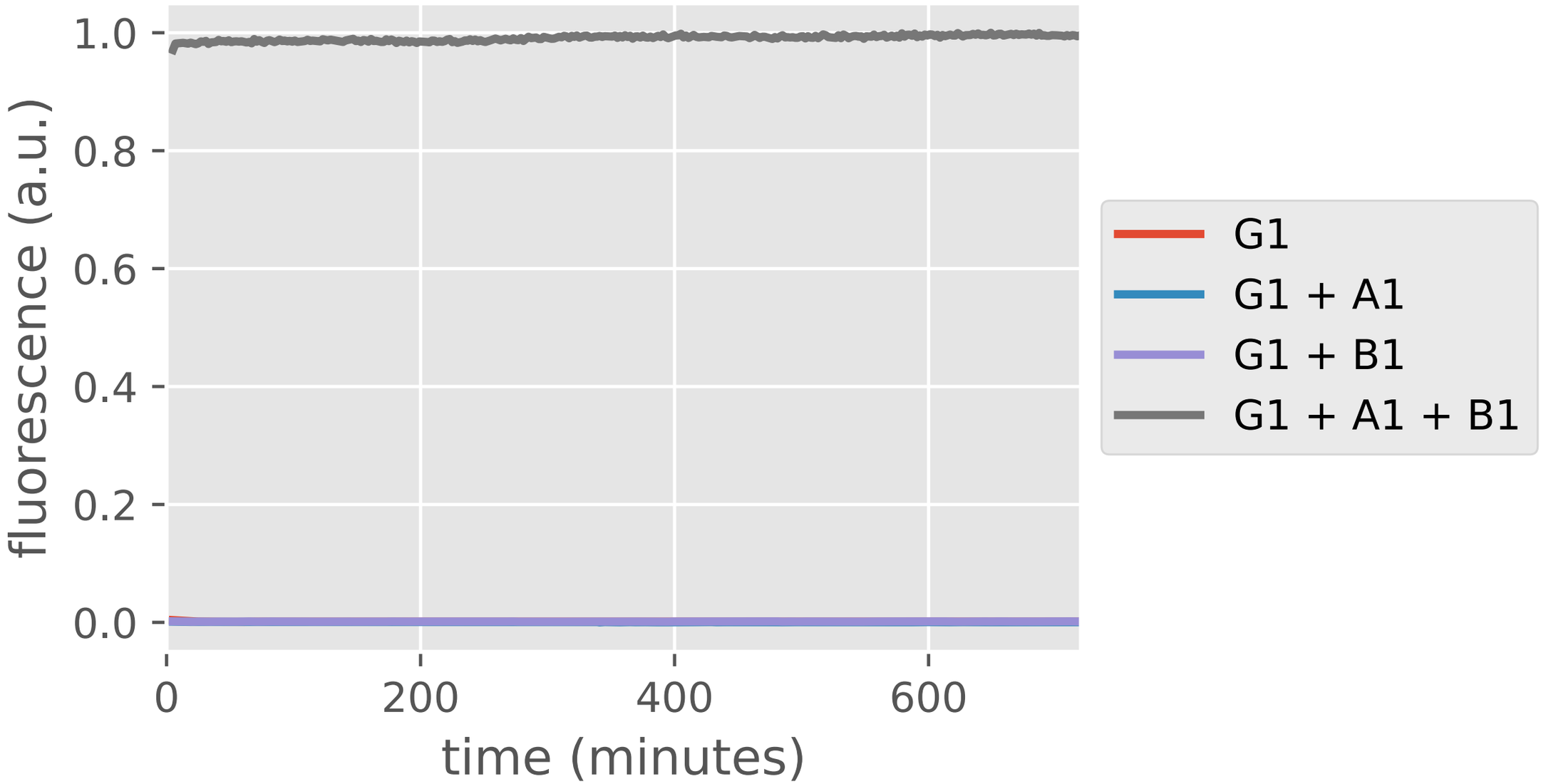




[fuel]=[input]=2uM, [reporter]=1uM

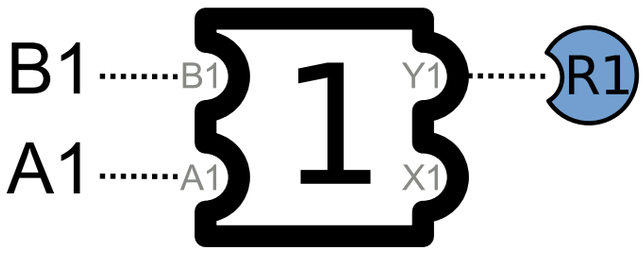
AND gate @ 2 μM (12 hours)

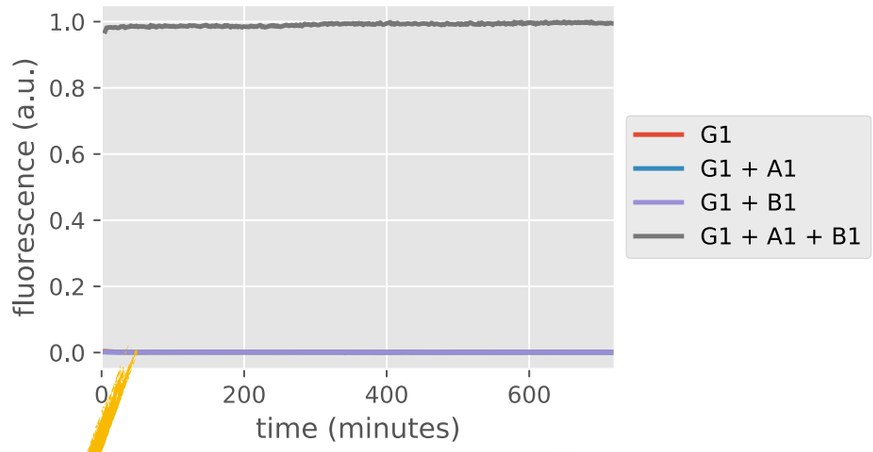


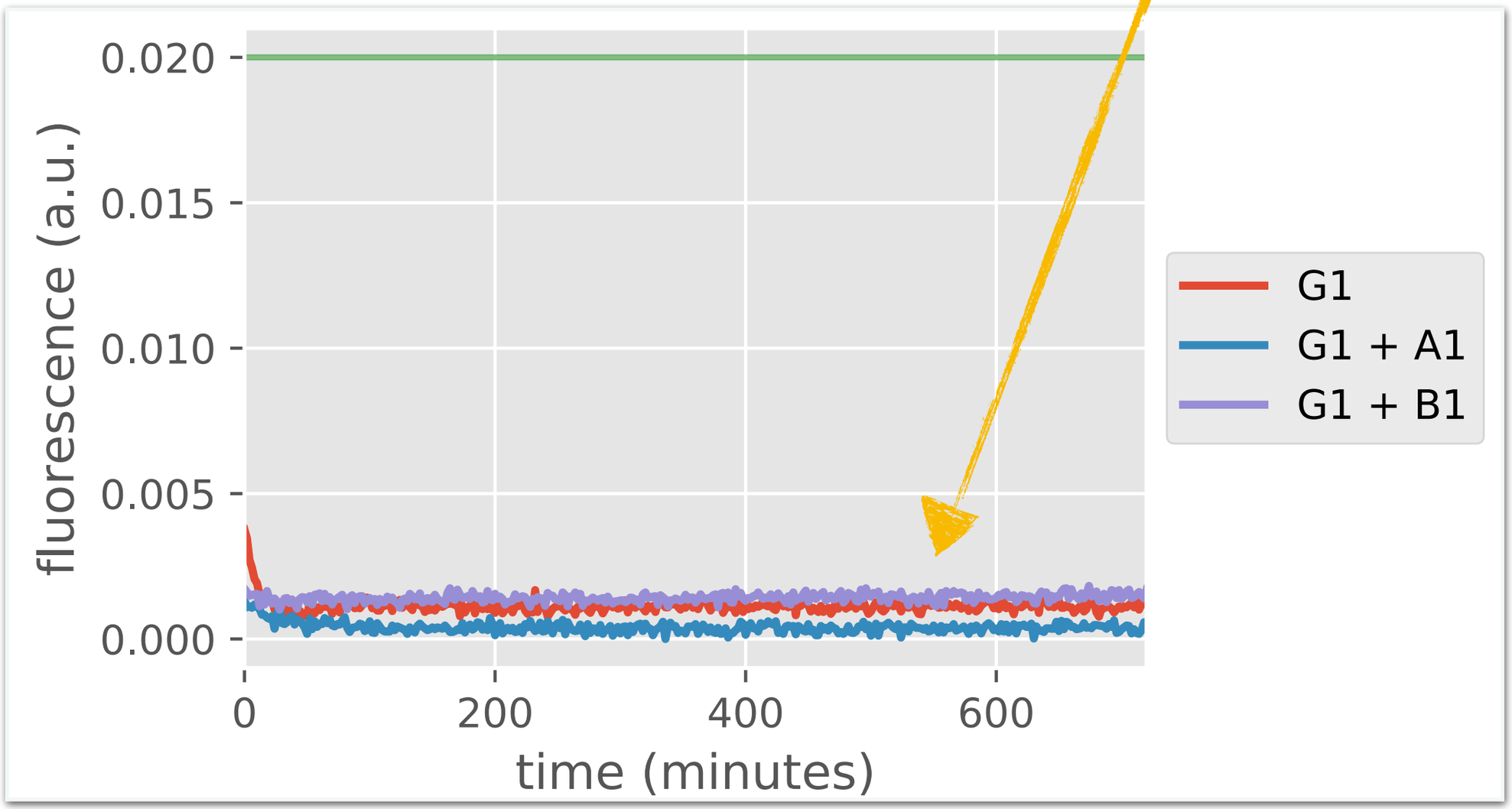


[fuel]=[input]=2uM, [reporter]=2.5uM

AND gate @ 2 μM (12 hours)

