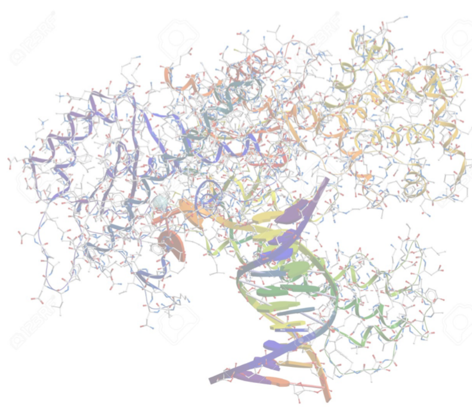
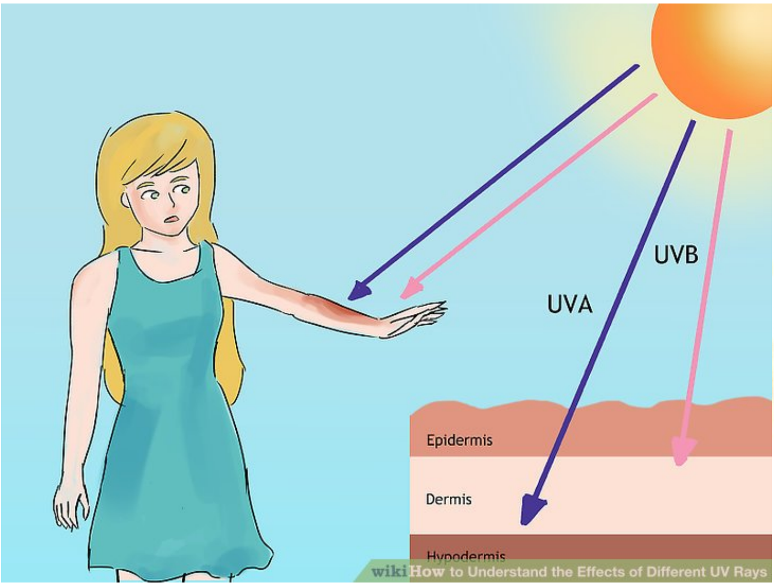
Using a strand displacing polymerase to program chemical reaction networks

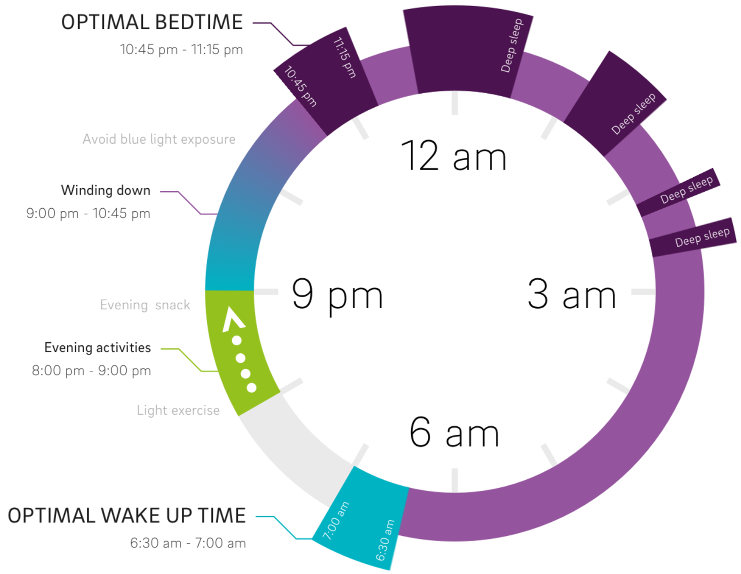
Shalin Shah



Motivation for synthetic biocontrollers

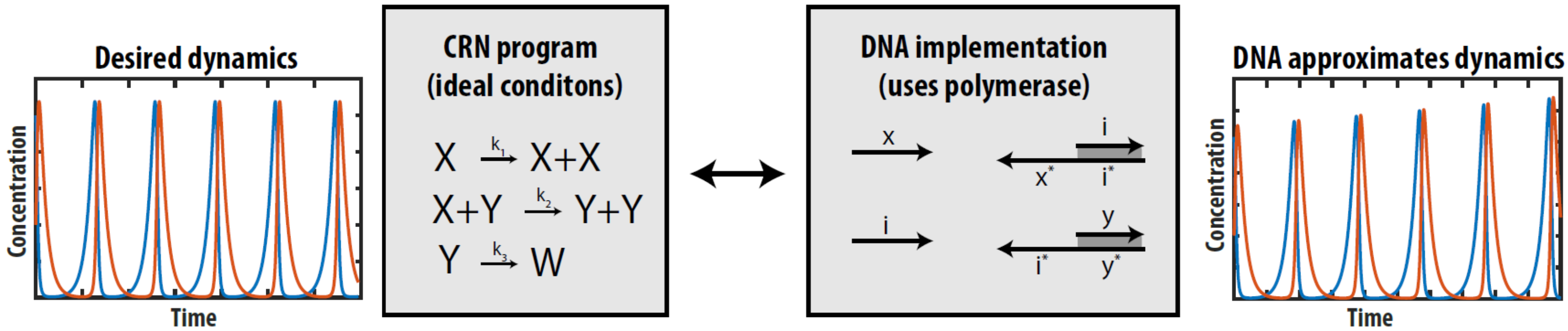
- Designing embedded systems that are compatible with life requires programming chemicals.





Soloveichik *et al.* PNAS (2010)
Shah *et al.* DNA25 (2018)

Synthetic biocontrollers



- Inverse problem is to use CRN (Turing universal) as a high-level programming language.

- Use DNA systems (highly programmable) to implement arbitrary chemical programs.

CRNs: Chemical reaction networks

Shah *et al.* JACS (2020) (under review)

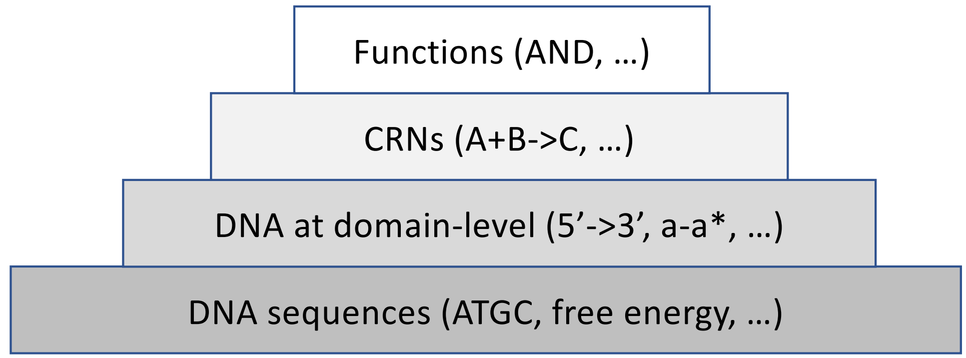
DNA-based architecture

Programming language

```
public static void main()
{
        O = I;
}
```

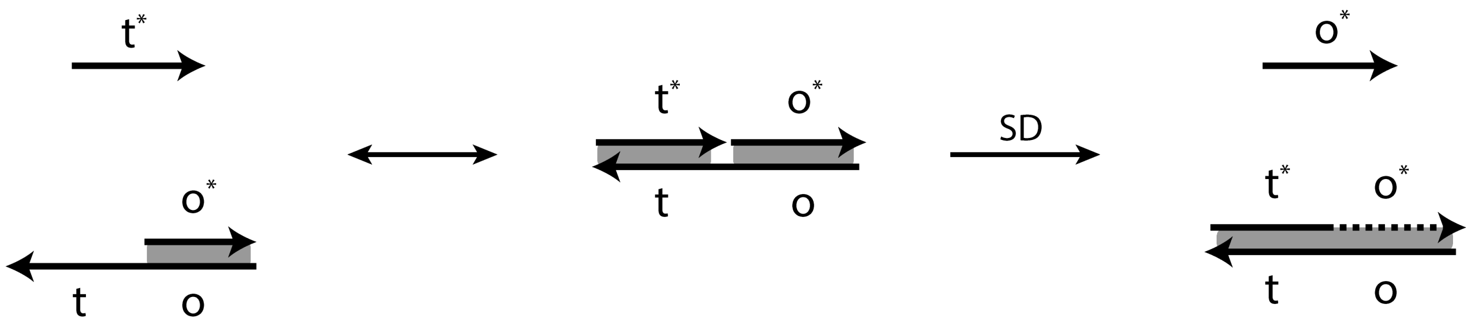
CRN language

$$I \xrightarrow{k} O$$



DNA-based full stack

Domain level

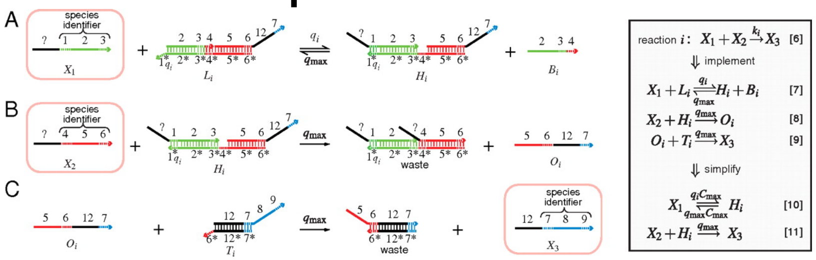


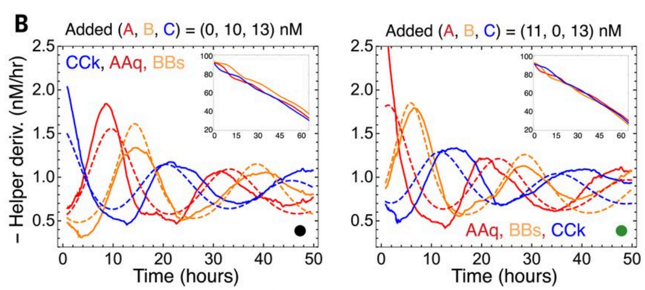
PEN toolbox: Polymerase Exonuclease nicking toolbox

