REACTION ENUMERATION & CONDENSATION OF DOMAIN-LEVEL STRAND DISPLACEMENT SYSTEMS

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33rd TBI Winterseminar, Bled, Slovenia

Grun, Badelt, Sarma, Shin, Wolfe, and Winfree (manuscript in preparation)

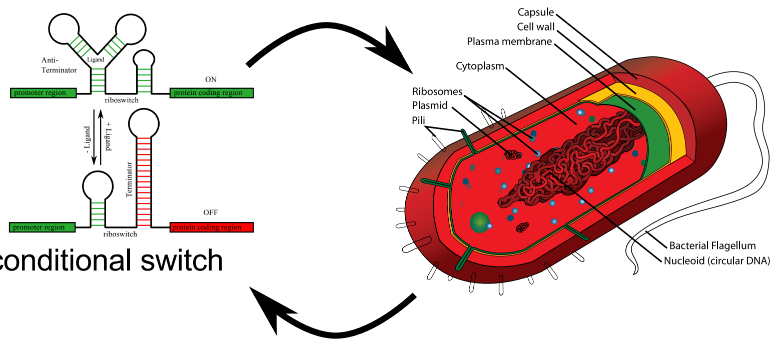
http://www.github.com/DNA-and-Natural-Algorithms-Group/peppercornenumerator

MOLECULAR PROGRAMMING

(in terms of the nuskell compiler project)

nucleic acids are architecture to implement algorithms
chemical reaction networks are a programming language
formal/experimental verification of correct implementation

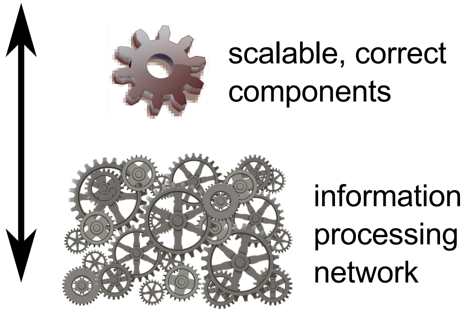
minimal/optimal components for biological systems