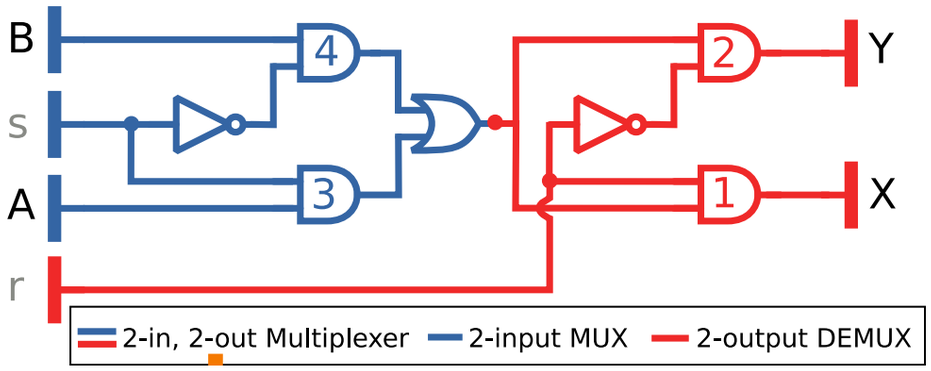


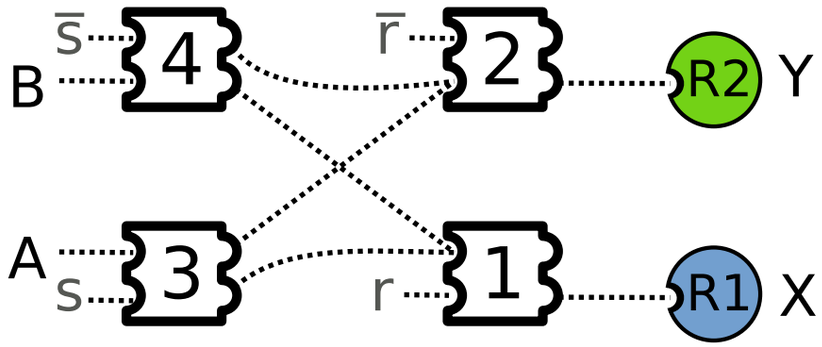


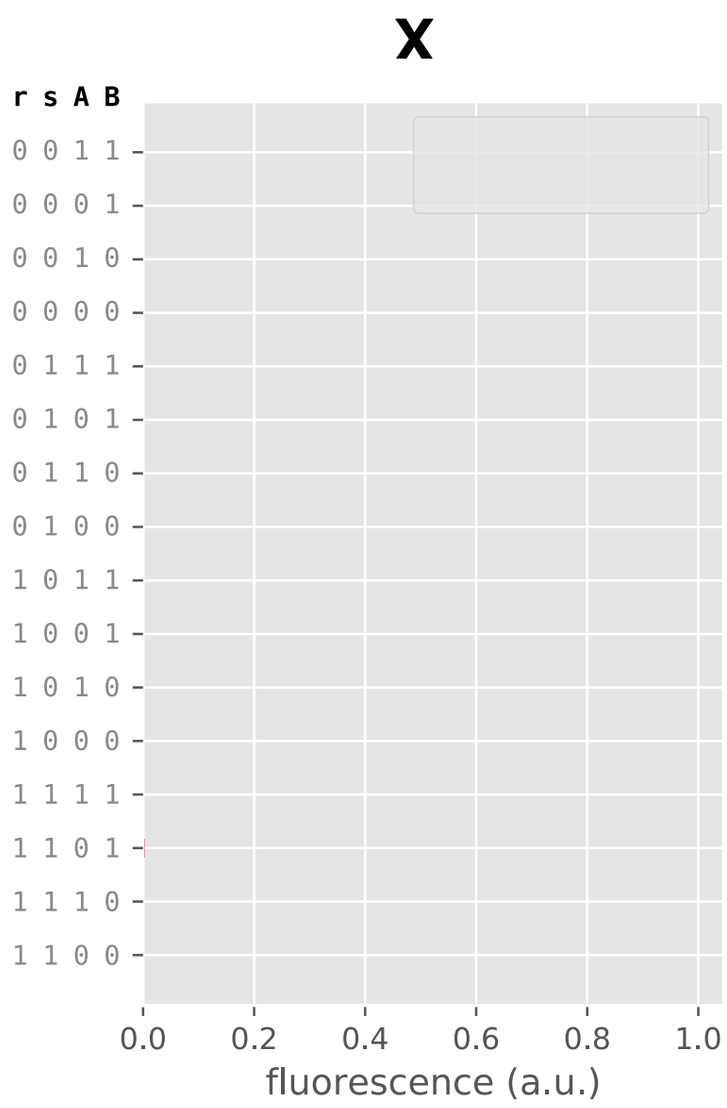


[fuel]=[input]=2uM, [reporter]=2.5uM

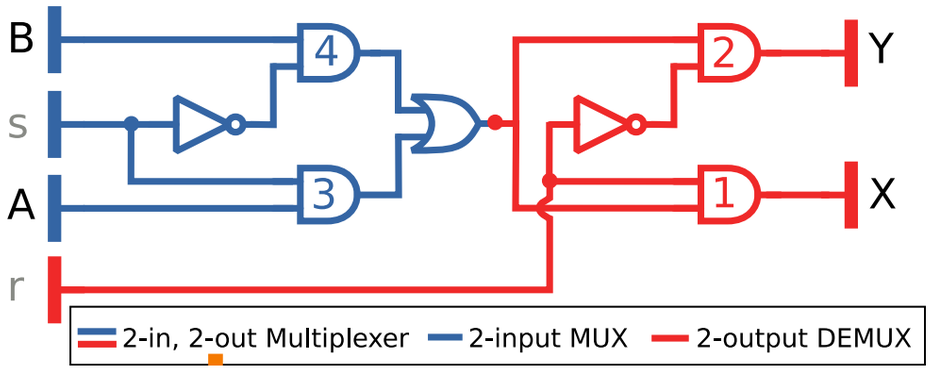
Multiplexer-Demultiplexer

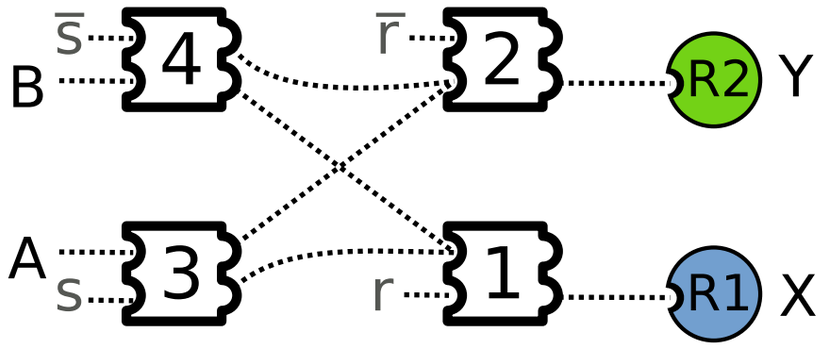


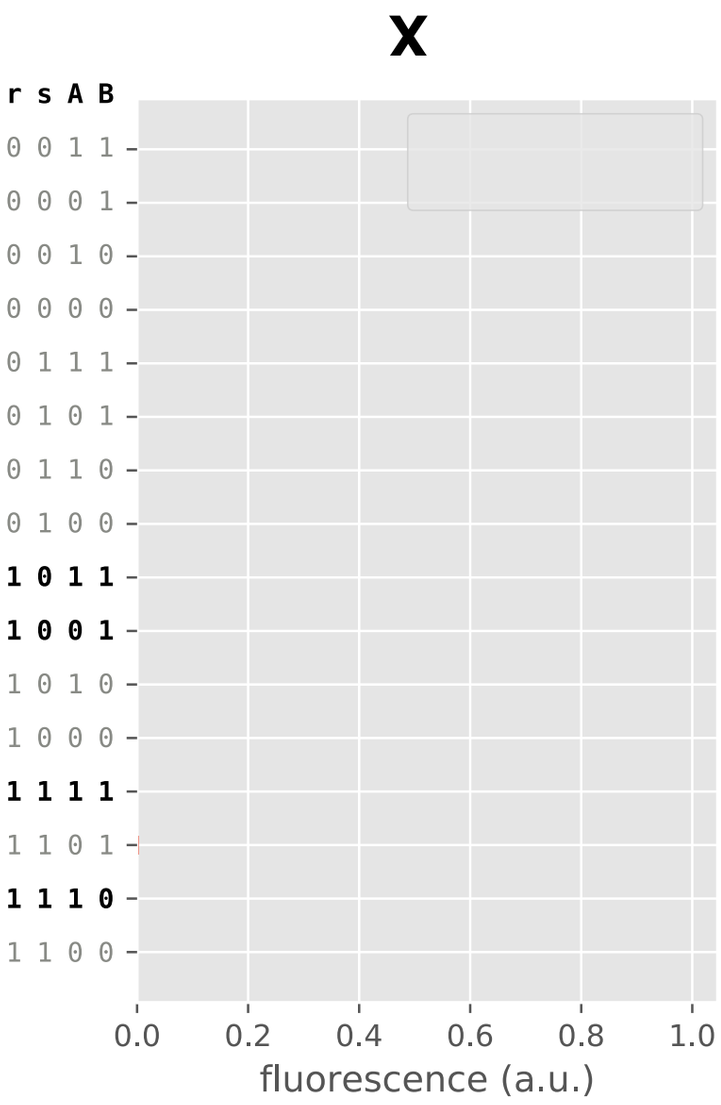




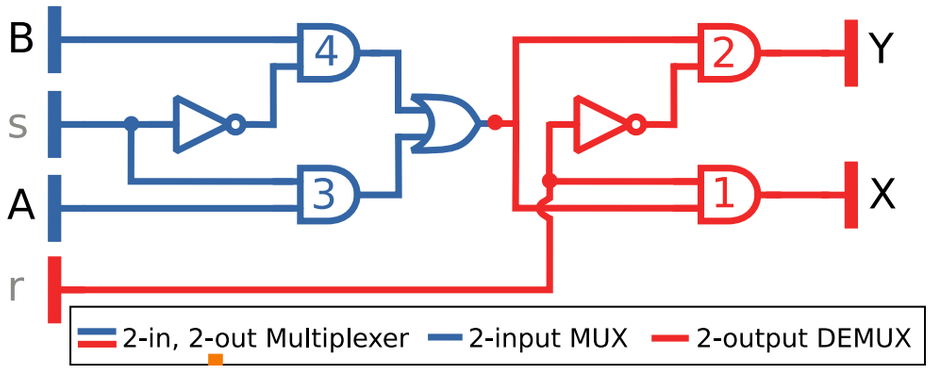
Multiplexer-Demultiplexer

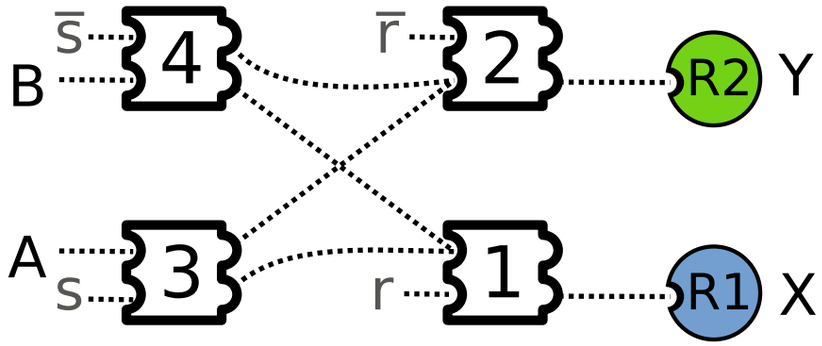