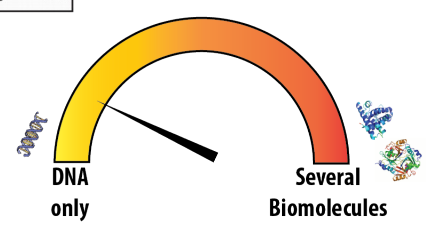
Soloveichik *et al.* PNAS (2010)
Fujii *et al.* ACS Nano (2012)
Srinivas *et al.* Science (2017)

DNA-based implementations of CRNs

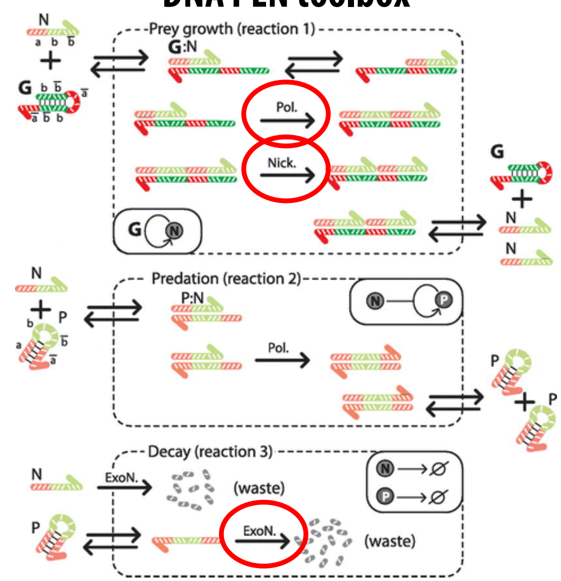
Strand displacement architecture







DNA PEN toolbox




Soloveichik


Srinivas


Winfree


Fujii


Rondelez

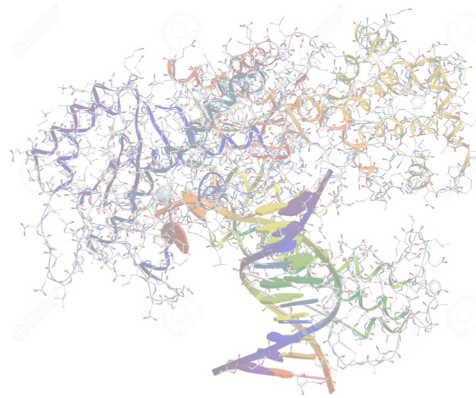
Problem statement

- **Several difficulties with existing techniques:**
  - DNA only systems are biologically simpler to design but they can be slow and leaky.
  - Multi-component systems are biologically more complex restricting the environmental conditions.

- **Problem statement:** Design synthetic bio-controllers that are biologically simple and fast.

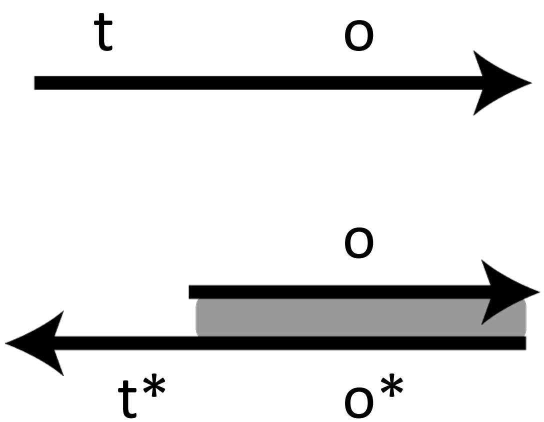
- **Our proposed solution: Polymerase strand displacement**

Outline

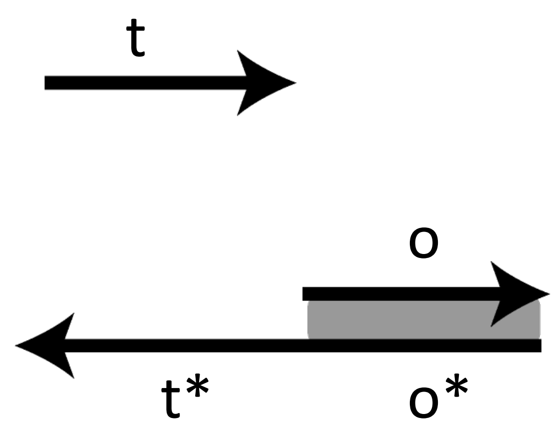


- Introduction to synthetic biocontrollers
- CRN implementation: Model and theory
- Towards *in vitro* implementation of PSD
- Closing remarks on PSD and future work

What is strand displacement?



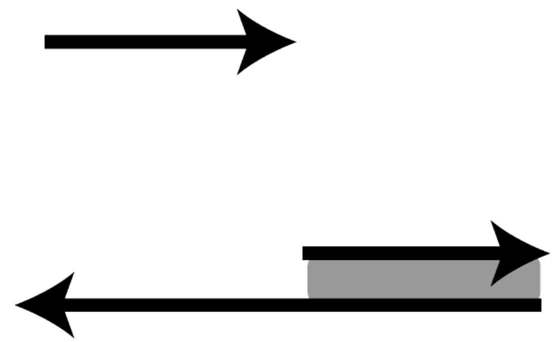
Toehold mediated strand displacement (TMSD)



Polymerase strand Displacement (PSD)