biological relevance is primary
➔ if experiments fail, refine the method

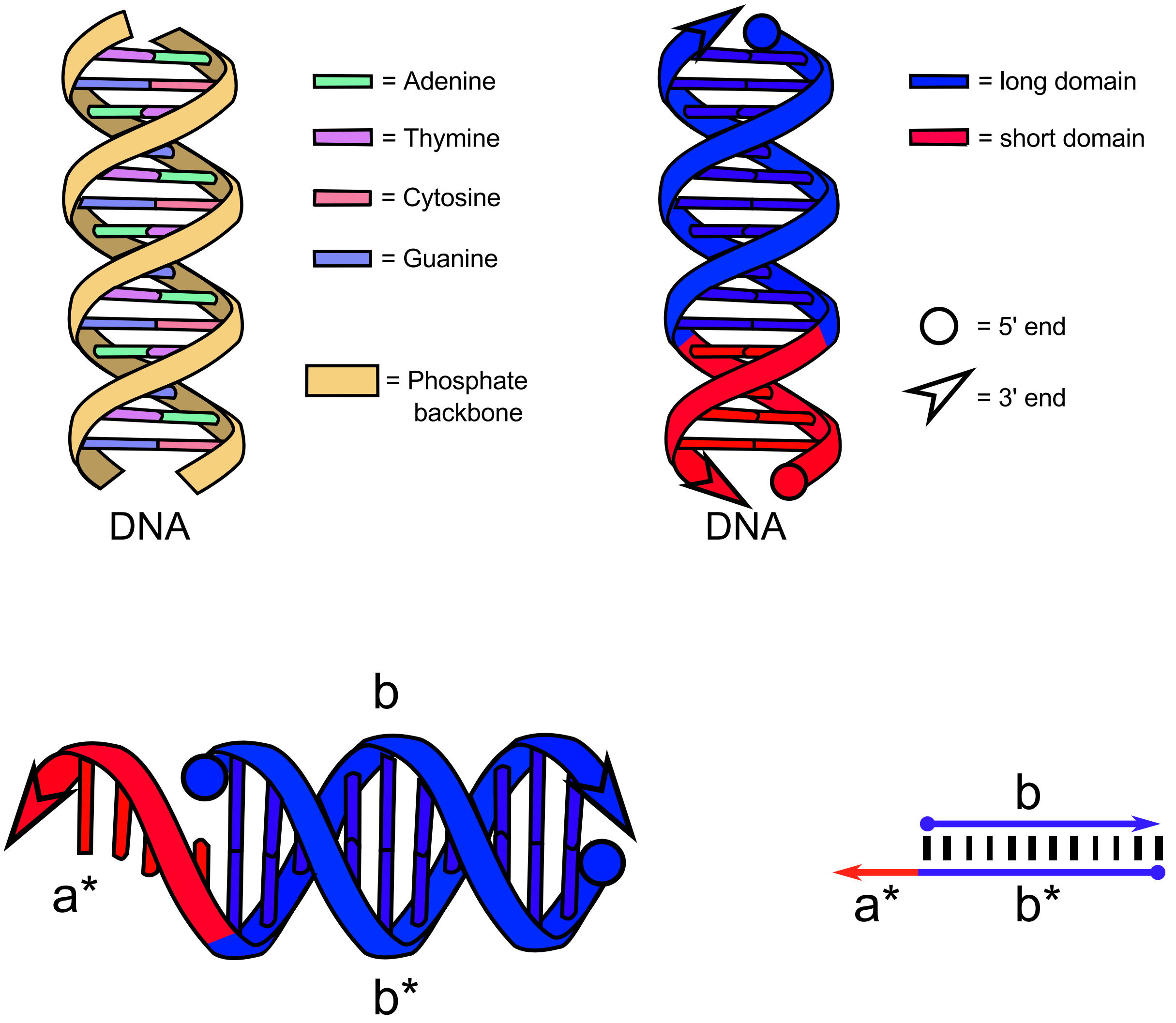
verifyably correct artificial systems

arbitrary algorithm



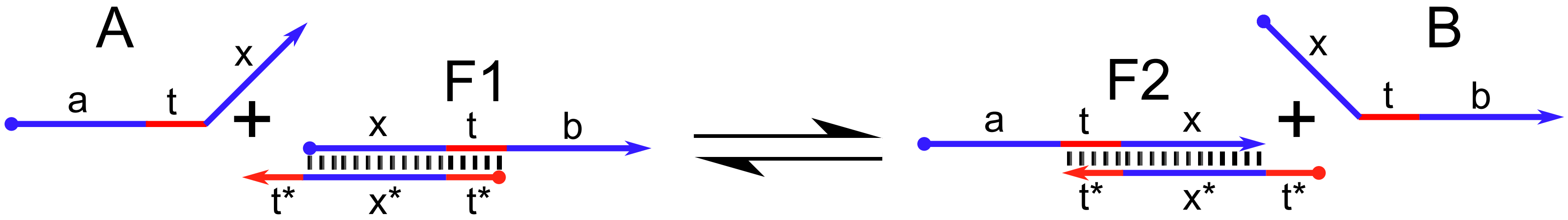
formal description is primary,
biological relevance secondary