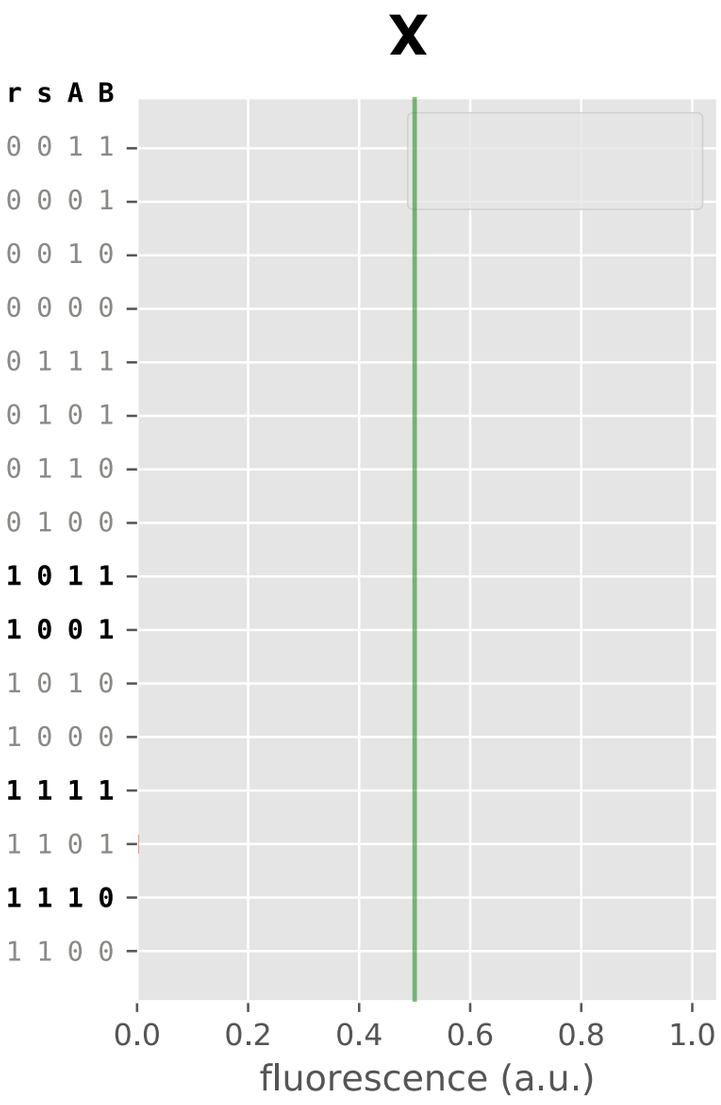




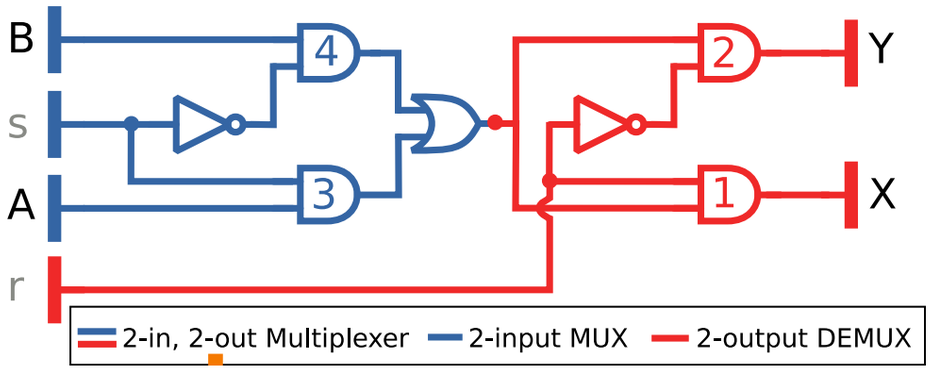
Multiplexer-Demultiplexer

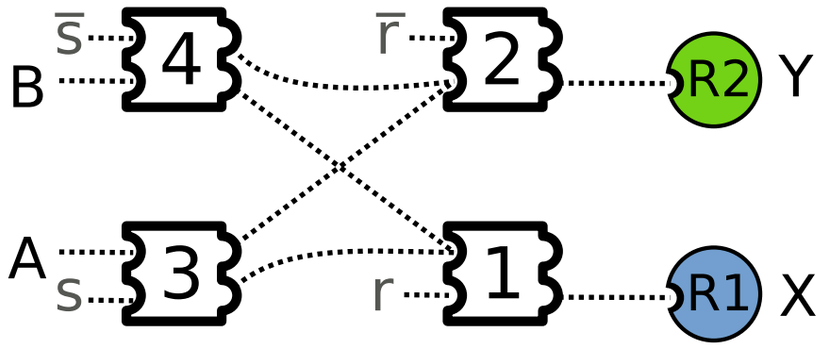


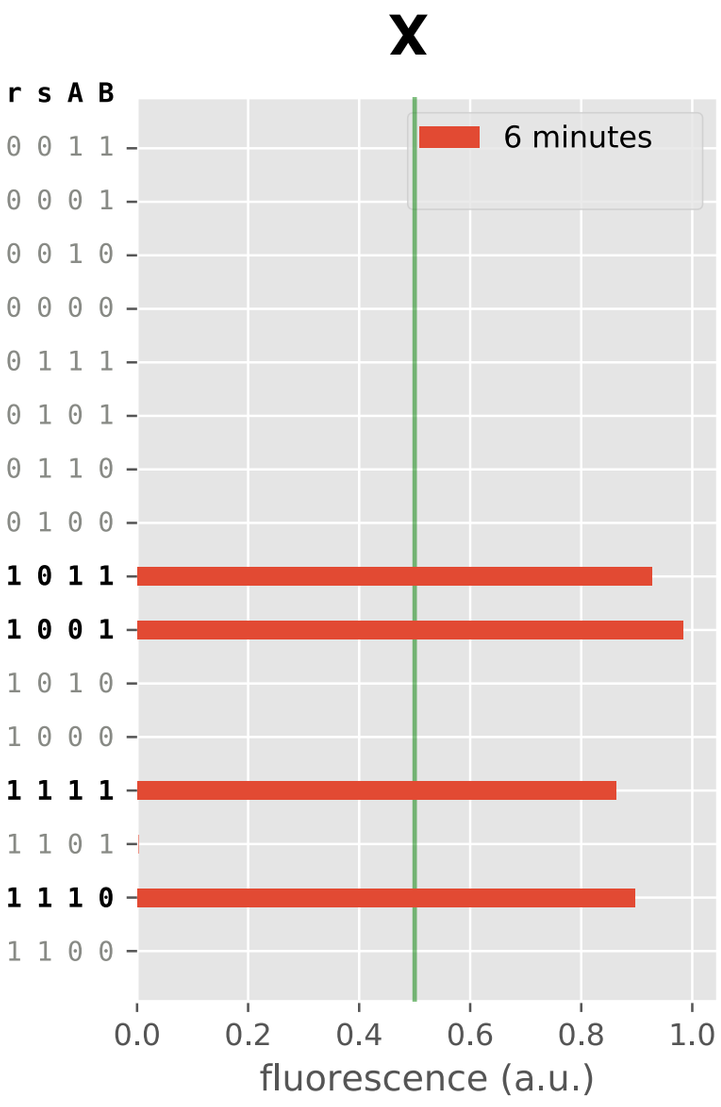




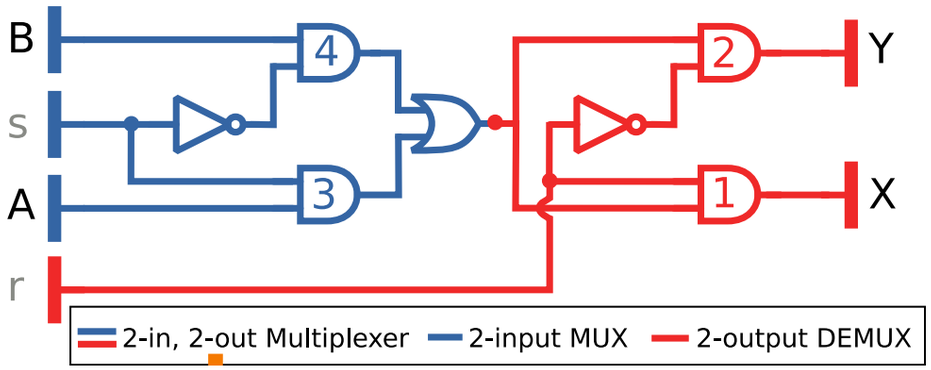
Multiplexer-Demultiplexer

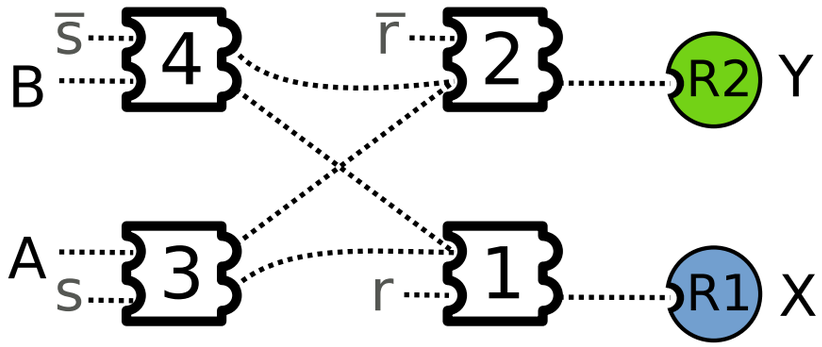


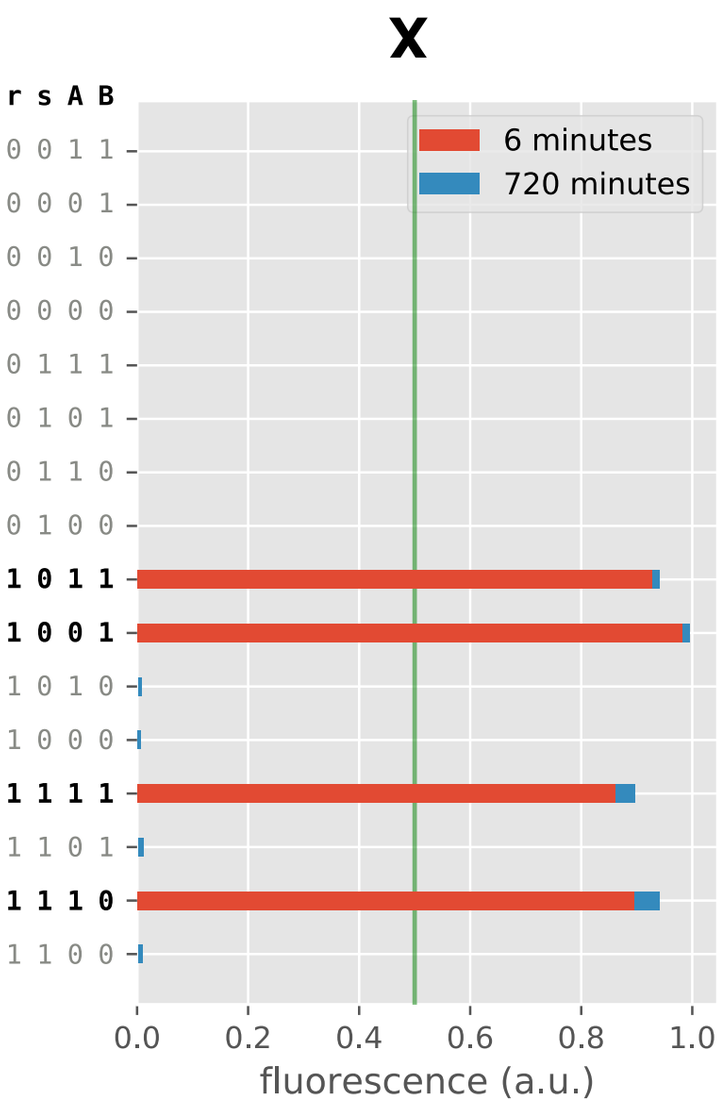




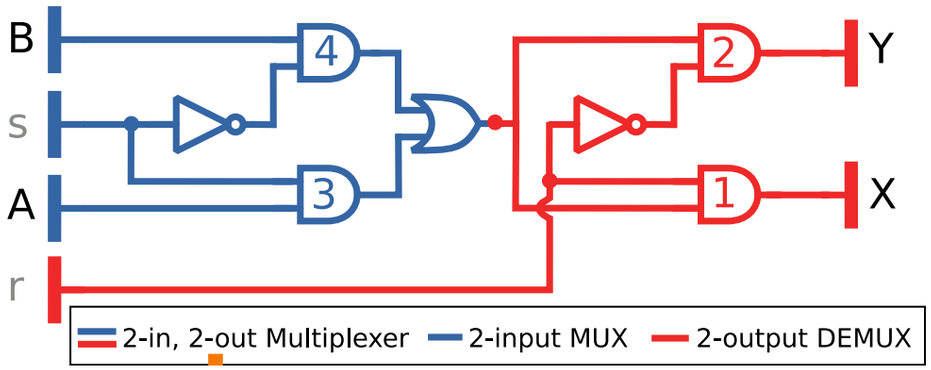
Multiplexer-Demultiplexer