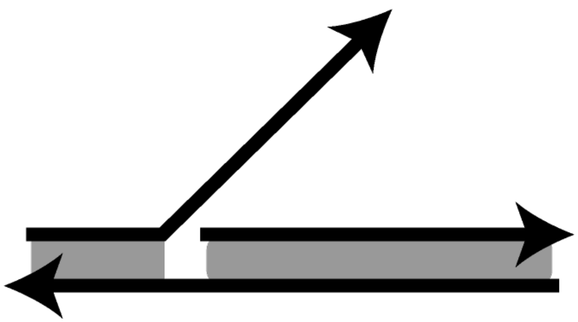
TMSD vs PSD: A comparison

Toehold mediated strand displacement

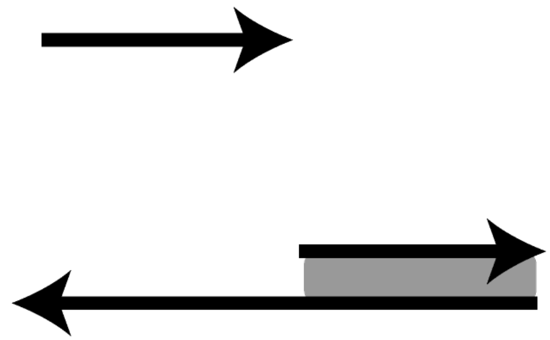


Polymerase strand displacement

TMSD vs PSD: A comparison

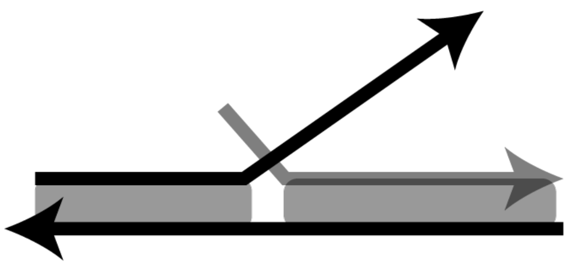


Toehold mediated strand displacement

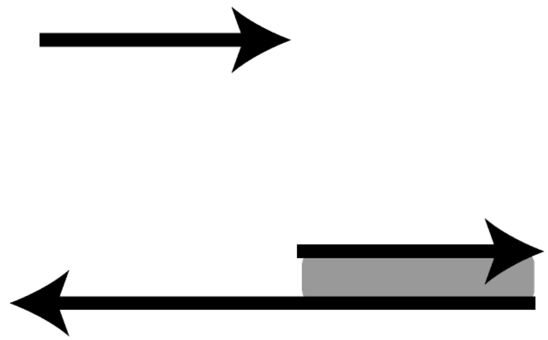


Polymerase strand displacement

TMSD vs PSD: A comparison

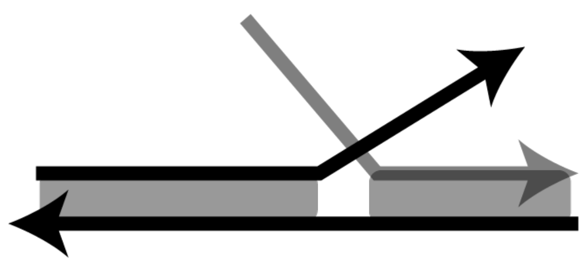


Toehold mediated strand displacement

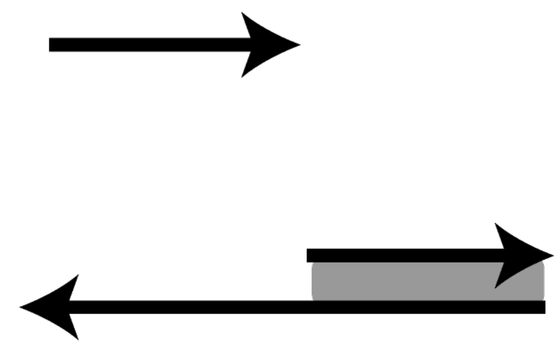


Polymerase strand displacement

TMSD vs PSD: A comparison

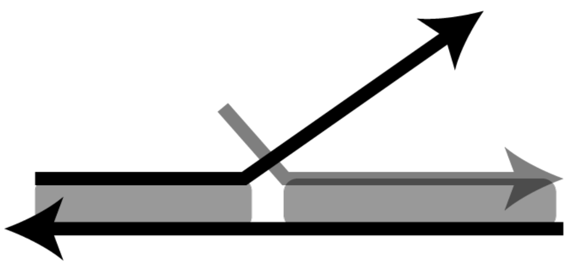


Toehold mediated strand displacement

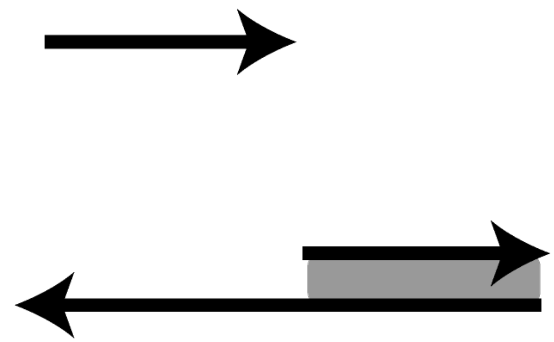


Polymerase strand displacement

TMSD vs PSD: A comparison

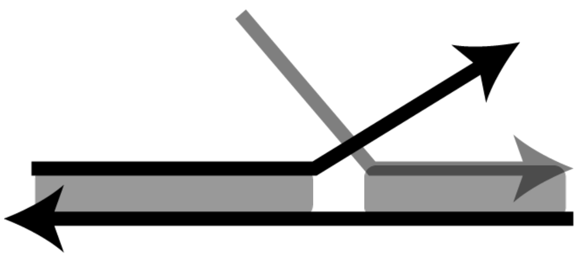


Toehold mediated strand displacement

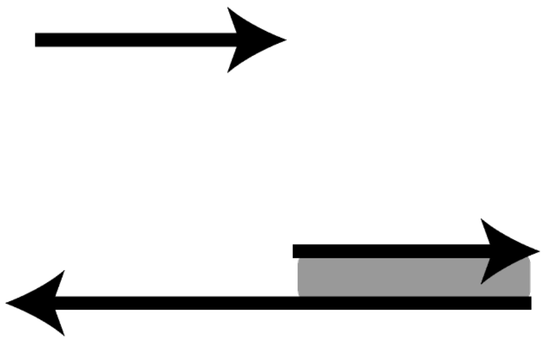


Polymerase strand displacement

TMSD vs PSD: A comparison



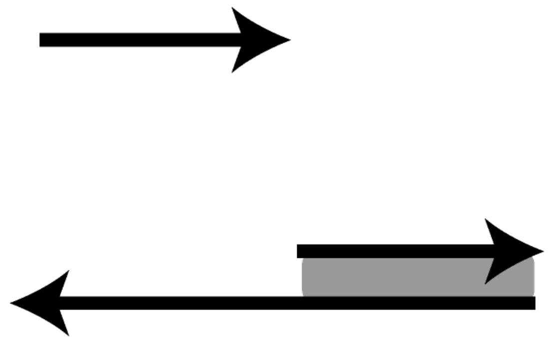
Toehold mediated strand displacement



Polymerase strand displacement

TMSD vs PSD: A comparison

Toehold mediated strand displacement



Polymerase strand displacement

TMSD vs PSD: A comparison

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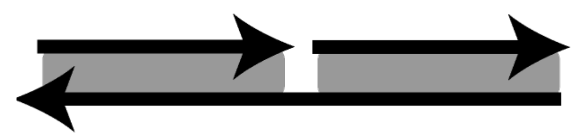

Polymerase strand displacement

TMSD vs PSD: A comparison

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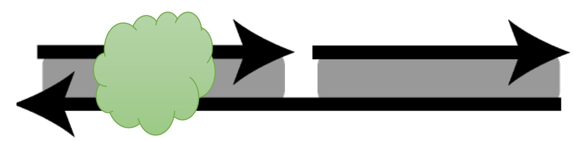

Polymerase strand displacement

TMSD vs PSD: A comparison

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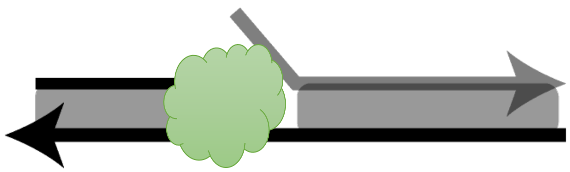

Polymerase strand displacement

TMSD vs PSD: A comparison

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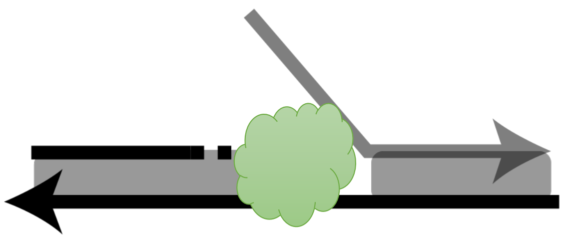
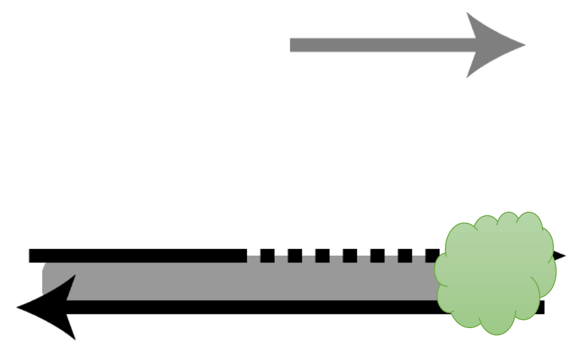


Polymerase strand displacement

TMSD vs PSD: A comparison

Toehold mediated strand displacement



Polymerase strand displacement

TMSD vs PSD: A comparison

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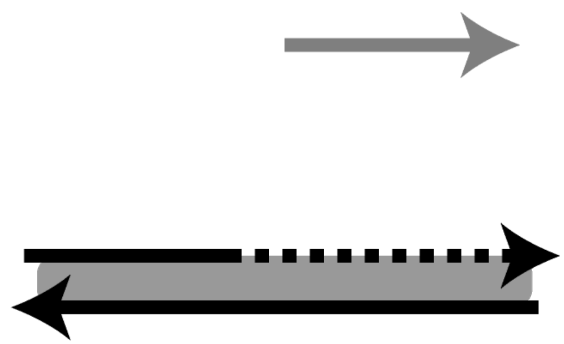
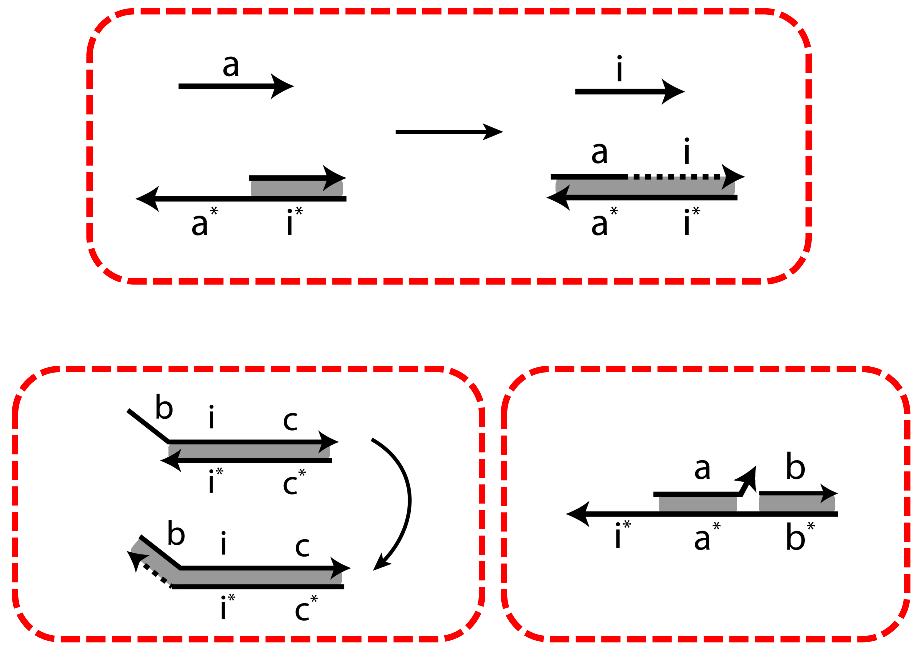


Polymerase strand displacement

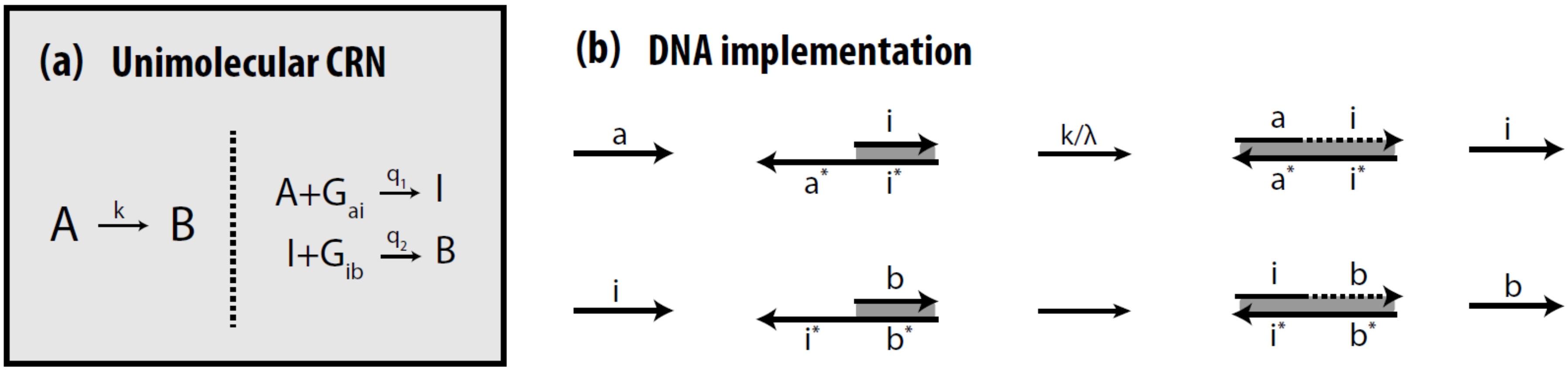
Strand displacing polymerase rules (Bst, Bsu …)