DNA STRAND DISPLACEMENT

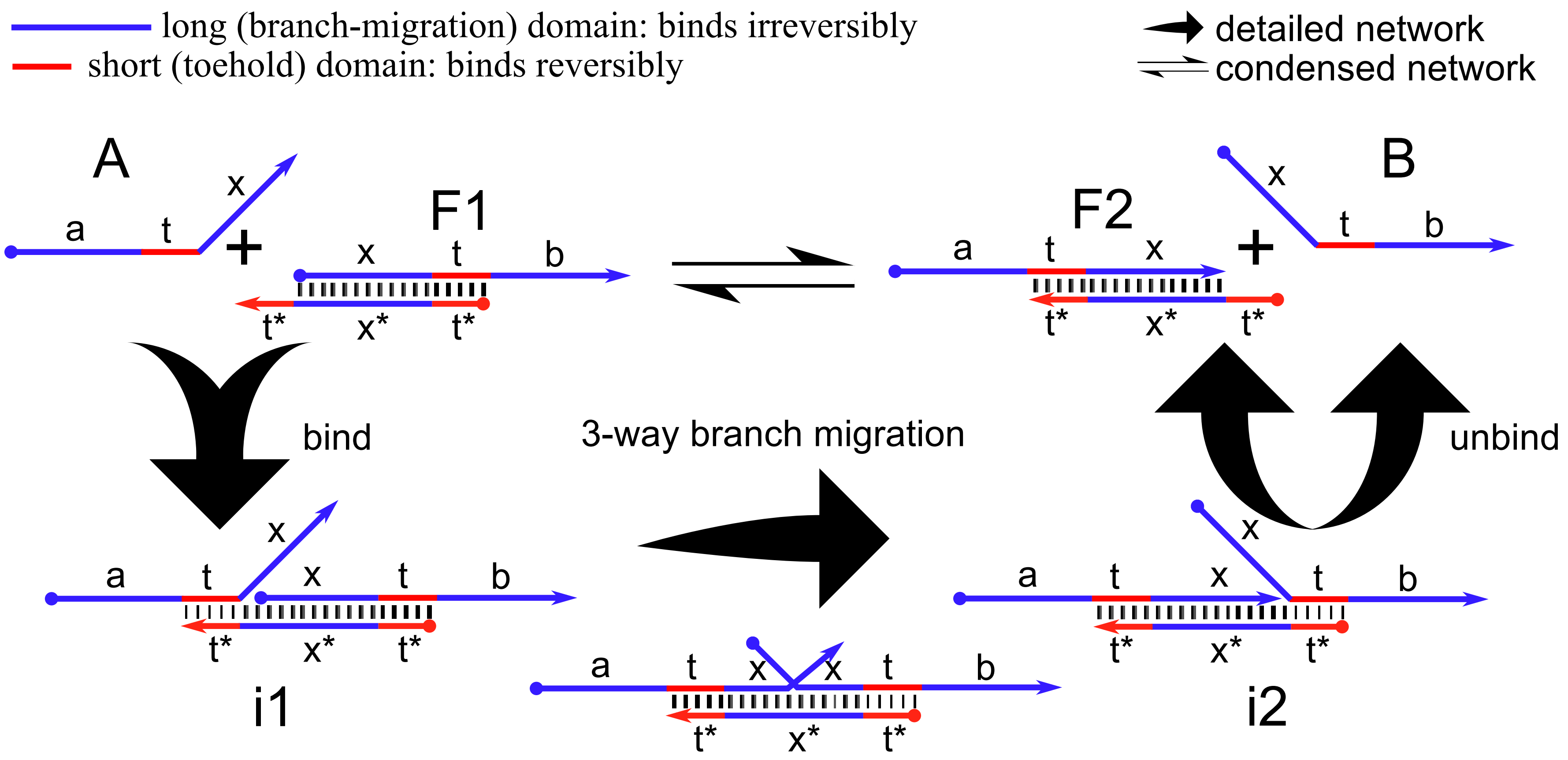


DOMAIN-LEVEL STRAND DISPLACEMENT

long (branch-migration) domain: binds irreversibly
short (toehold) domain: binds reversibly