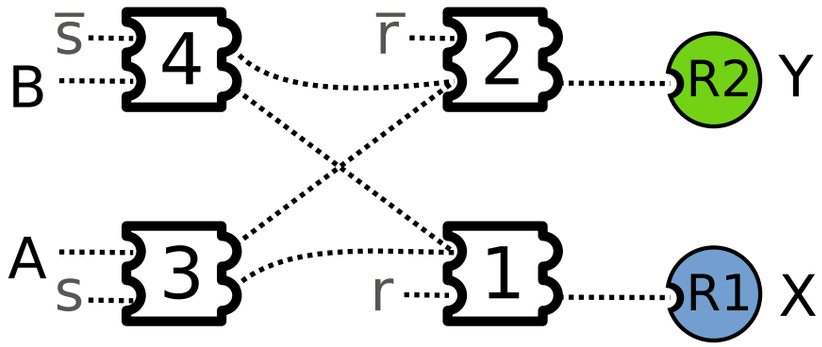


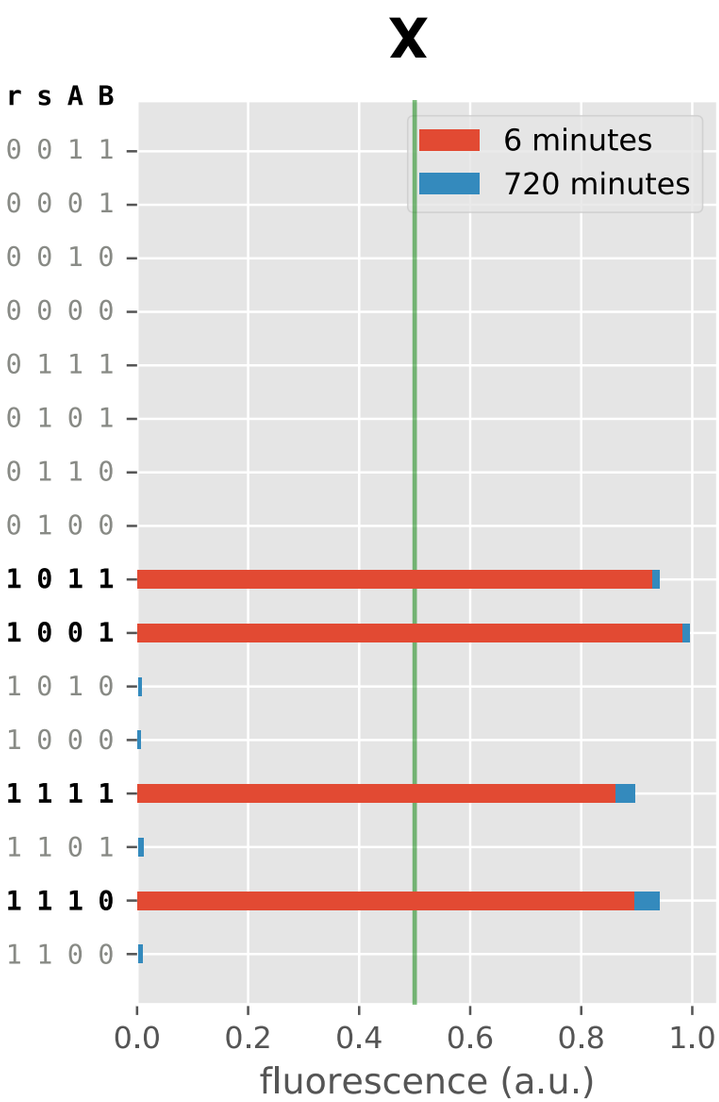


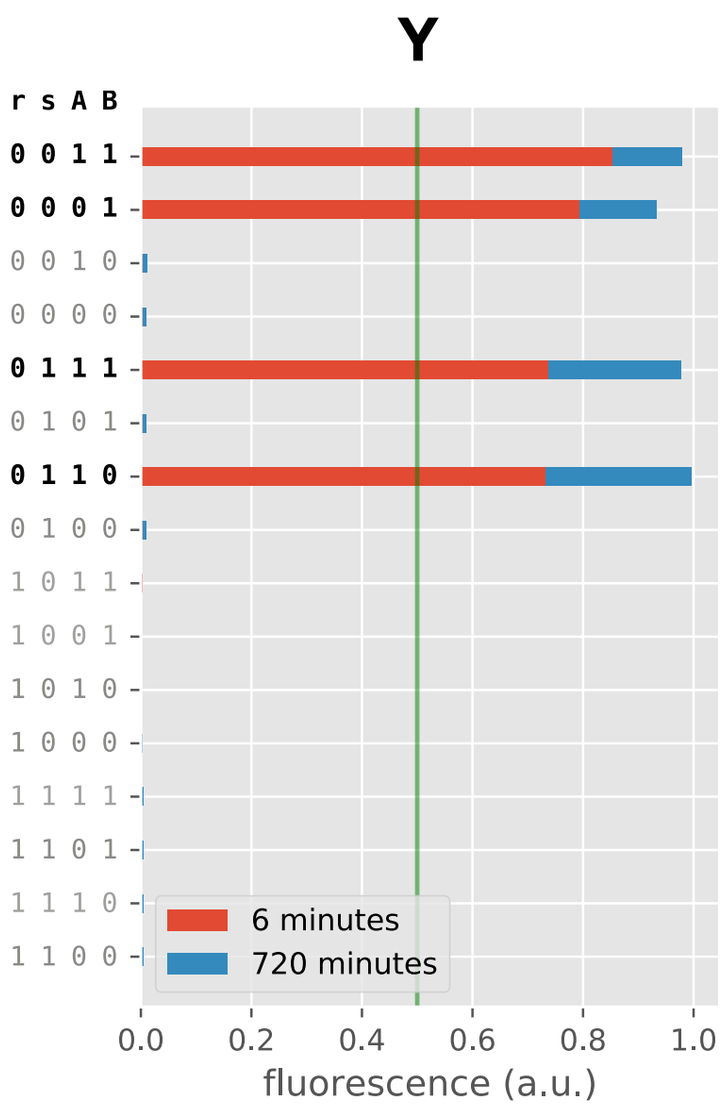


Multiplexer-Demultiplexer

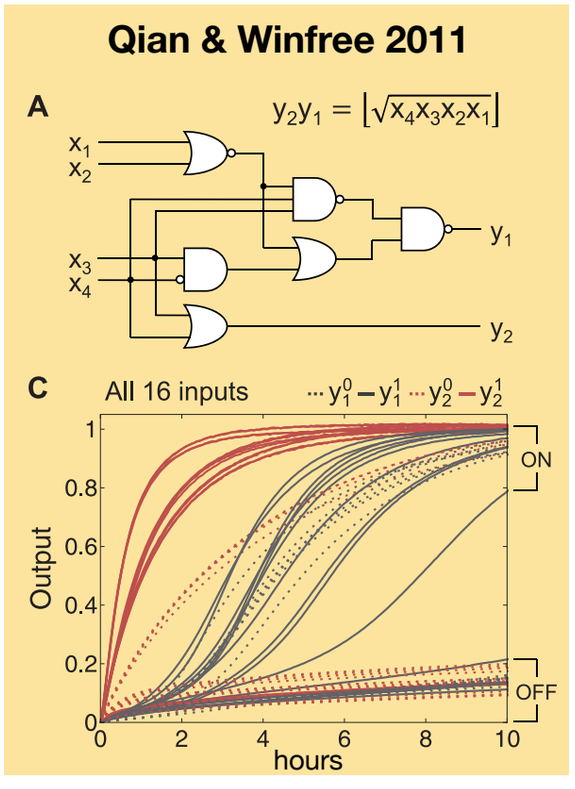




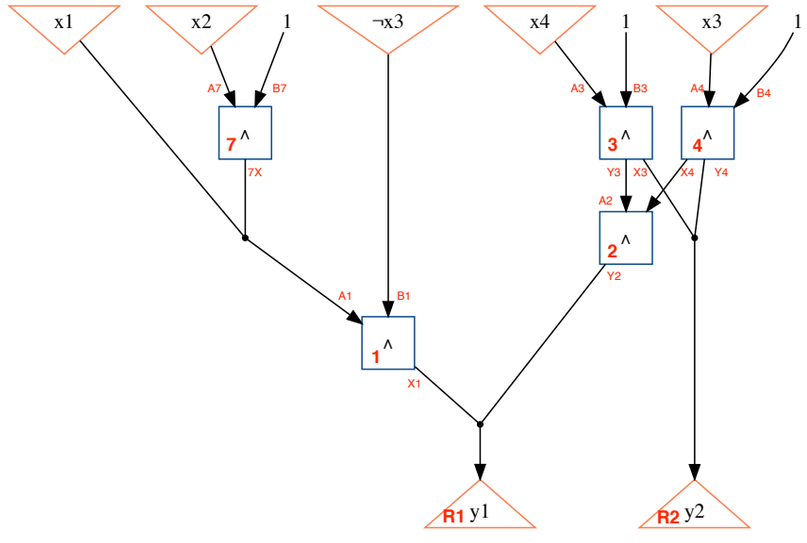




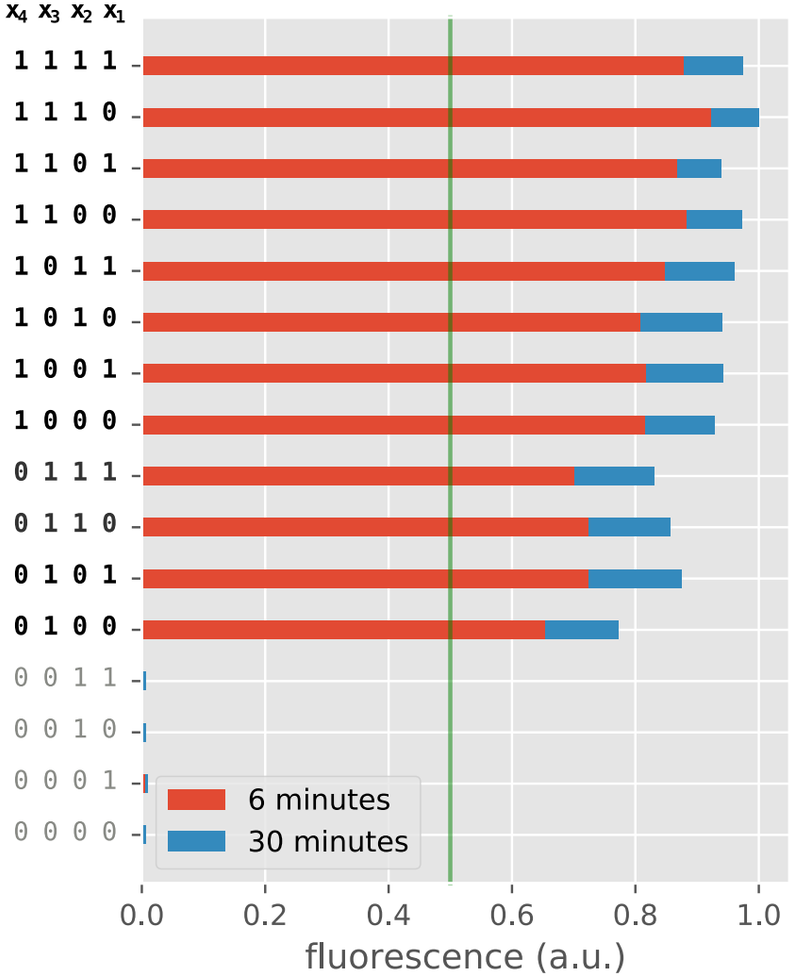
Large circuits that are fast



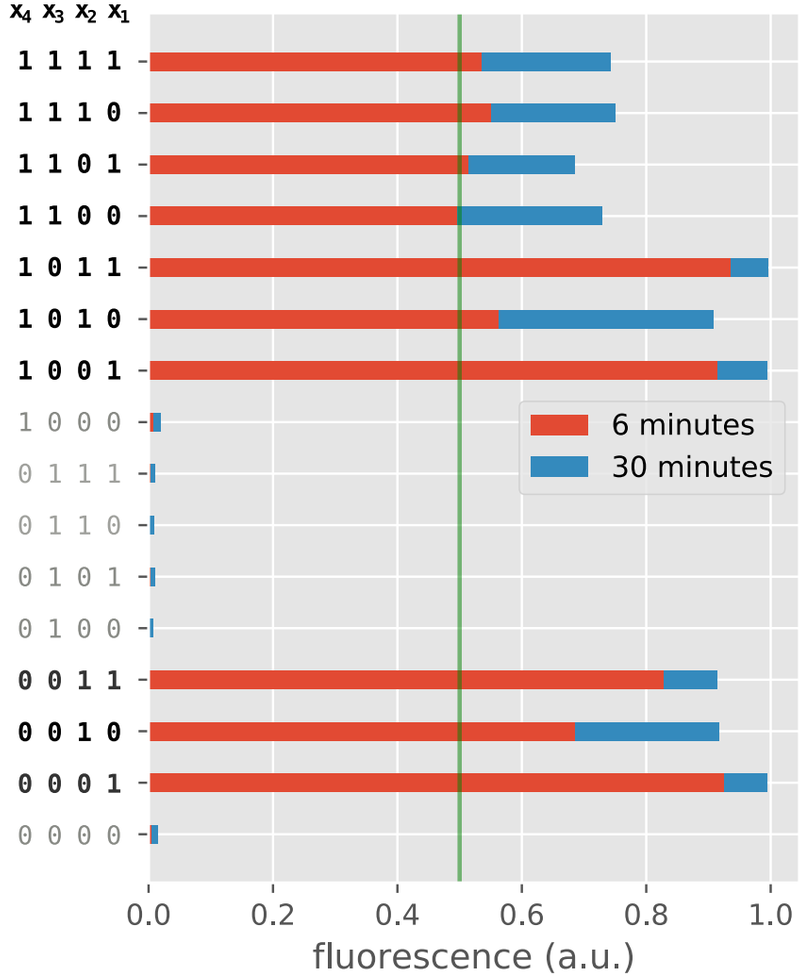
Reaction half-time improved from ~6 hours to < 6 minutes