- For polymerization, a double strand region is required
- Polymerase goes 5′ -> 3′ direction, displaces the incumbent strand
- Polymerization blunts 5′ overhangs
- Polymerization stops if there are 3′ overhangs (or mismatching bases)

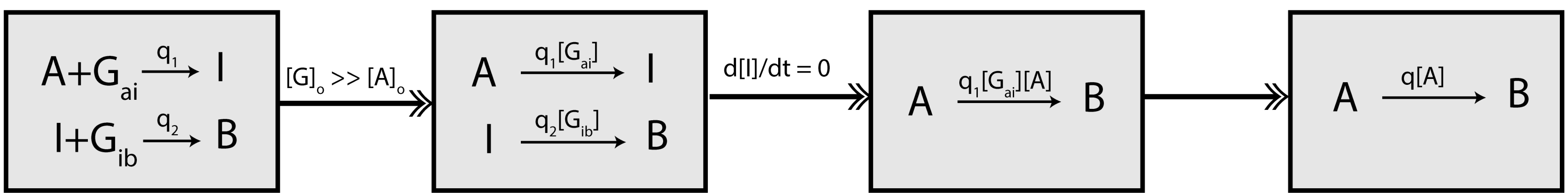


Shah *et al.* DNA25 (2019)

Unimolecular CRN

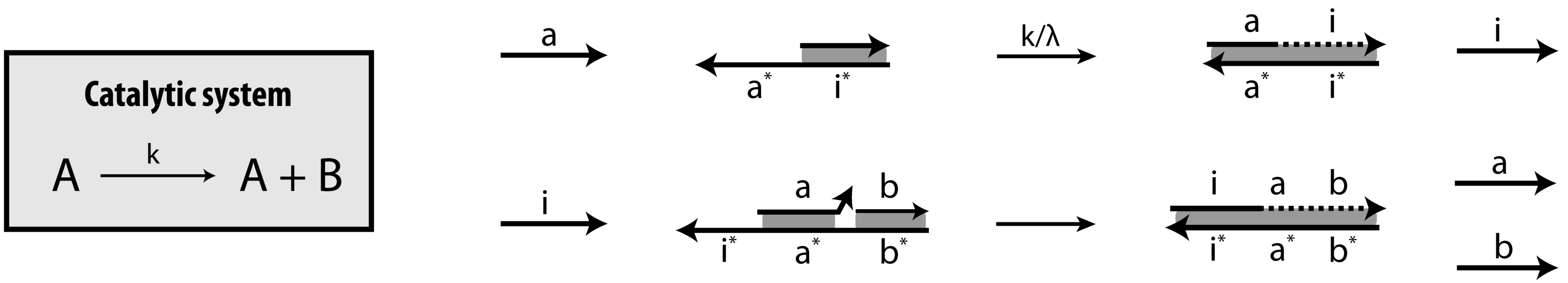


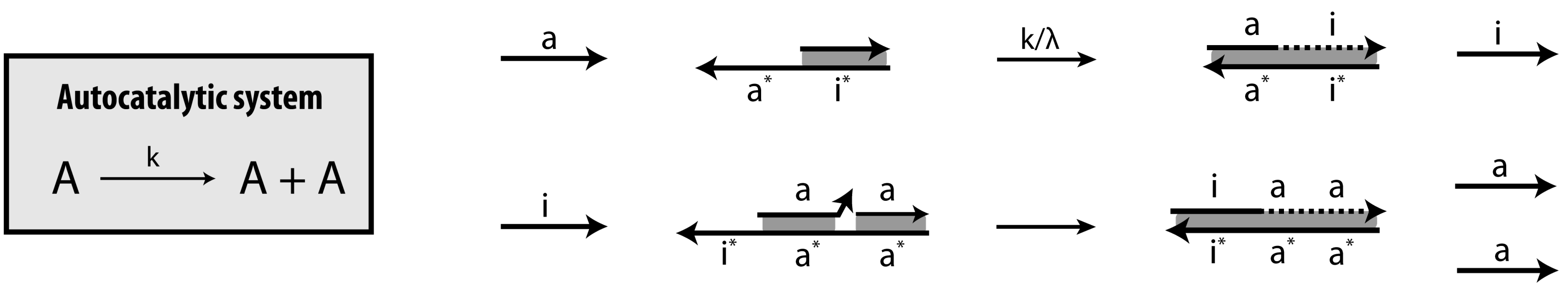
Mini proof:



Shah *et al.* DNA25 (2019)

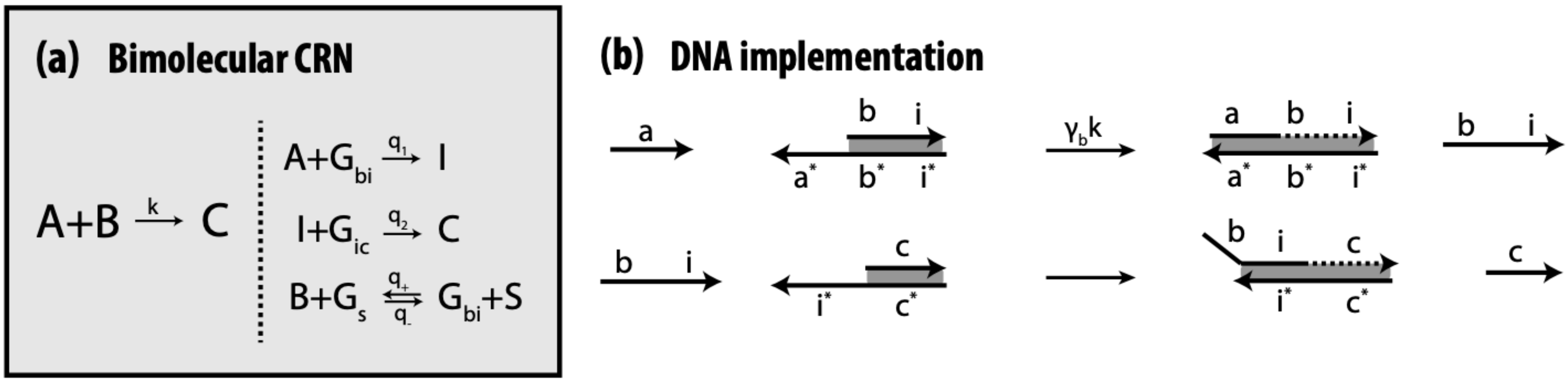
Easy modification to catalytic or autocatalytic



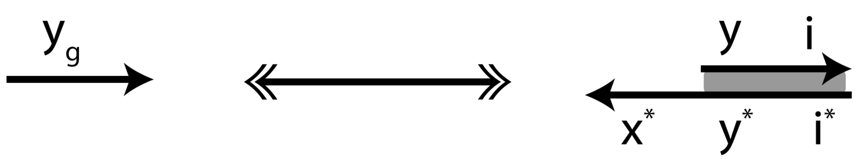


Shah *et al.* DNA25 (2019)

Bimolecular CRN



How to related unimolecular and bimolecular gates?



unimolecular CRNs

bimolecular CRNs

Shah *et al.* DNA25 (2019)

Unimolecular <-> Bimolecular

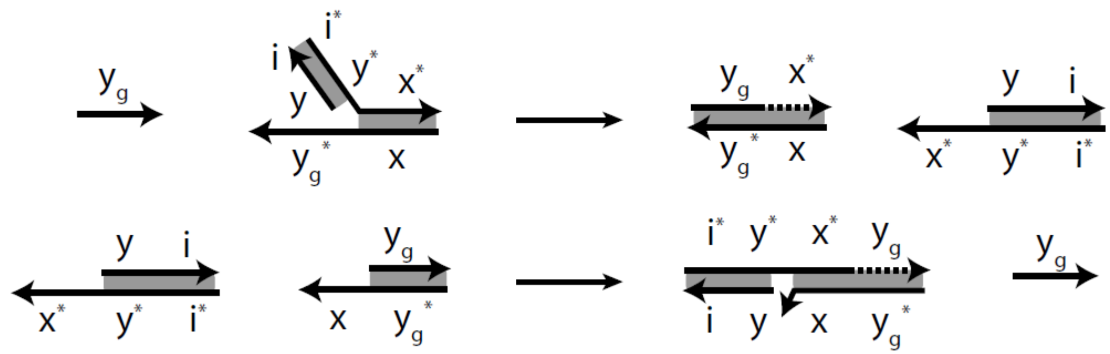
(a) Linker gate CRN

$$Y_g \underset{k_-}{\overset{k_+}{\rightleftharpoons}} G_y$$

$$Y_g + L_g \xrightarrow{k_1} G_y$$

$$G_y + L_y \xrightarrow{k_2} Y_g$$

(b) DNA implementation



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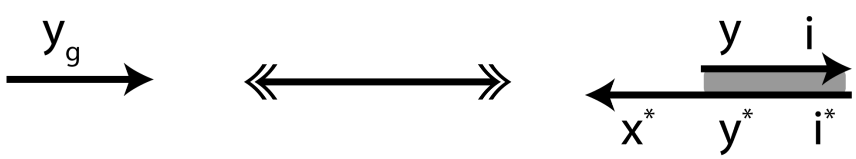

unimolecular CRNs

bimolecular CRNs

Shah *et al.* DNA25 (2019)

Unimolecular <-> Bimolecular

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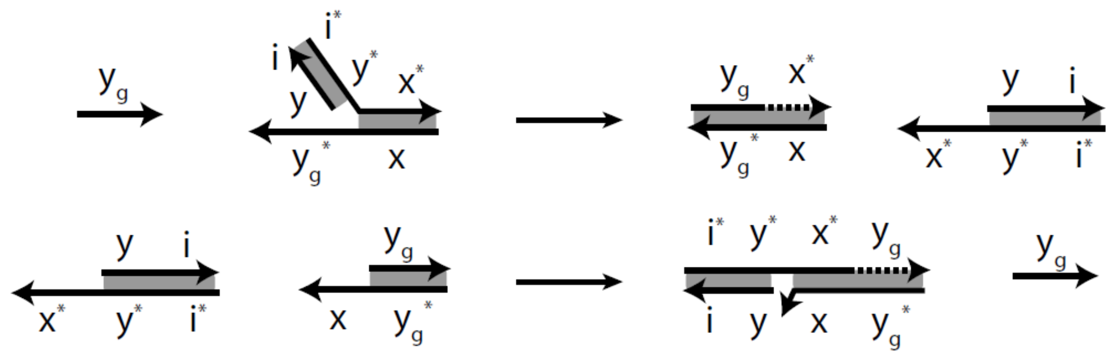