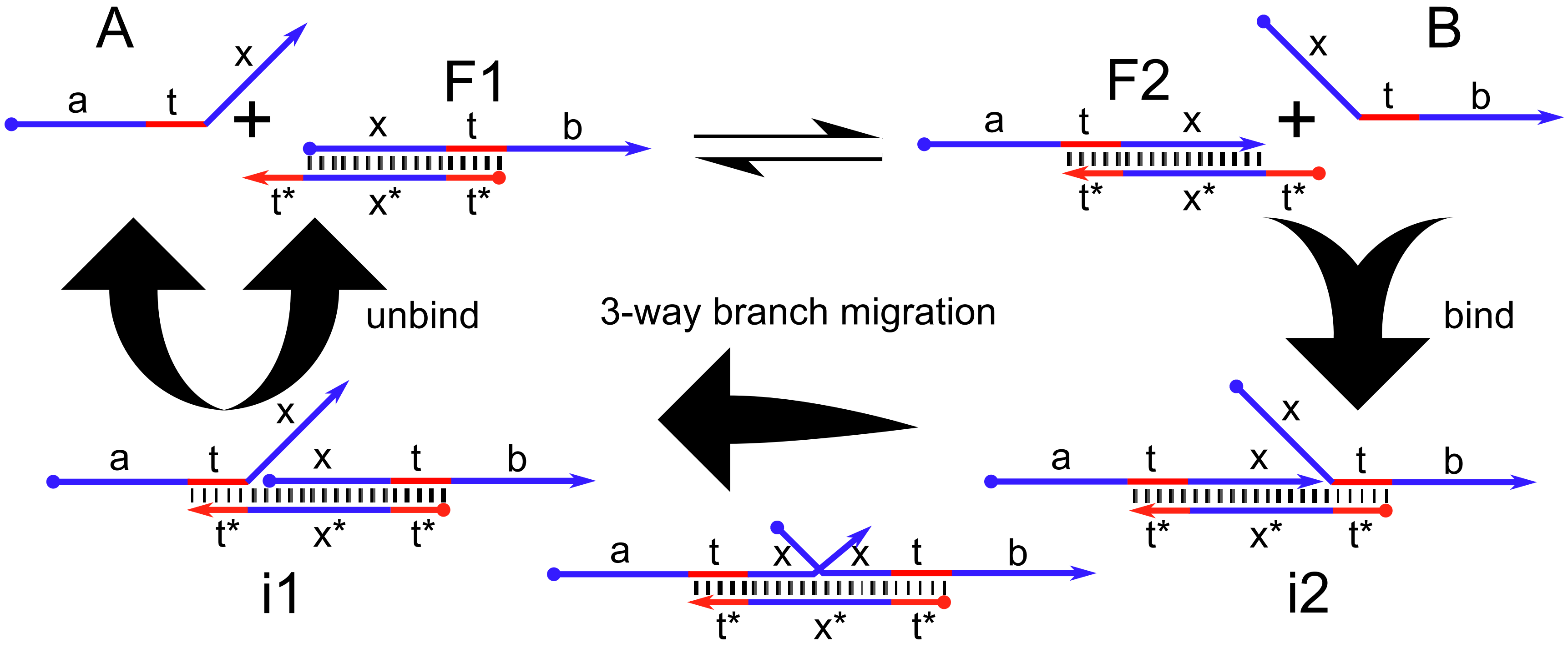
DOMAIN-LEVEL STRAND DISPLACEMENT



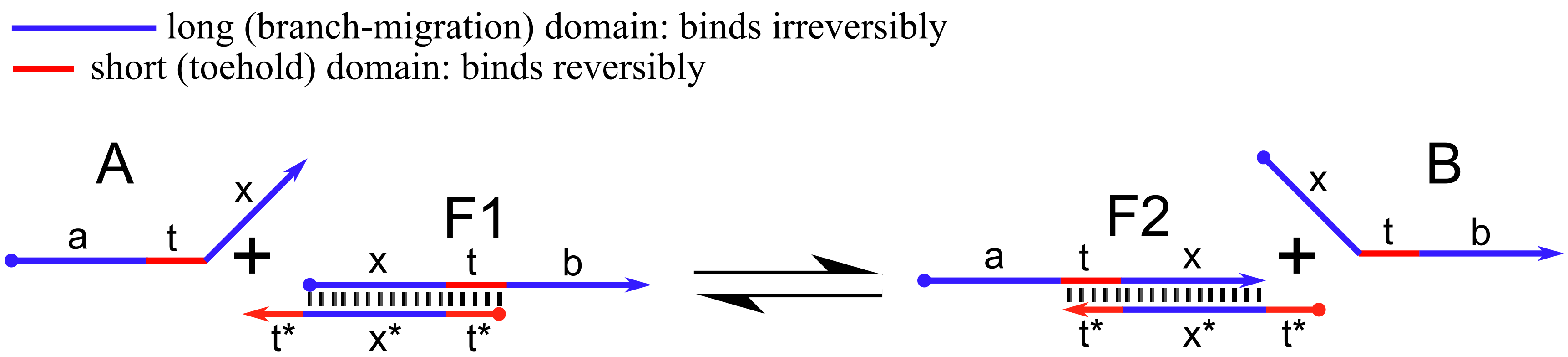
DOMAIN-LEVEL STRAND DISPLACEMENT

long (branch-migration) domain: binds irreversibly
short (toehold) domain: binds reversibly

detailed network
condensed network



DOMAIN-LEVEL STRAND DISPLACEMENT



formal CRN

$$A \rightleftharpoons B$$

formal species: {A, B}

DSD sytem specification

$$A + F1 \rightleftharpoons F2 + B$$

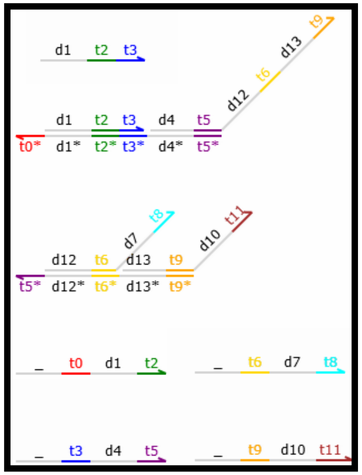
signal species (low concentation): {A, B}

fuel species (high concentration): {F1, F2}

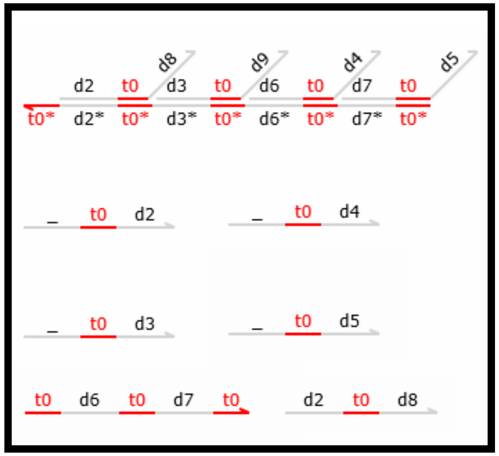
FROM CRN TO DSD SYSTEMS

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

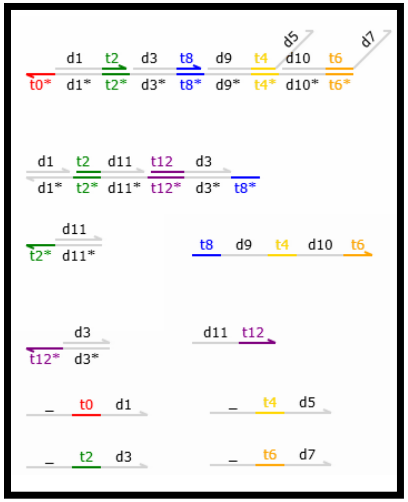
Soloveichik et al. (2010)