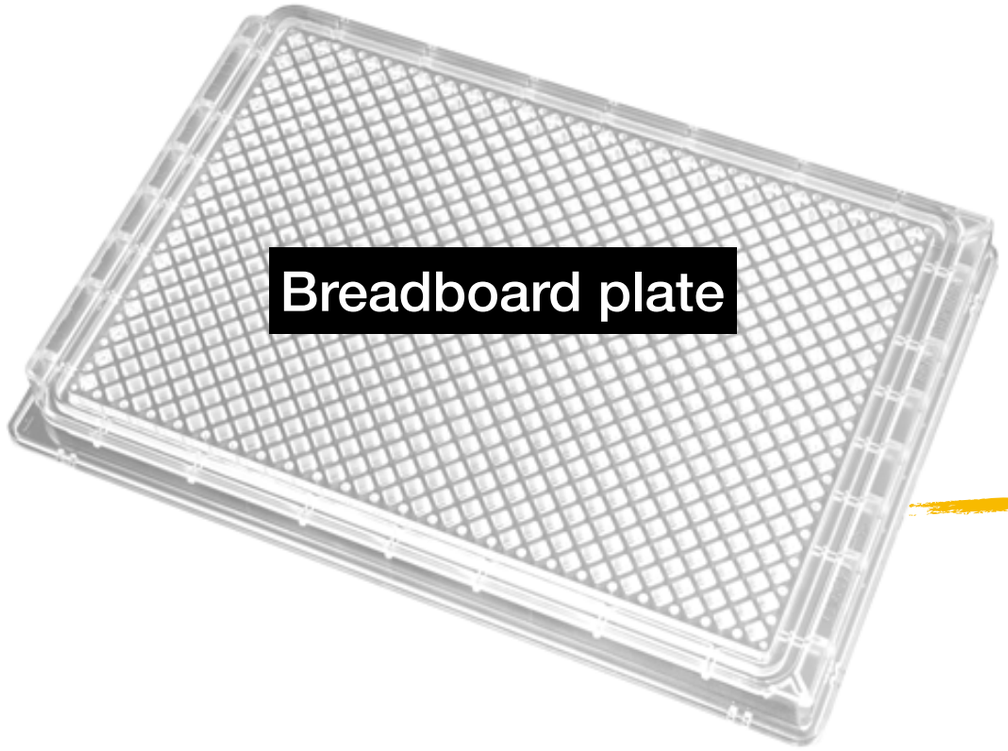


Y2



Y1

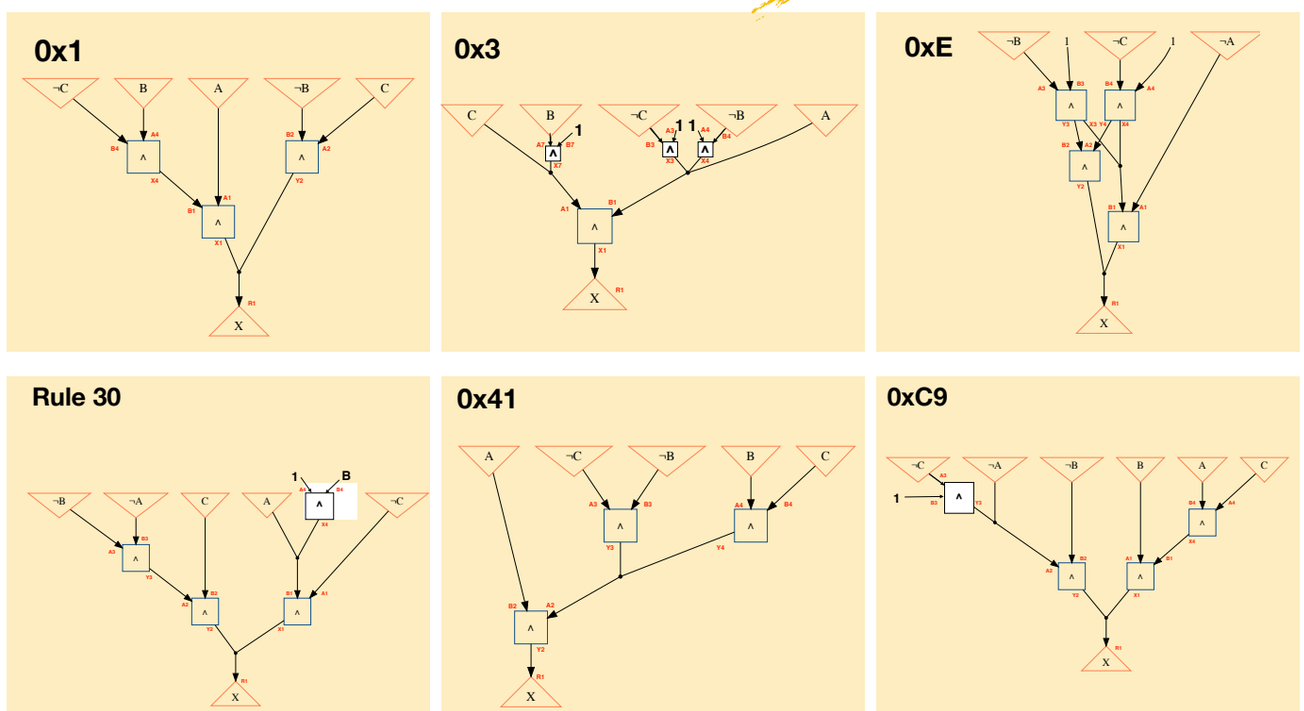


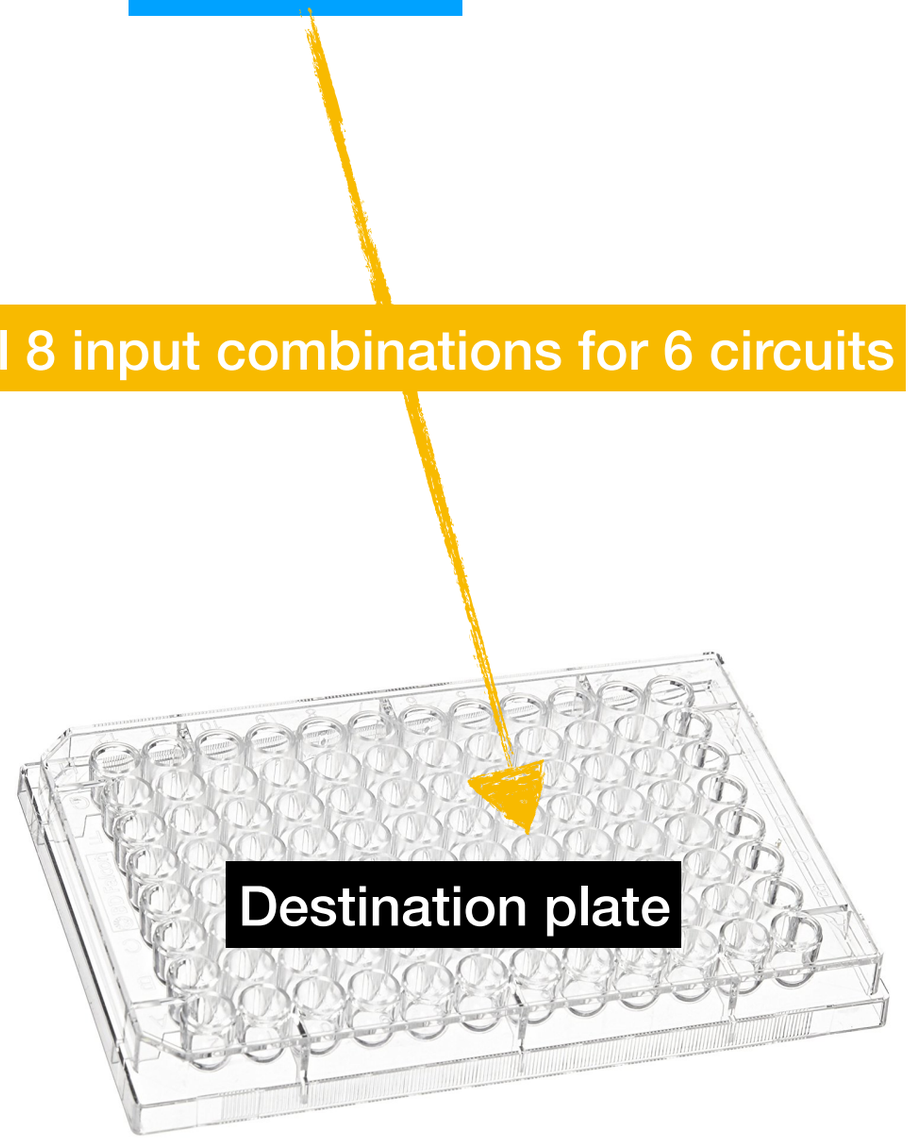


Acoustic Liquid Handler

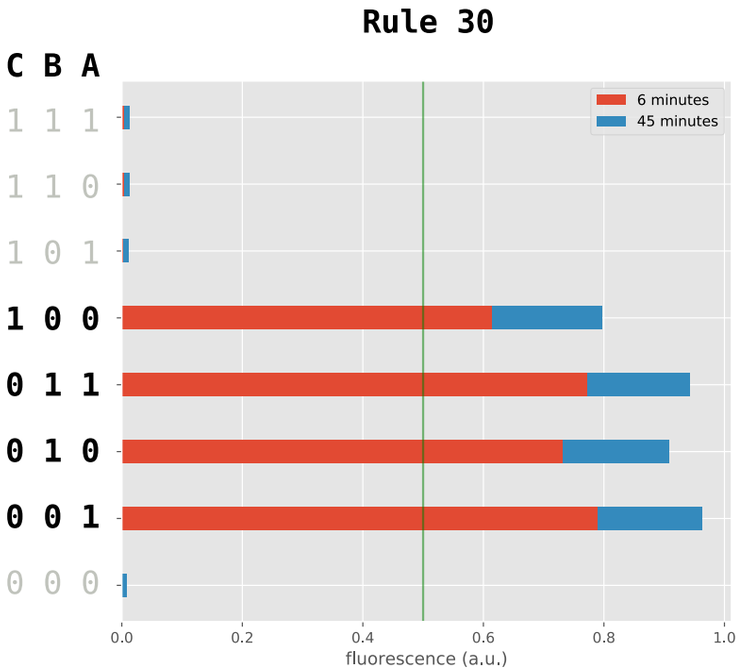
Breadboard compiler produces a mixing protocol

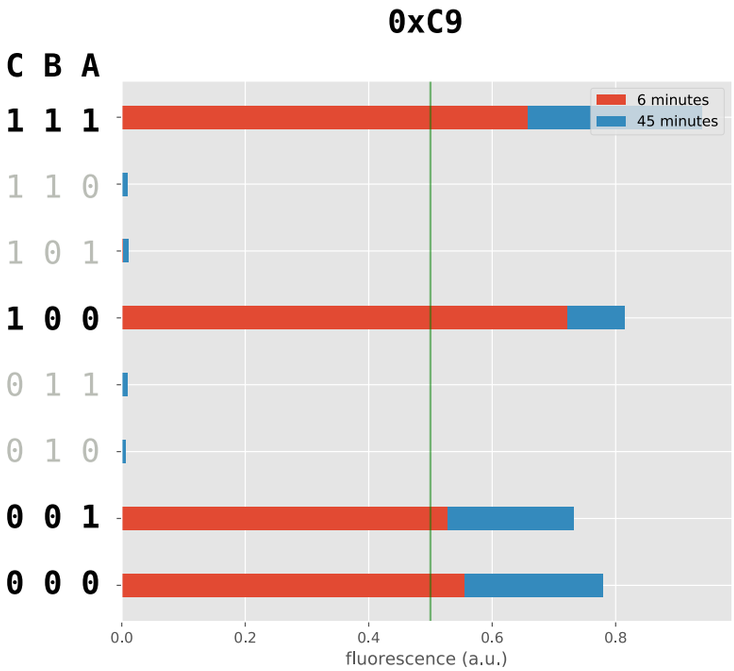
All 8 input combinations for 6 circuits