Mini proof:

$$Y_g + L_g \xrightarrow{q_1} G_y \qquad G_y + L_y \xrightarrow{q_2} Y_g$$

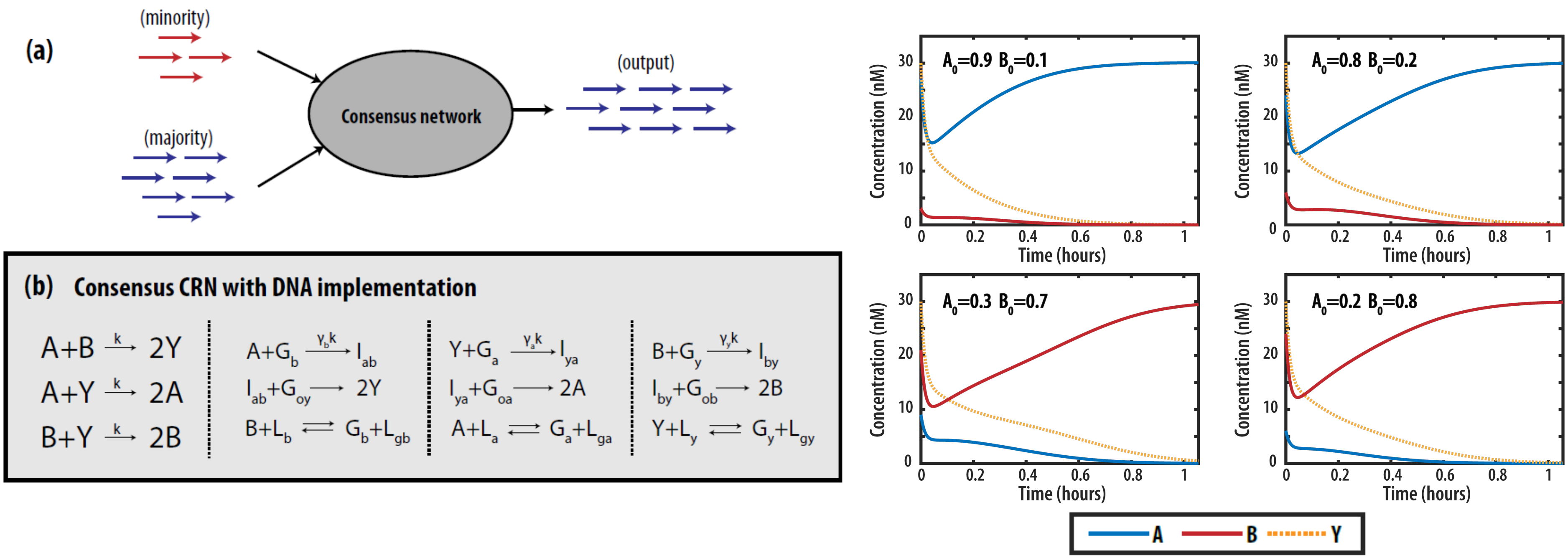
$\xrightarrow{[L]_o >> [Y]_o}$

$$Y_g \xrightarrow{q_1[L_g]} G_y \qquad G_y \xrightarrow{q_2[L_y]} Y_g$$

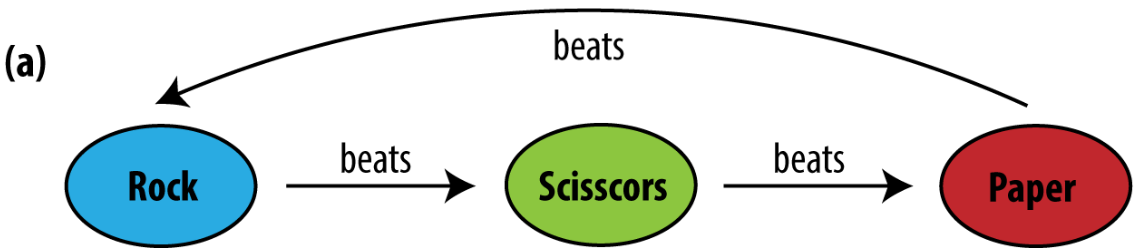
$\longrightarrow$

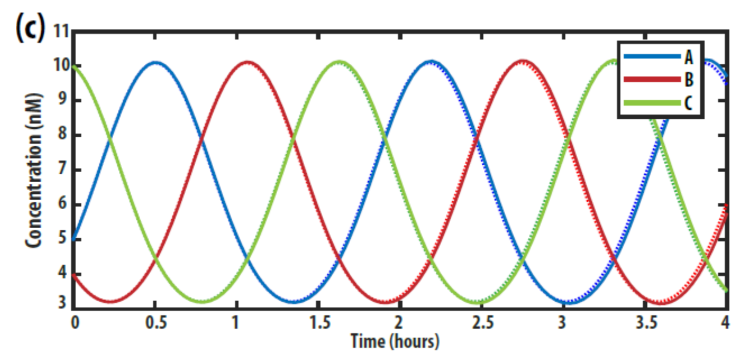
$$Y_g \underset{k_-}{\overset{k_+}{\rightleftharpoons}} G_y$$

Applications – molecular democracy



Applications – rock, paper, scissors oscillator





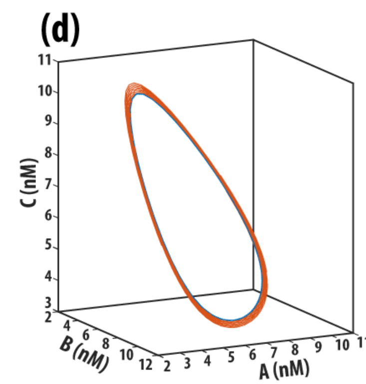
(b) Rock-paper-scissor CRN with DNA implementation

$$A+B \xrightarrow{k} 2B \qquad B+C \xrightarrow{k} 2C \qquad C+A \xrightarrow{k} 2A$$

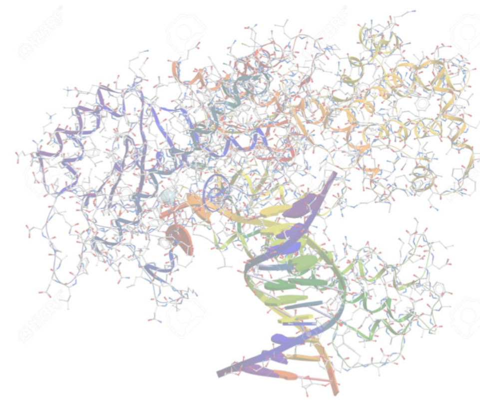
$$A+G_b \xrightarrow{\gamma_b k} I_{ab} \qquad I_{ab}+G_{ob} \longrightarrow 2B \qquad B+L_b \rightleftharpoons G_b+L_{gb}$$

$$B+G_c \xrightarrow{\gamma_c k} I_{bc} \qquad I_{bc}+G_{oc} \longrightarrow 2C \qquad C+L_c \rightleftharpoons G_c+L_{gc}$$

$$C+G_a \xrightarrow{\gamma_a k} I_{ca} \qquad I_{ca}+G_{oa} \longrightarrow 2A \qquad A+L_a \rightleftharpoons G_a+L_{ga}$$



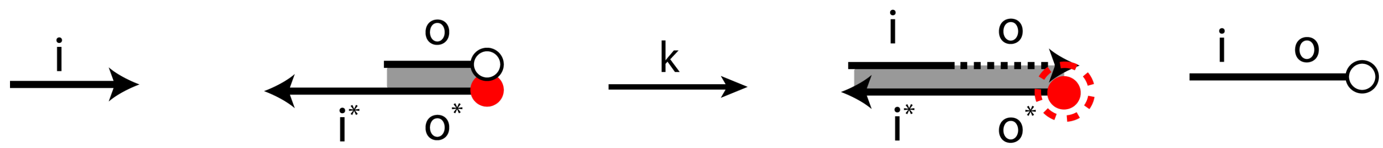
Outline

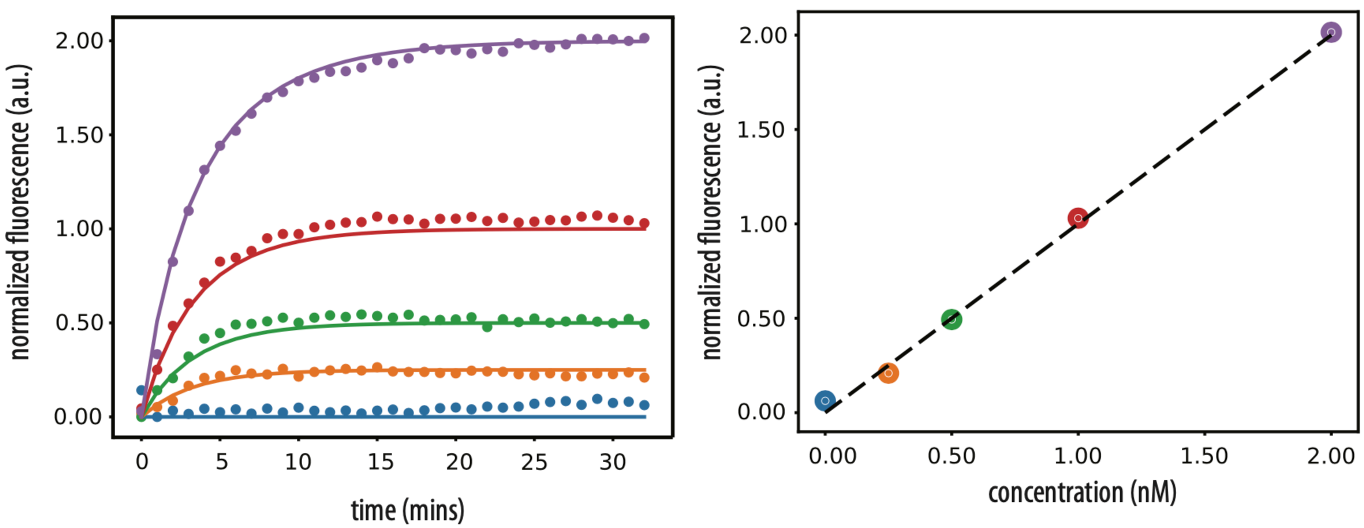


- **Introduction to synthetic biocontrollers**
- **CRN implementation: Model and theory**
- **Towards *in vitro* implementation of PSD**
- **Closing remarks on PSD and future work**