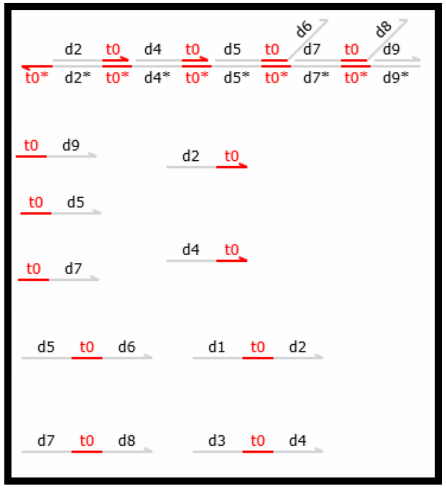
Lakin et al. (2012)



Cardelli (2011)



Qian et al. (2011)

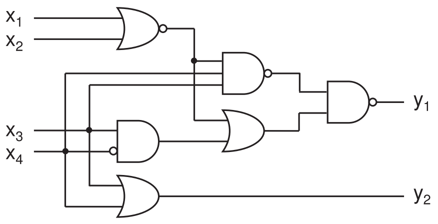


Chen et al. (2012), Cardelli (2013), Srinivas (2015), Lakin et al. (2016), ...

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

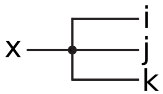
FROM A DIGITAL CIRCUIT TO DSD

$$y_2 y_1 = \lfloor \sqrt{x_4 x_3 x_2 x_1} \rfloor$$



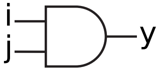
Qian et al. (2011)

fanout:



$$x_{on} \rightarrow i_{on} + j_{on} + k_{on}$$
$$x_{off} \rightarrow i_{off} + j_{off} + k_{off}$$

AND gate:



$$i_{off} + j_{off} \rightarrow y_{off}$$
$$i_{off} + j_{on} \rightarrow y_{off}$$
$$i_{on} + j_{off} \rightarrow y_{off}$$
$$i_{on} + j_{on} \rightarrow y_{on}$$

```
# The CRN computes the floor of the squareroot of a four-bit binary number.

# Fanout X3 -> F5 + F6 + F7
X3_OFF -> F5_OFF + F6_OFF + F7_OFF
X3_ON  -> F5_ON  + F6_ON  + F7_ON
# Fanout X4 -> F8 + F9 + F10
X4_OFF -> F8_OFF + F9_OFF + F10_OFF
X4_ON  -> F8_ON  + F9_ON  + F10_ON
# G11 = NOT(X1 OR X2) + Fanout G11 -> F14 + F15
X1_OFF + X2_OFF -> F14_ON  + F15_ON
X1_OFF + X2_ON  -> F14_OFF + F15_OFF
X1_ON  + X2_OFF -> F14_OFF + F15_OFF
X1_ON  + X2_ON  -> F14_OFF + F15_OFF
# G12 = F6 AND (NOT F9)
F6_OFF + F9_OFF -> G12_OFF
F6_OFF + F9_ON  -> G12_OFF
F6_ON  + F9_OFF -> G12_ON
F6_ON  + F9_ON  -> G12_OFF
# Y2 = F7 OR F10
F7_OFF + F10_OFF -> Y2_OFF
F7_OFF + F10_ON  -> Y2_ON
F7_ON  + F10_OFF -> Y2_ON
F7_ON  + F10_ON  -> Y2_ON

# G16b = F5 AND F8
F5_OFF + F8_OFF -> G16b_OFF
F5_OFF + F8_ON  -> G16b_OFF
F5_ON  + F8_OFF -> G16b_OFF
F5_ON  + F8_ON  -> G16b_ON
# G16 = NOT(F14 AND G16b)
F14_OFF + G16b_OFF -> G16_ON
F14_OFF + G16b_ON  -> G16_ON
F14_ON  + G16b_OFF -> G16_ON
F14_ON  + G16b_ON  -> G16_OFF
# G17 = F15 OR G12
F15_OFF + G12_OFF -> G17_OFF
F15_OFF + G12_ON  -> G17_ON
F15_ON  + G12_OFF -> G17_ON
F15_ON  + G12_ON  -> G17_ON
# Y1 = NOT(G16 AND G17)
G16_OFF + G17_OFF -> Y1_ON
G16_OFF + G17_ON  -> Y1_ON
G16_ON  + G17_OFF -> Y1_ON
G16_ON  + G17_ON  -> Y1_OFF
```

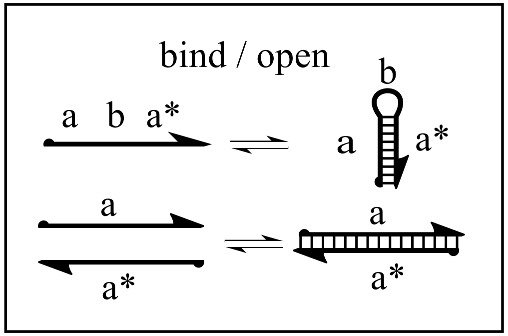
Input for the nuskell compiler: **32** formal reactions.