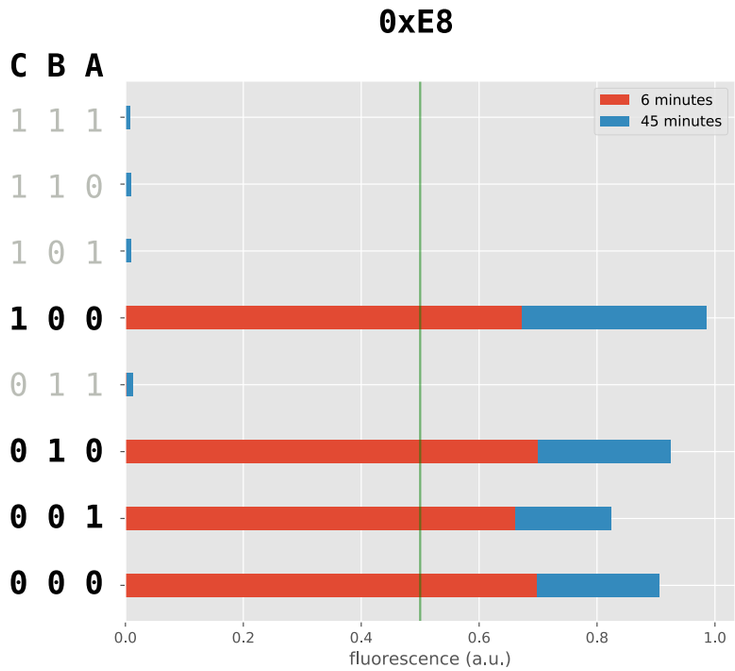


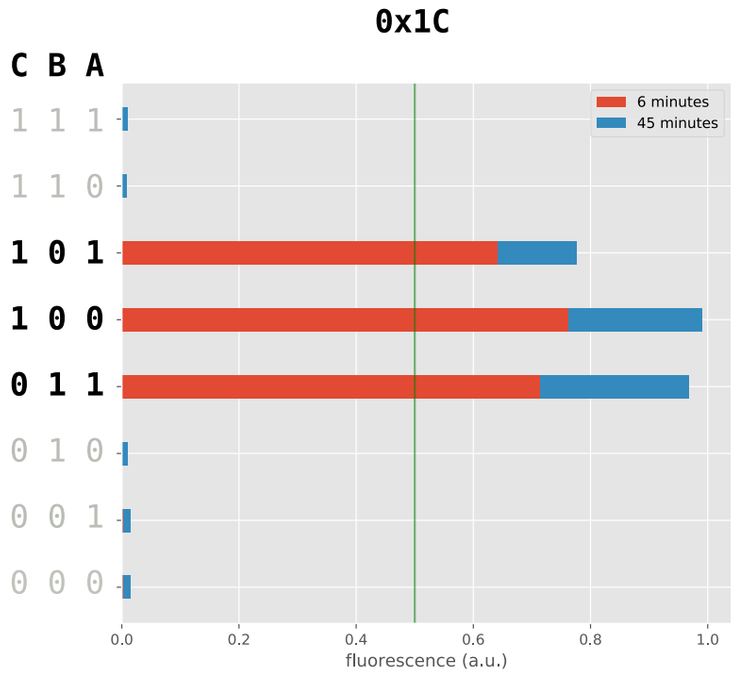


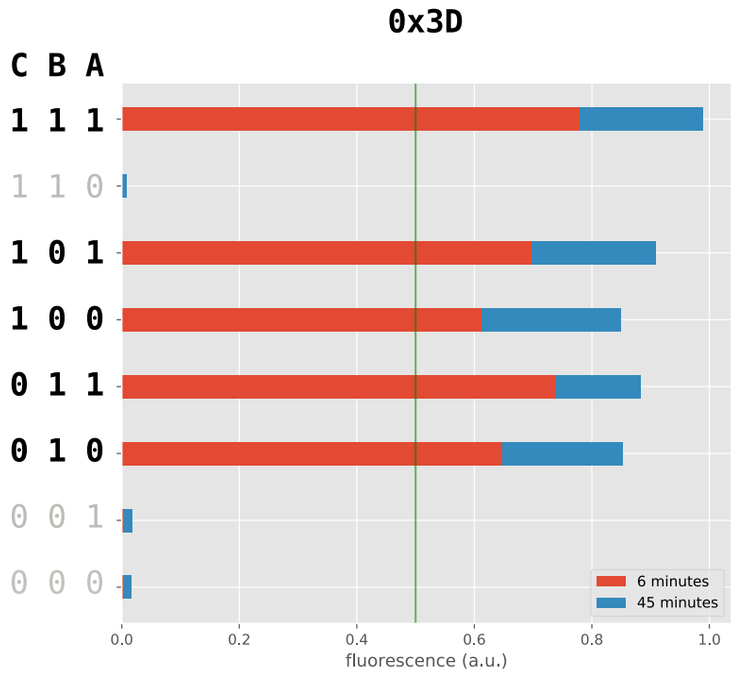
First measurement 6 minutes after mixing start time

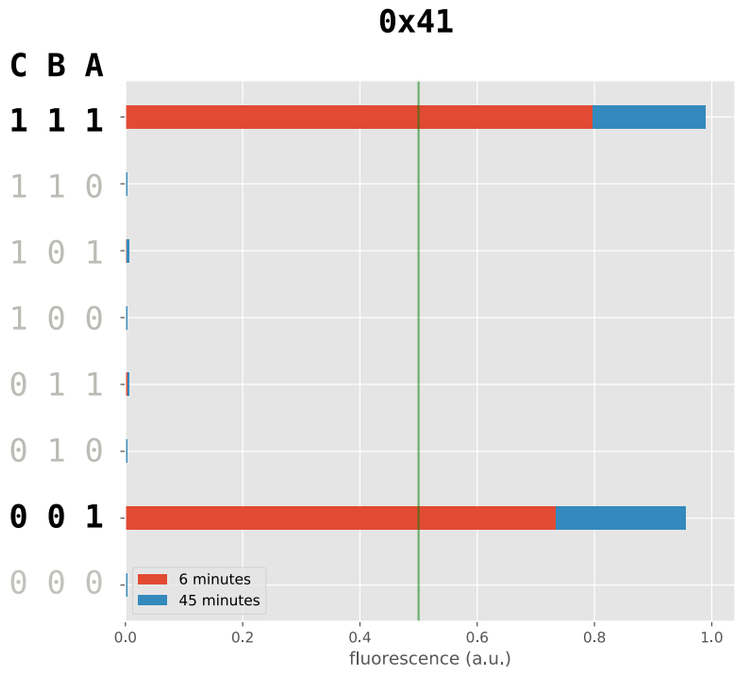












Molecular Circuit Breadboard

Roadmap

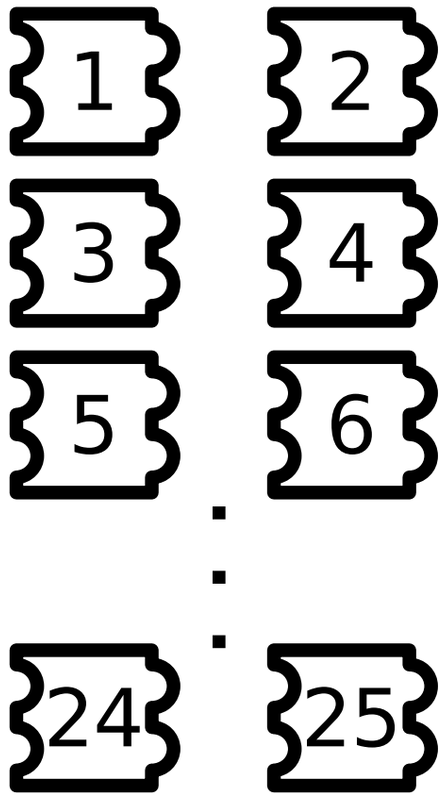
Molecular Breadboard 2.0:

More components

input signals

A1 B1
A2 B2
A3 B3
A4 B4
A5 B5
⋮
A25 B25

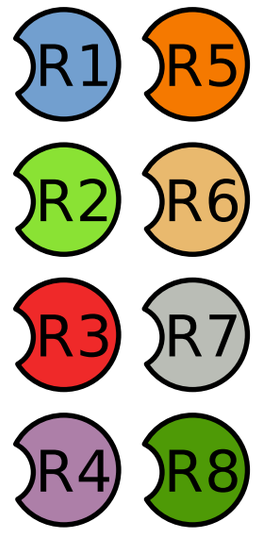
reaction gates



wires

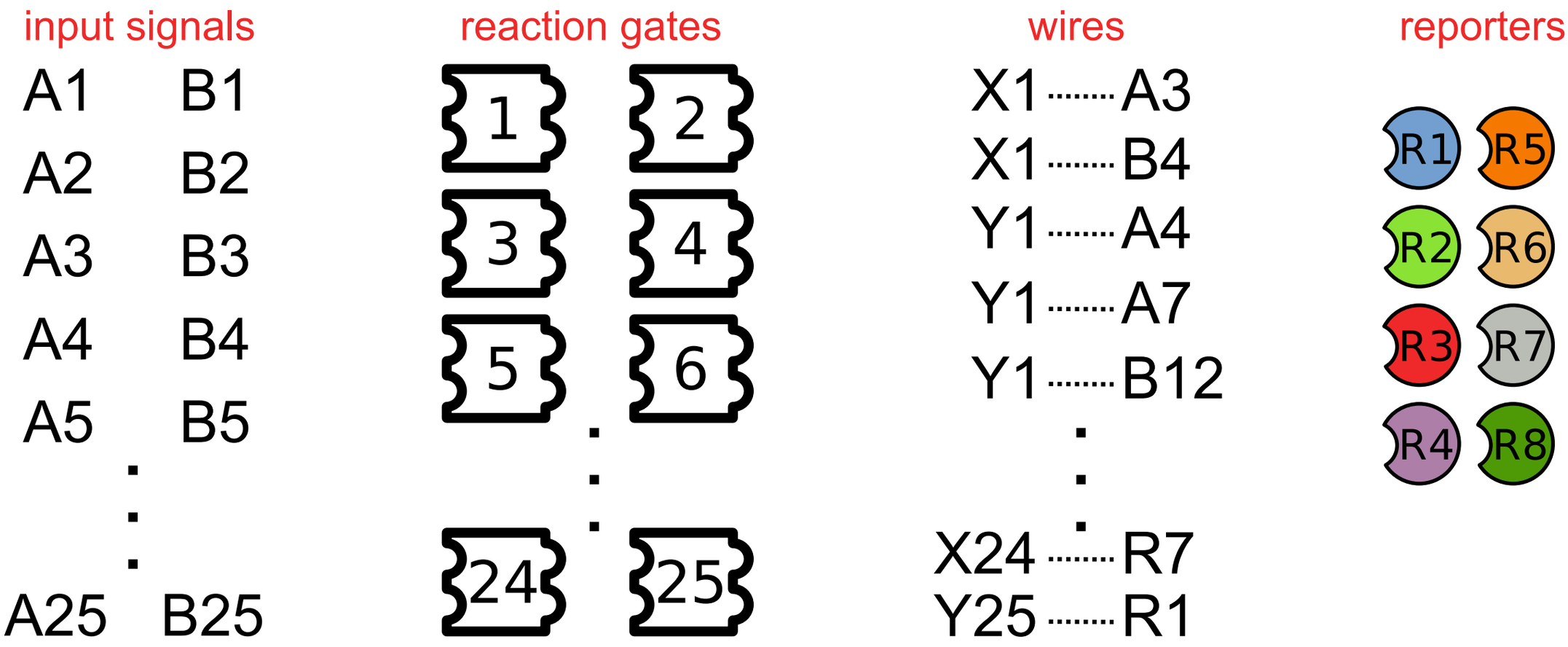
X1 ..... A3
X1 ..... B4
Y1 ..... A4
Y1 ..... A7
Y1 ..... B12
⋮
X24 ..... R7
Y25 ..... R1

reporters



Molecular Breadboard 2.0:

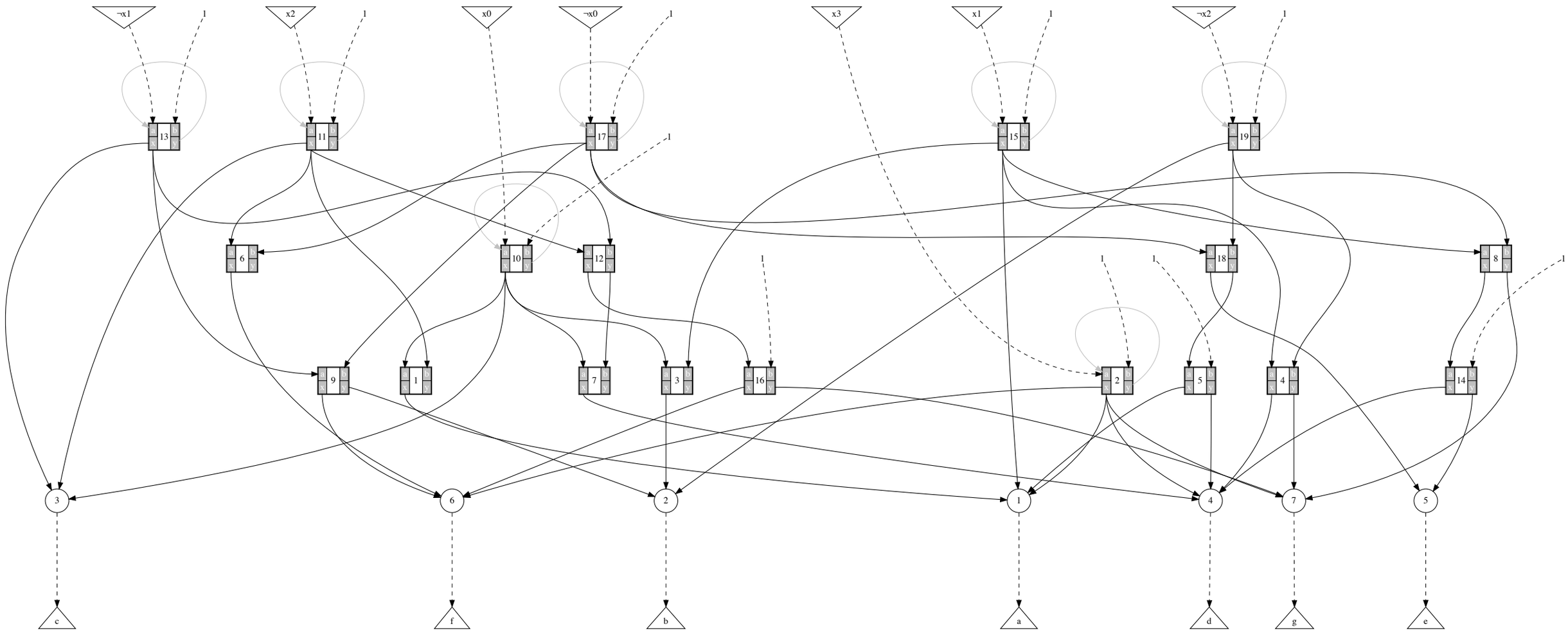
More circuits



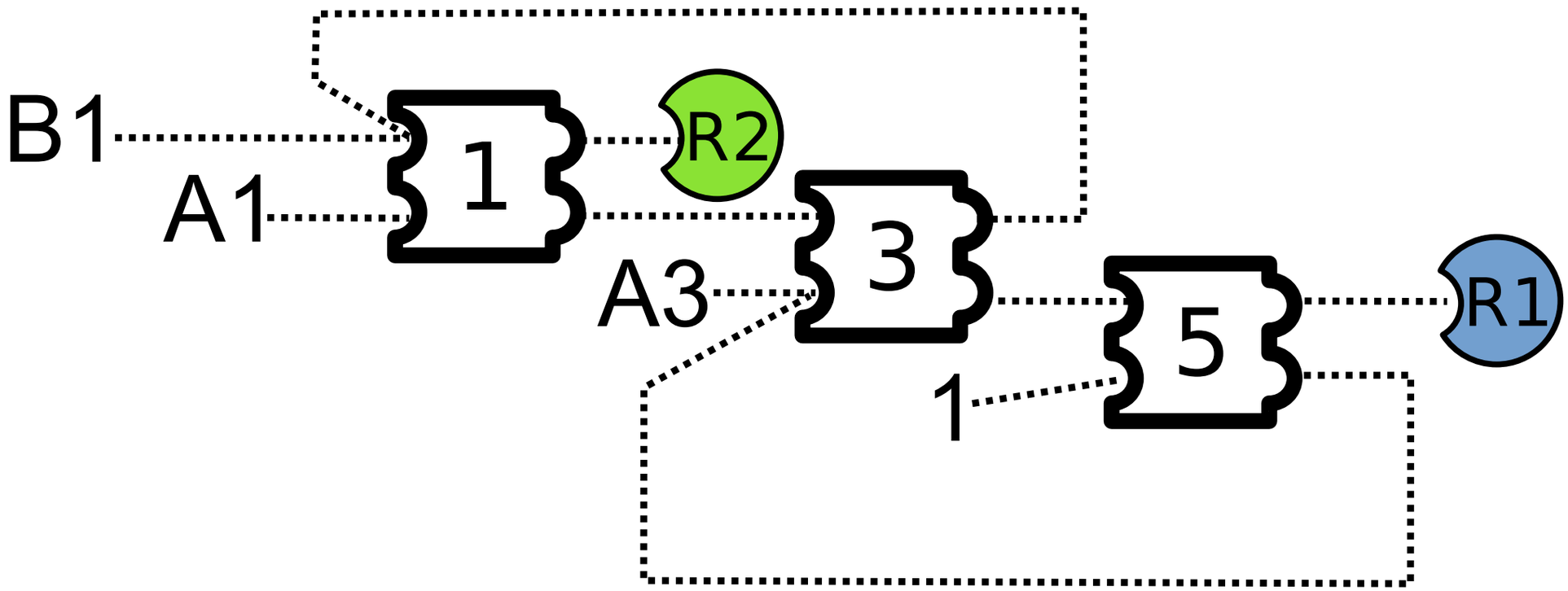
Breadboard 2.0 can realize > 130 K circuits

Molecular Breadboard 2.0:

Larger circuits



Building circuits with feedback loops

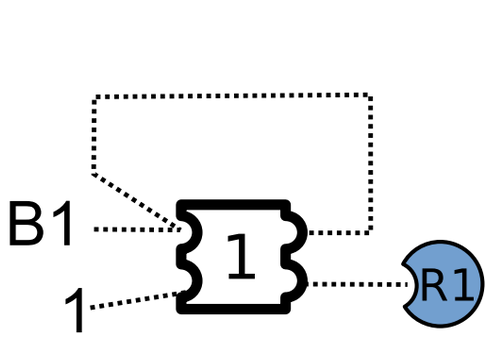


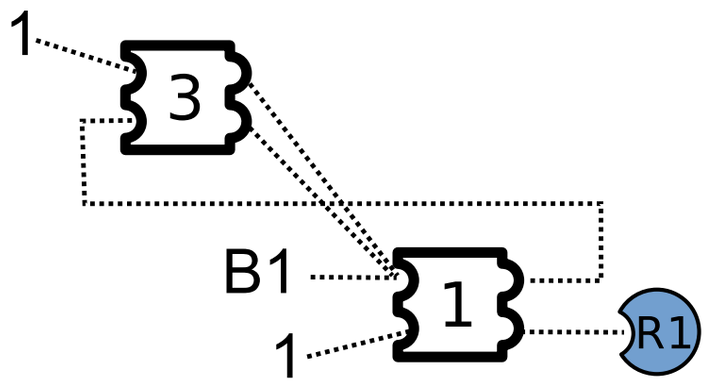
Chemical Reaction Networks

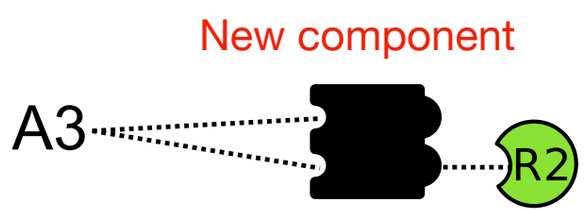
Asynchronous Sequential Logic Circuits

→ **Finite state machines**

Providing input amplifiers & output signal restoration







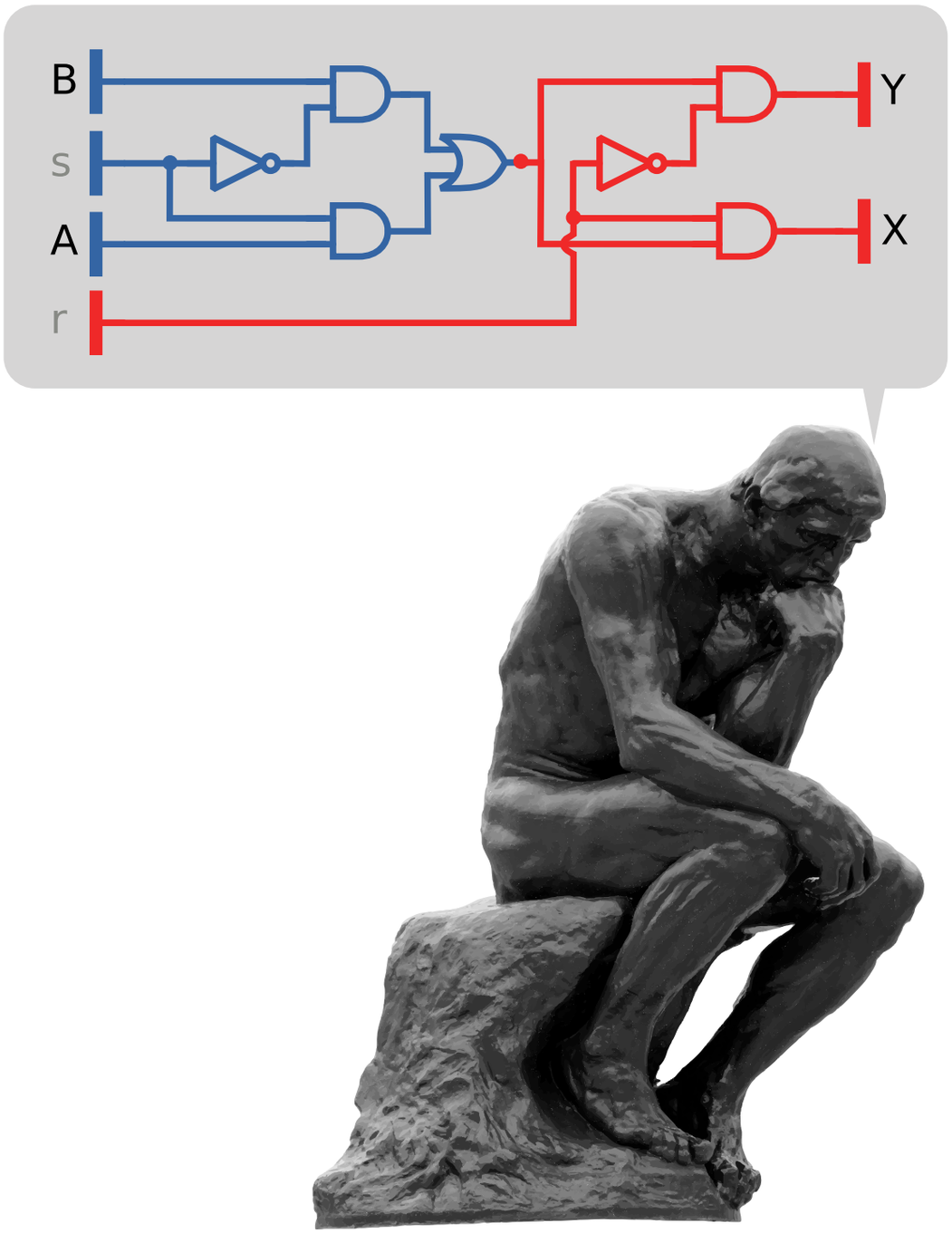
Linear input amplifier

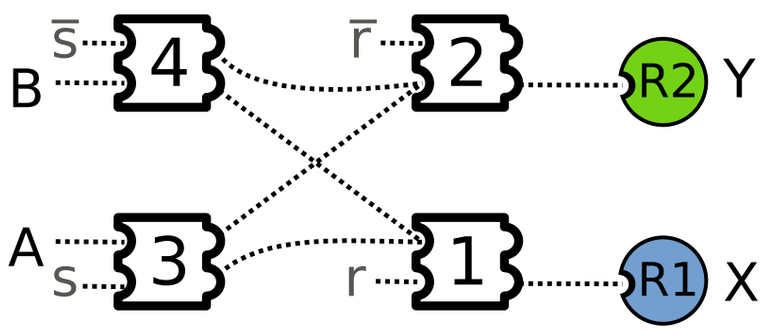
Exponential input amplifier

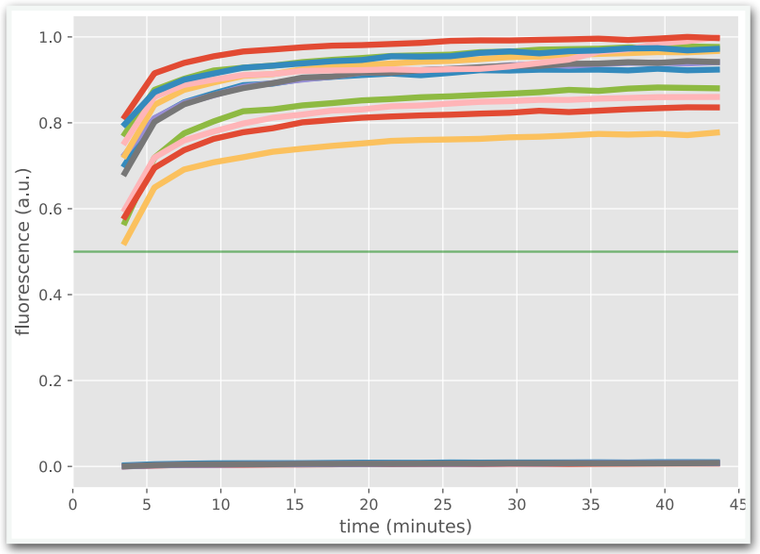
Output signal restoration

http://DSDbreadboard.org

Increased speed	Robustness to error	Automation







Related talks & posters @ DNA 24

Dominic Scalise, Nisita Dutta and Rebecca Schulman
DNA strand-displacement buffers

Si-Ping Han, Lisa Scherer, Matt Gethers, Marwa Ben Hadj Salah, Rebecca Mancusi, Sahil Sagar, Robin Hu, Julia Derogatis, Ya-Huei Kuo, Guido Marcucci, John Rossi and William A. Goddard Iii
Development and optimization of strand displacement based conditional small interfering RNAs for operation inside mammalian cells

Eyal Nir, Yaron Berger and Miran Liber
Computer Controlled DNA Bipedal Walker that Perform Several Steps a Minute

Abhinav Singh and Manoj Gopalkrishnan
EM Algorithm with DNA Molecules

Wooli Bae, Thomas Ouldridge and Guy-Bart Stan
Autonomous generation of multi-stranded RNA complexes for synthetic molecular circuits

Yan Shan Ang and Lin-Yue Lanry Yung
Design of Split Proximity Circuit as a Plug-and-Play Translator for Discriminating Single Nucleotide Mutation

Yan Shan Ang and Lin-Yue Lanry Yung
Dynamically Elongated Association Toehold for Tuning Circuit Kinetics and Thermodynamics

Patrick Irmisch and Ralf Seidel
Modelling DNA-strand displacement reactions in the presence of base-pair mismatches

Boya Wang and David Soloveichik
Experimentally characterizing the design space of strand displacement translators with toehold-size clamps

Allison Tai and Anne Condon
Error-free stable computation with stack-supplemented chemical reaction networks

Kevin Cherry, Gokul Gowri and Lulu Qian
DNA-based neural networks that learn from their molecular environment

Robert F. Johnson and Erik Winfree
Using Bisimulation for Verification of Polymer Reaction Networks

Acknowledgments

- **Winfree lab** (Caltech)
- **Soloveichik lab** (University of Texas at Austin)
- **Qian lab** (Caltech)
- **Murray lab** (Caltech)
- Thanks to DNA 24 organizers for the invitation

thachuk@caltech.edu

Tools discussed in tutorial

ABC: logic synthesis and verification
https://people.eecs.berkeley.edu/~alanmi/abc

VisualDSD
https://lepton.research.microsoft.com/webdna

Nuskell compiler framework
https://github.com/DNA-and-Natural-Algorithms-Group

DSD breadboard
http://dsdbreadboard.org *(online later this year)*

(a)

SLD intended reaction

X

F1

F2

reporter

F1.top

waste1

waste2

Y

reporter.top

waste3

CTAACTAATCTTTCCTACAATACACTACCTT

AAGGATGTTATGTGATGGAATG

ACAATACACTACCTTACATATCCAAACTCT

(b)

SLD leak reaction

F1

F2

reporter

waste1

Y

reporter.top

waste2

SLD leak	before		after
number of base pairs	49	≈	50
separate components	3	=	3

(a)

DLD intended reaction

X

F1

F2

reporter

waste1

F1.top

waste2

Y

reporter.top

waste3

(b)

DLD leak reaction

F1

F2

reporter

Ycomplex

reporter.top

waste

DLD leak	before		after
number of base pairs	79	≈	80
separate components	3	>	2

entropic penalty

(a) **TLD intended reaction**

(b) **TLD leak reaction**

TLD leak	before		after
number of base pairs	166	≈	165
separate components	4	>	2

entropic penalty