Shah *et al.* JACS (2020) (under review)

Calibration curve a.k.a sanity check





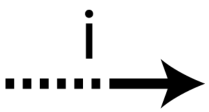
k_f (/M /s)
$1.03\ (\pm 0.047) \times 10^6$

1.0 x = 1nM
RC conc. = 2.5x

input concentration at 50 ℃ — 2.00 x, 1.00 x, 0.50 x, 0.25 x, 0.00 x

Shah *et al.* JACS (2020) (under review)

Tuning reaction speed with primer length

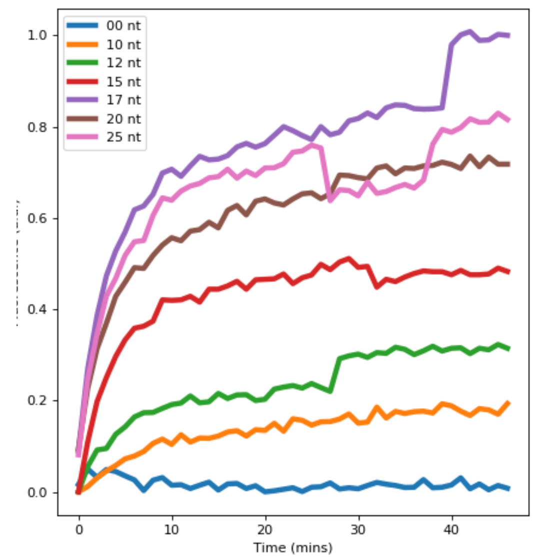


Truncate input from 5' side

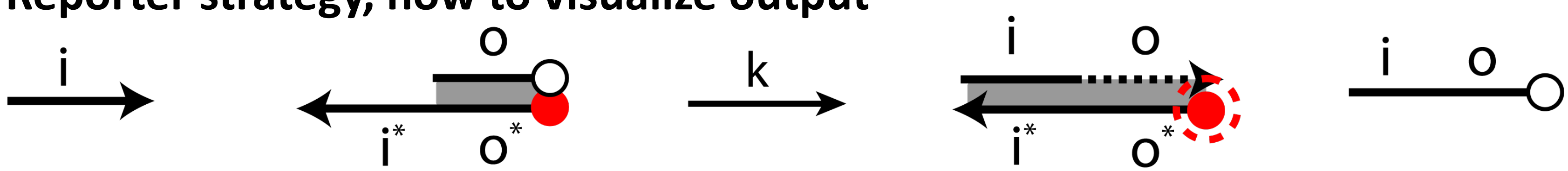
Assumed model

$$I + RC \xrightarrow{k} O$$

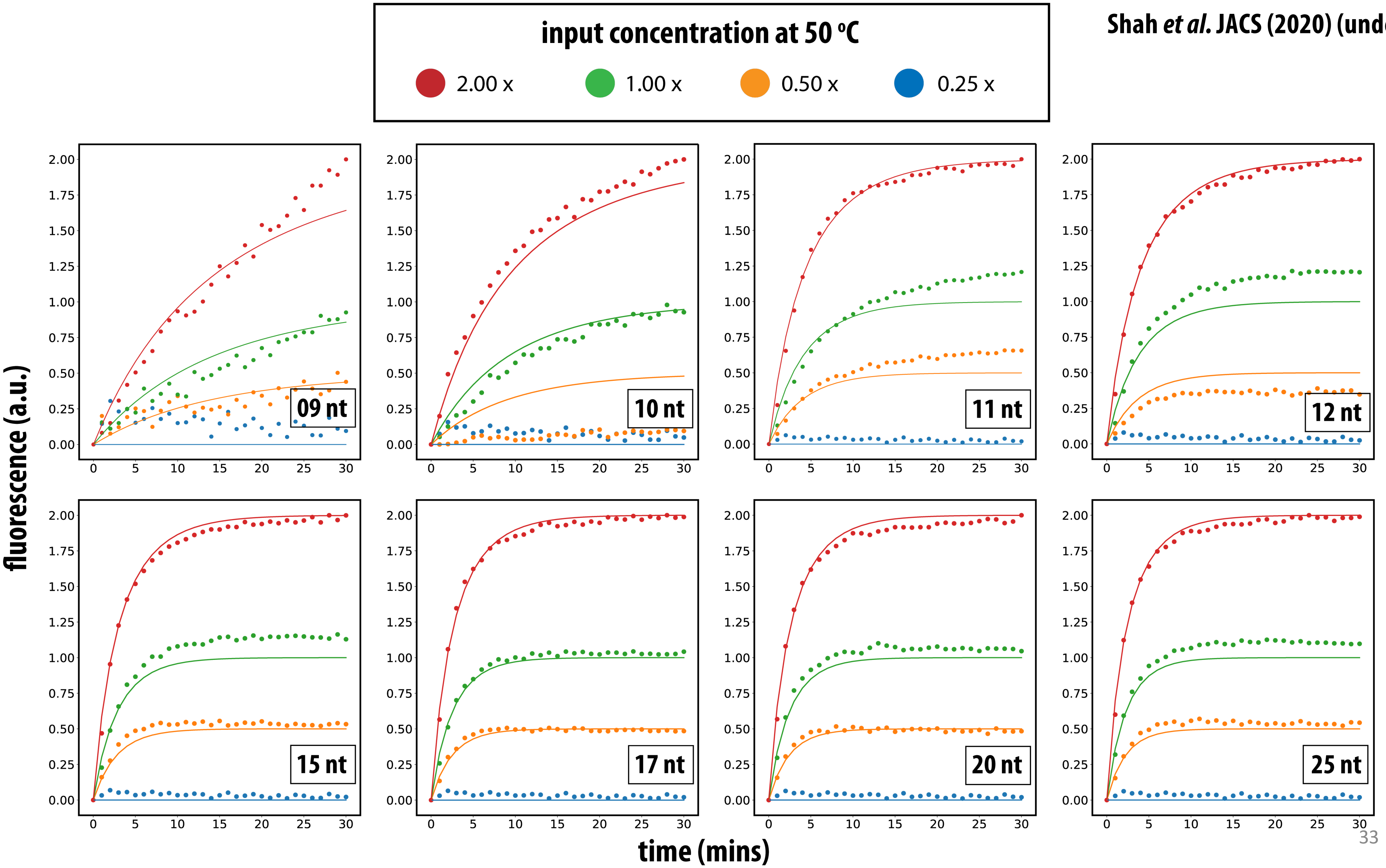
Input length
09 nt
10 nt
11 nt
12 nt
15 nt
17 nt
20 nt
25 nt



Reporter strategy, how to visualize output



Shah *et al.* JACS (2020) (under review)



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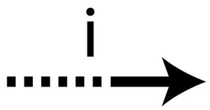
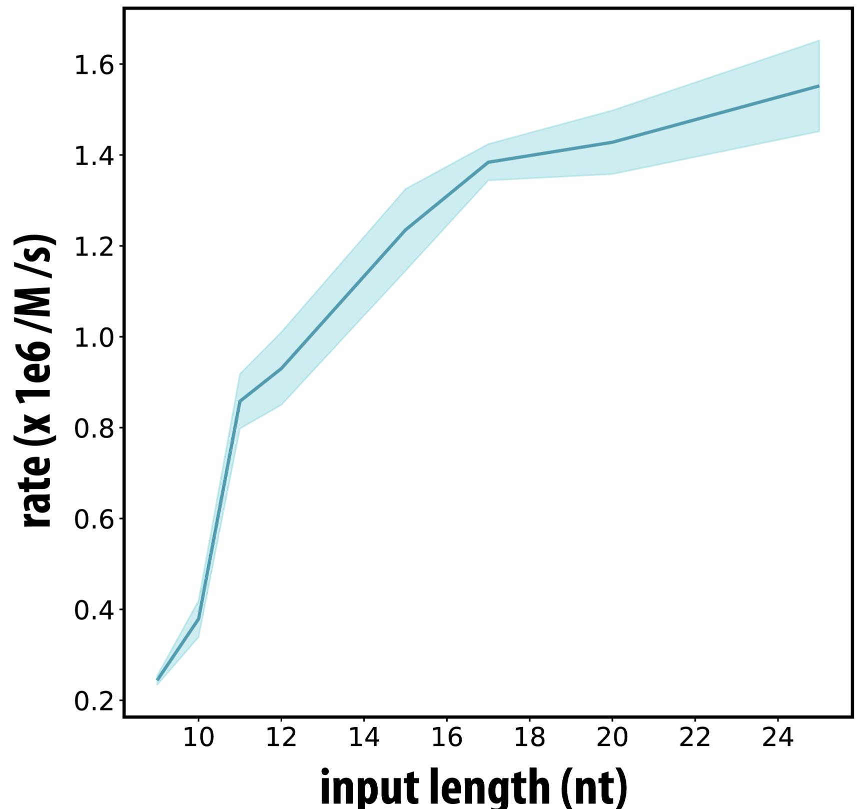