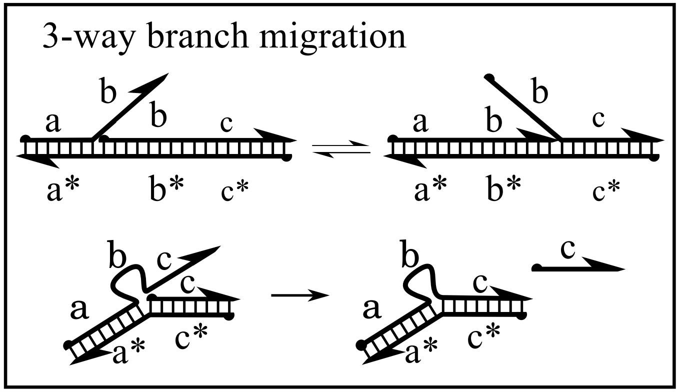
soloveichik2010.ts: **52** signal species, **92** fuel species, **172** intermediate species, **180** reactions.

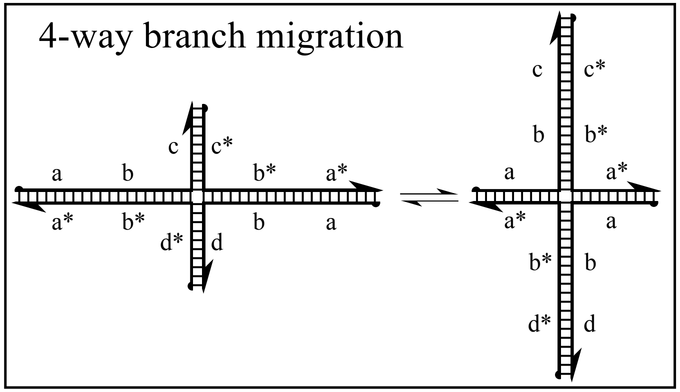
verifies as **correct** according to the **pathway decomposition** and **CRN bisimulation** equivalence

Badelt, Johnson, Dong, Shin, Thachuk and Winfree: A general-purpose CRN-to-DSD compiler with formal verification, optimization, and simulation capabilities. LNCS (2017)

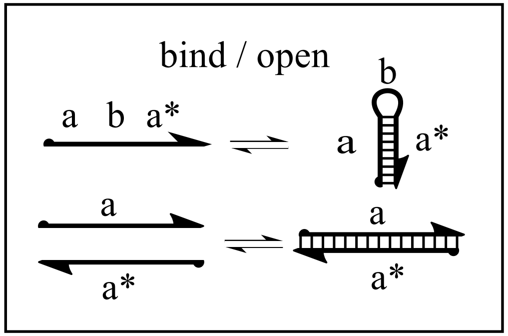
REACTION TYPES







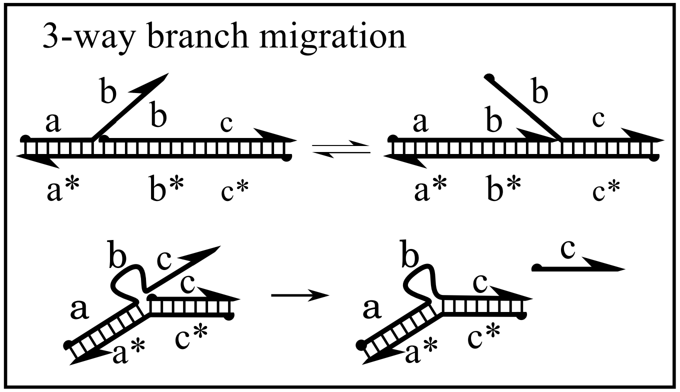
REACTION TYPES

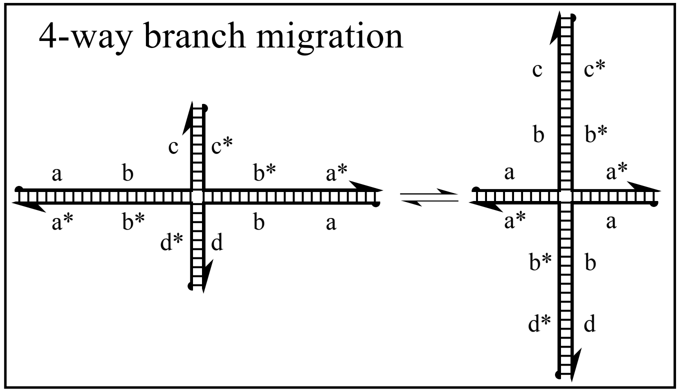


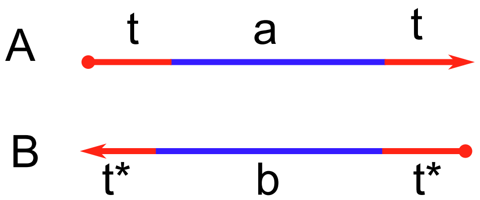
allows all secondary structures (pseudoknots excluded)
open reactions of domains with length > L are forbidden

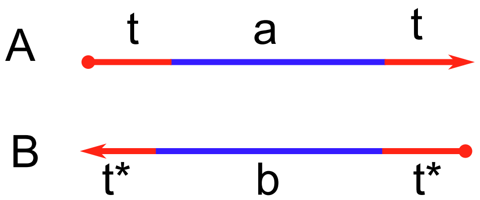
open & branch migration reactions are always unimolecular, but may lead to dissociation.

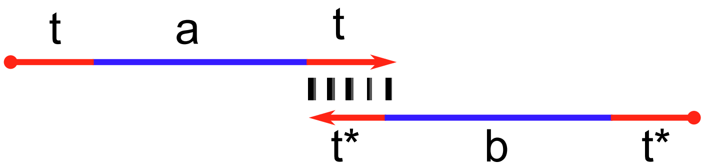
bind reactions are the only valid bimolecular reactions

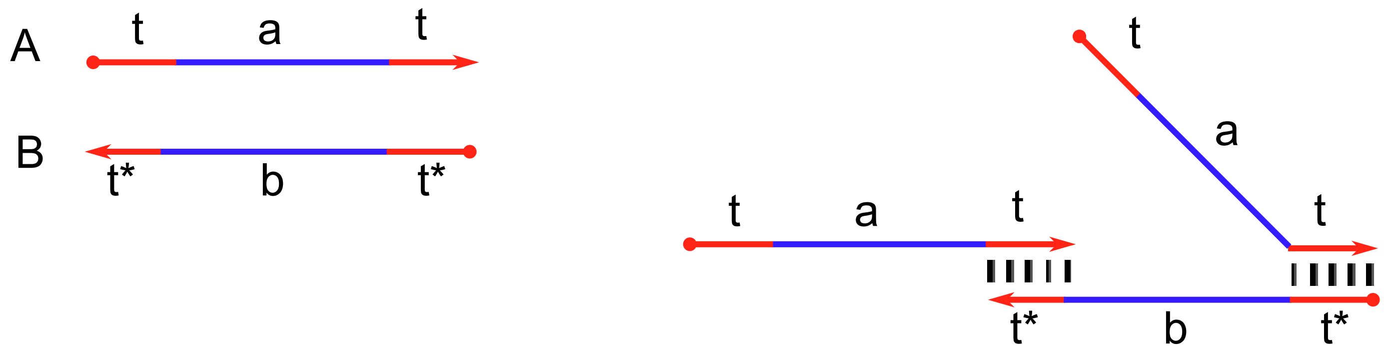





A
t
a
t
B
t*
b
t*


A
t
a
t
B
t*
b
t*


t
a
t
t*
b
t*


A
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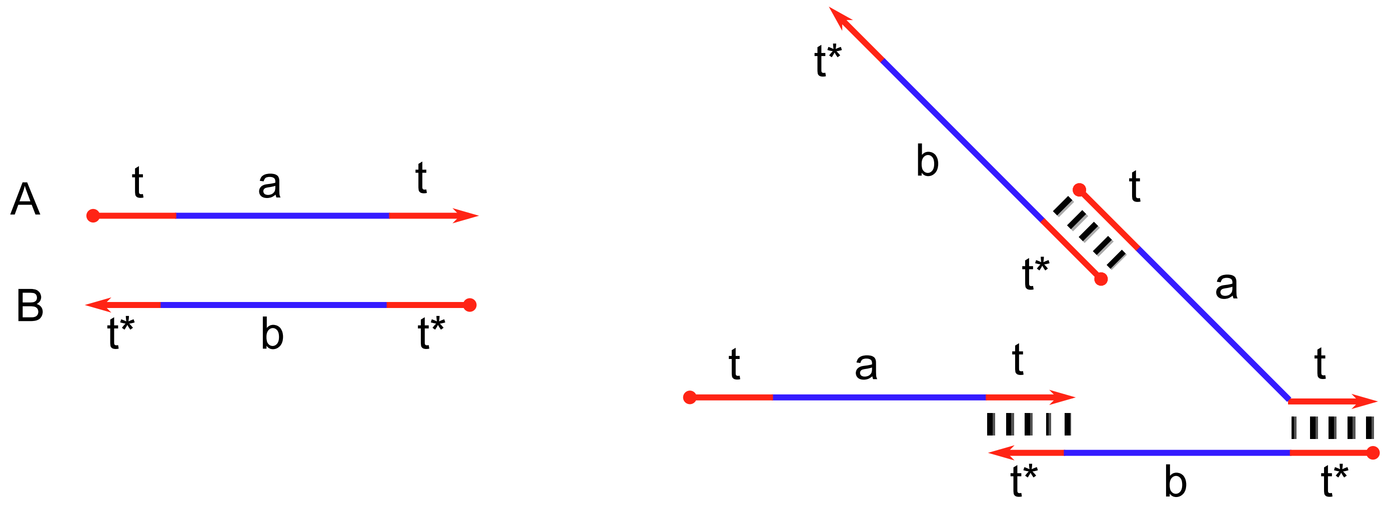
A