Assumed model

$$I + RC \xrightarrow{k} O$$

Input length	rate (/M /s)
09 nt	$0.24\ (\pm 0.01) \times 10^6$
10 nt	$0.37\ (\pm 0.04) \times 10^6$
11 nt	$0.85\ (\pm 0.06) \times 10^6$
12 nt	$0.93\ (\pm 0.08) \times 10^6$
15 nt	$1.23\ (\pm 0.09) \times 10^6$
17 nt	$1.38\ (\pm 0.04) \times 10^6$
20 nt	$1.42\ (\pm 0.07) \times 10^6$
25 nt	$1.55\ (\pm 0.10) \times 10^6$



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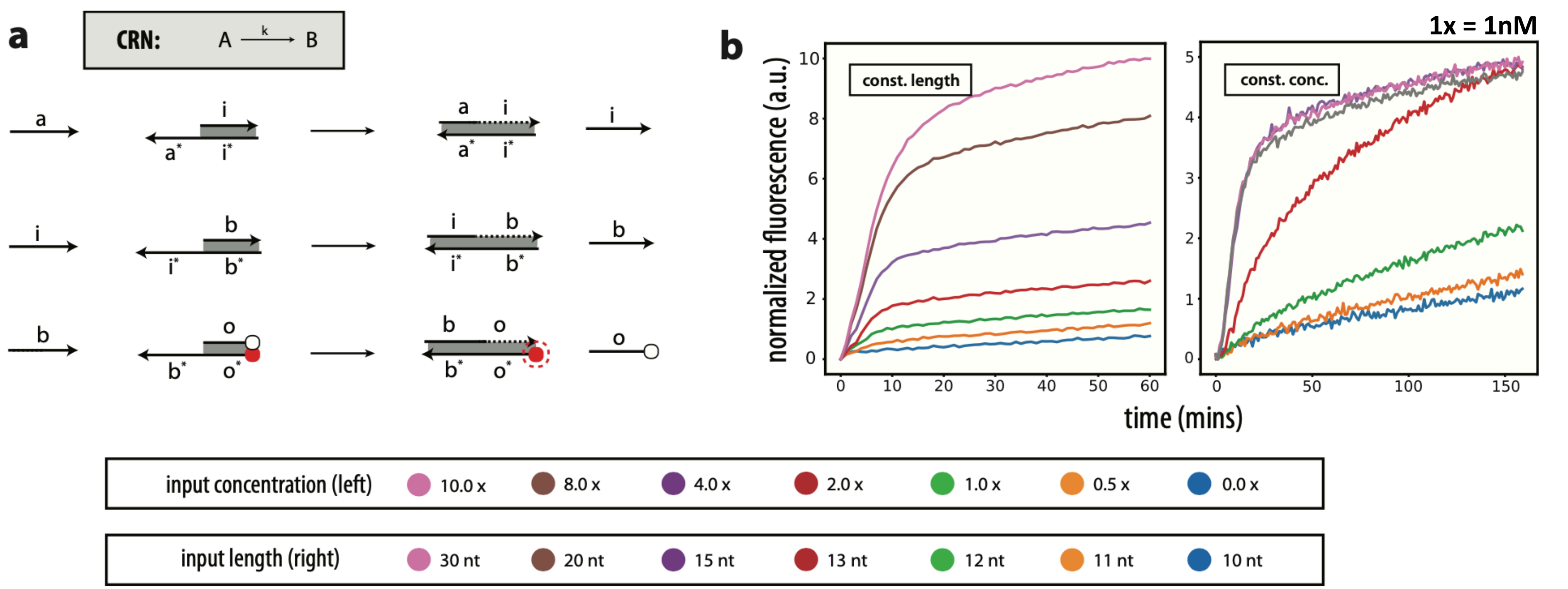
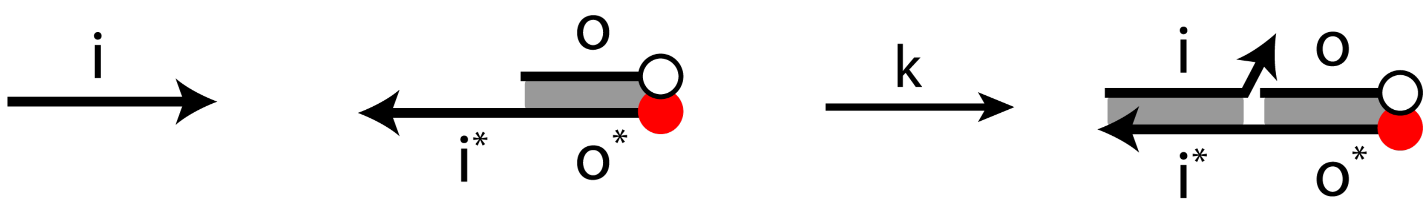
Single-output CRN system



Optimize 3' mismatch to stop polymerase



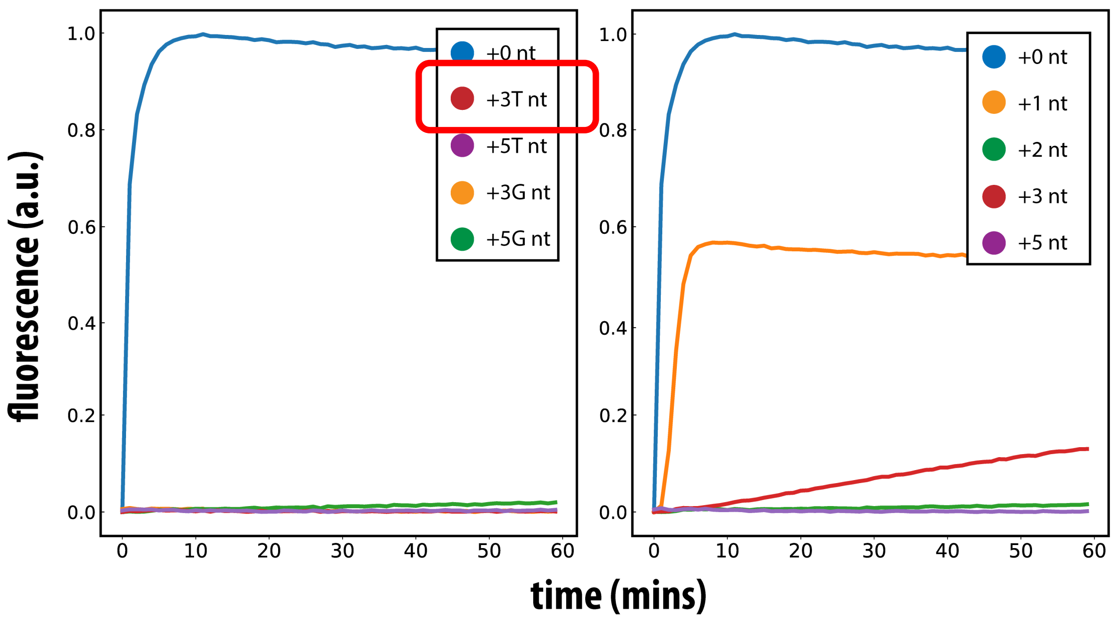
- Such polymerase stopper strategy is required for any complex with more than one strand. For example, catalytic reaction A -> A + B

- Two strategies to stop polymerase:
  - Random mismatch sequence with ~ 50% GC content
  - Poly-T tail/ poly-G tail

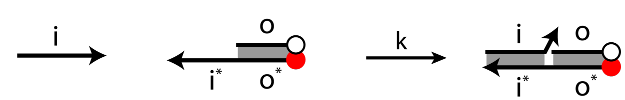
Optimize 3’ mismatch to stop polymerase

3’ mismatches (overhang)

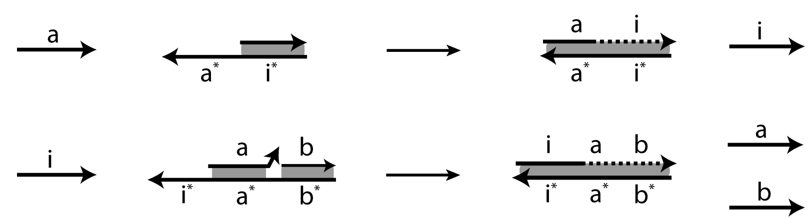
Input (5’-3’): TTCAAACTCACAACTCAAACATACA **GGGGG**
Input (5’-3’): TTCAAACTCACAACTCAAACATACA **TTTTT**
Input (5’-3’): TTCAAACTCACAACTCAAACATACA **TCATA**
Fluor (3’-5’): AAGTTTGAGTGTTGAGTTTGTATGT **GTGAAGTGTTGATGTGTTGTTTGGTAA**



Control: 3’ mismatch to stop PSD

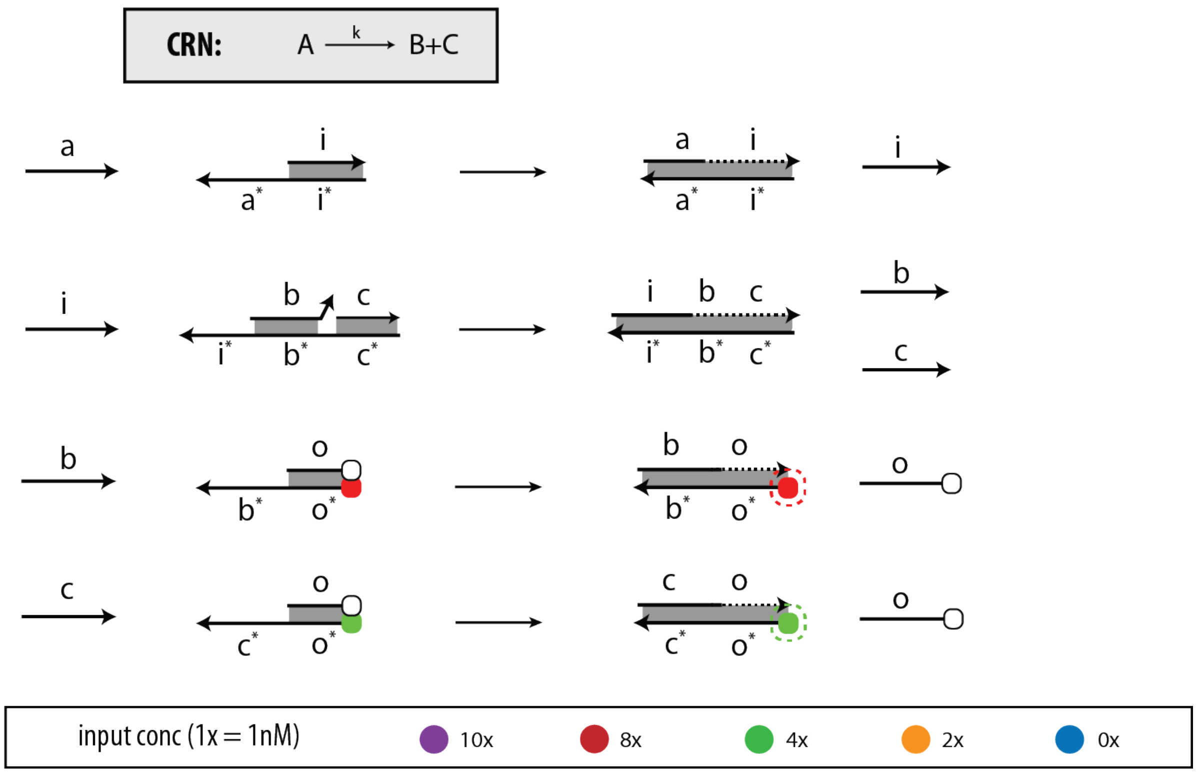


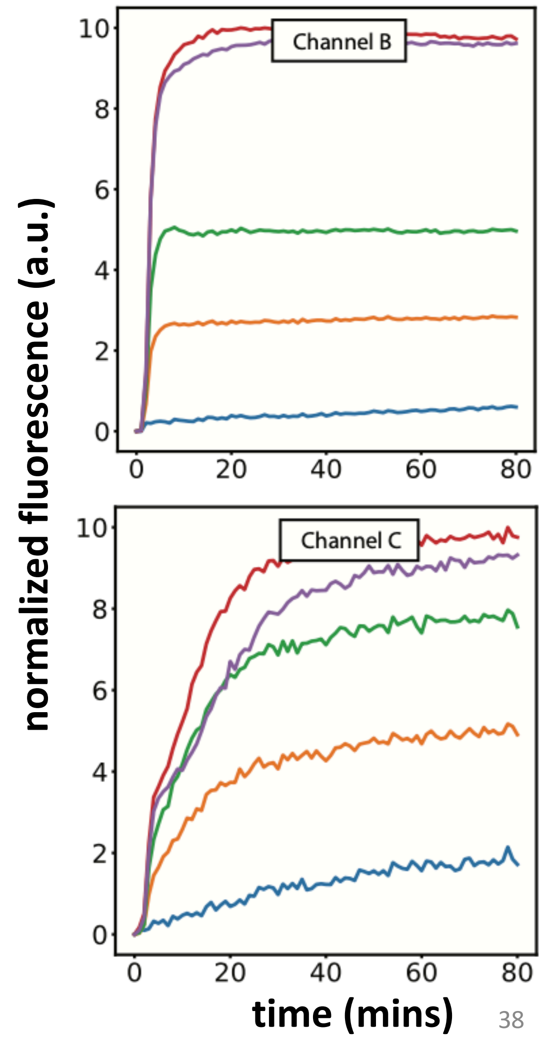
Required for catalytic systems, for example



Shah *et al.* JACS (2020) (under review)

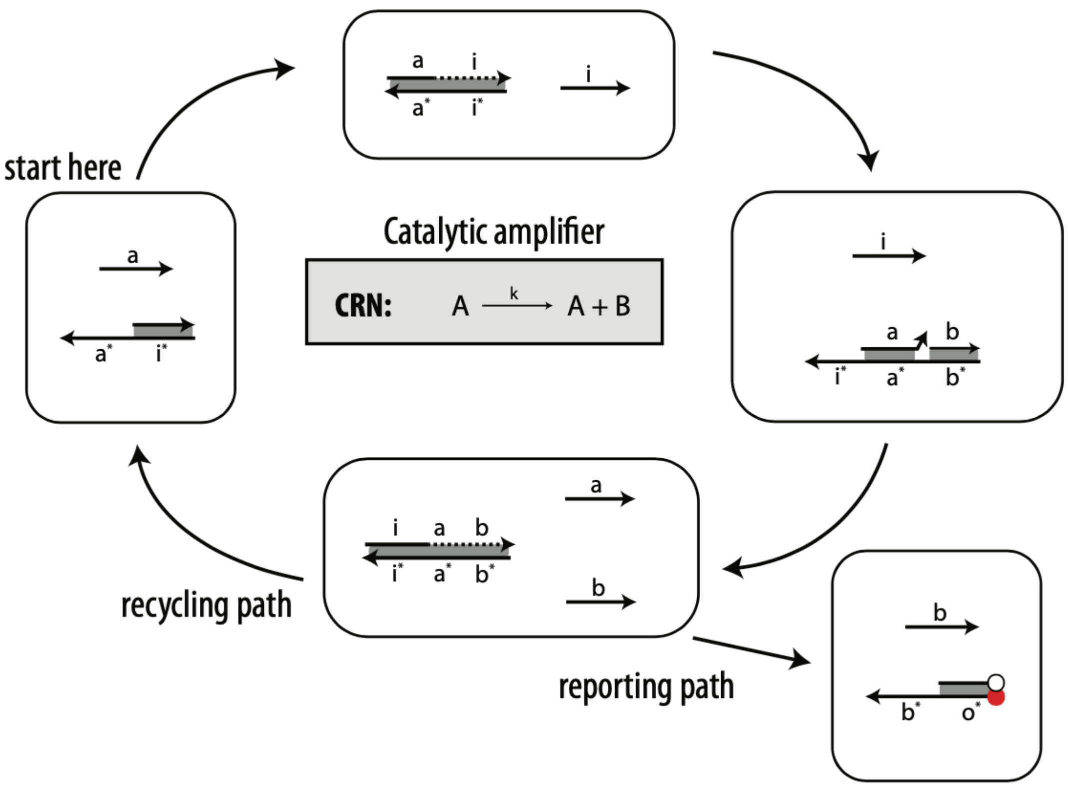
Multi-output CRN system

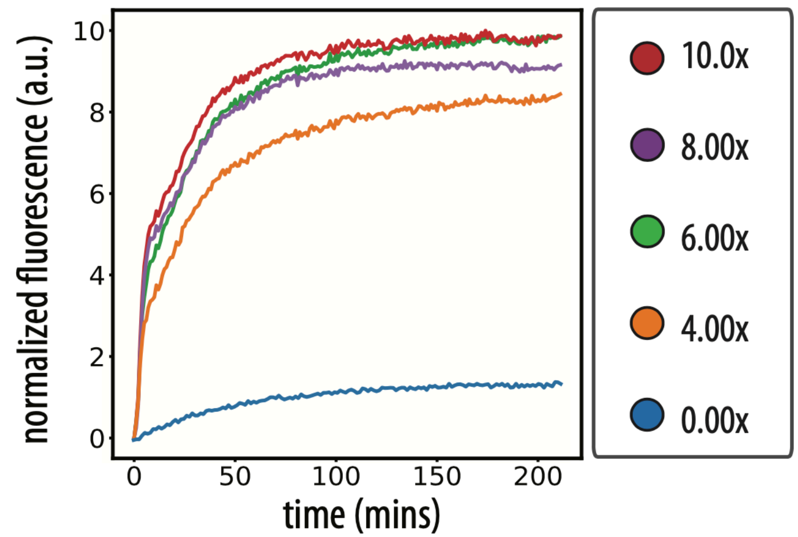




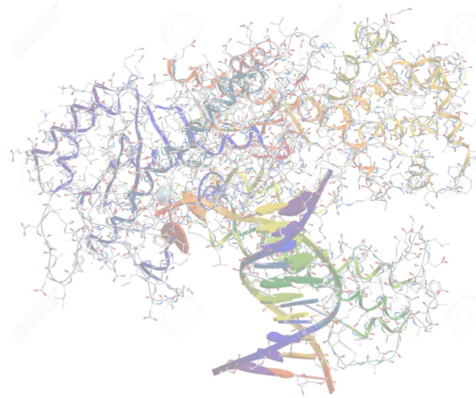
Shah *et al.* JACS (2020) (under review)

Catalytic amplifier



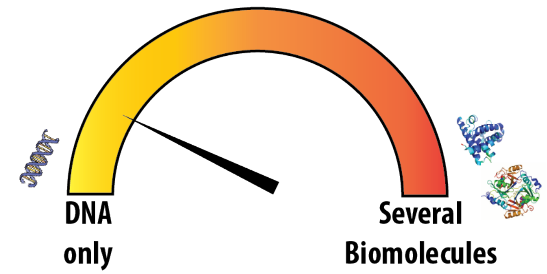


Outline



- Introduction to synthetic biocontrollers
- CRN implementation: Model and theory
- Towards *in vitro* implementation of PSD
- **Closing remarks on PSD and future work**

Summary of our work



- Proposed a new framework to implement arbitrary CRNs.
- Our design uses a fast strand displacing polymerase enzyme as an energy source.
- The reaction rate can be tuned by length of input (primer), concentration of input/ gates.
- Clamps at the 5′ end can effectively reduce circuit leak due to fraying.

Discussion and future work

- Experimental demonstration of complex reaction networks such as oscillators.
- Development of a simple compiler that can convert a set of CRNs to our DNA-based CRNs.
- Theoretical closed-form solution to test divergence error w.r.t gate conc. and time.