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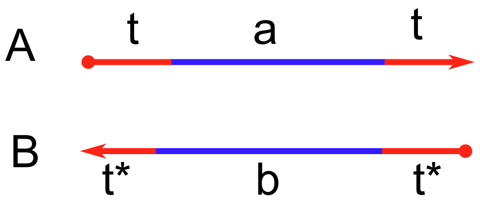
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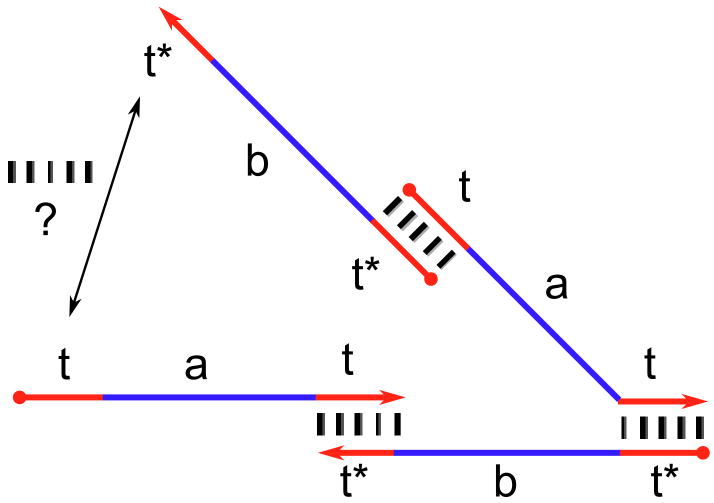
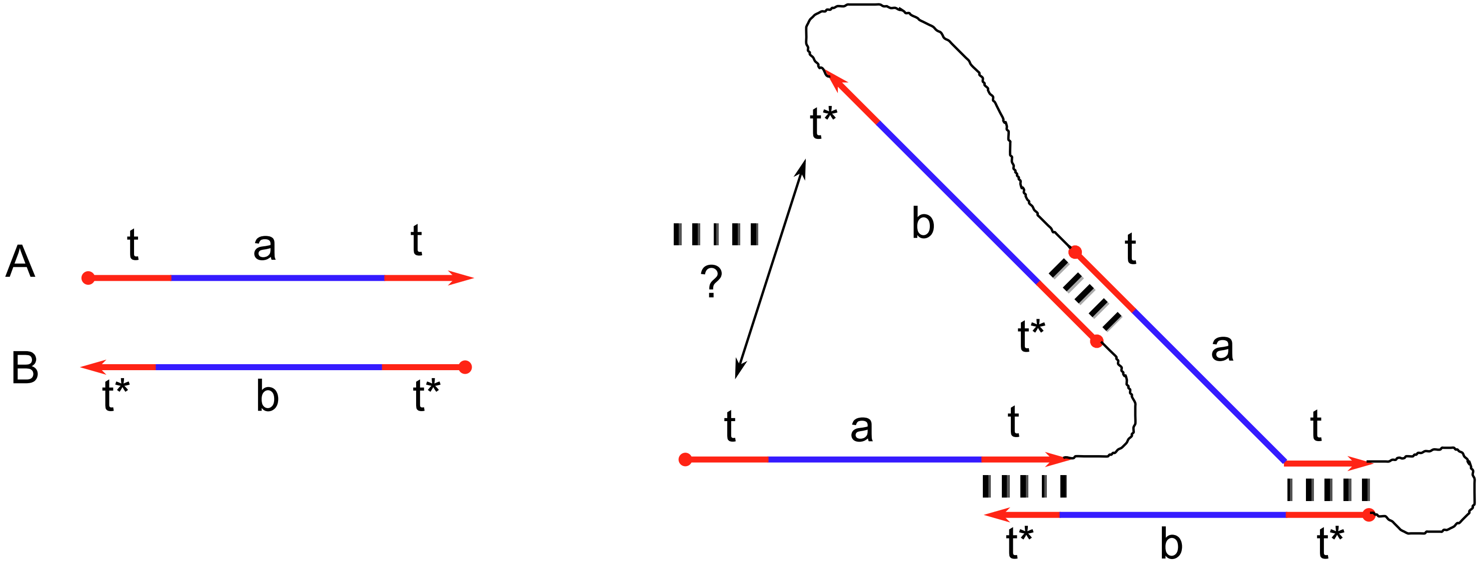
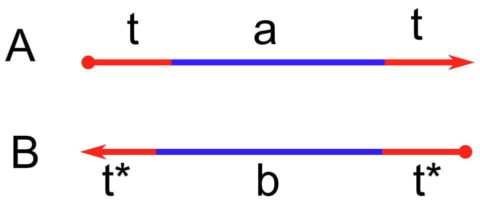

t*
b
?
t
t*
a
t
a
t
t
t*
b
t*

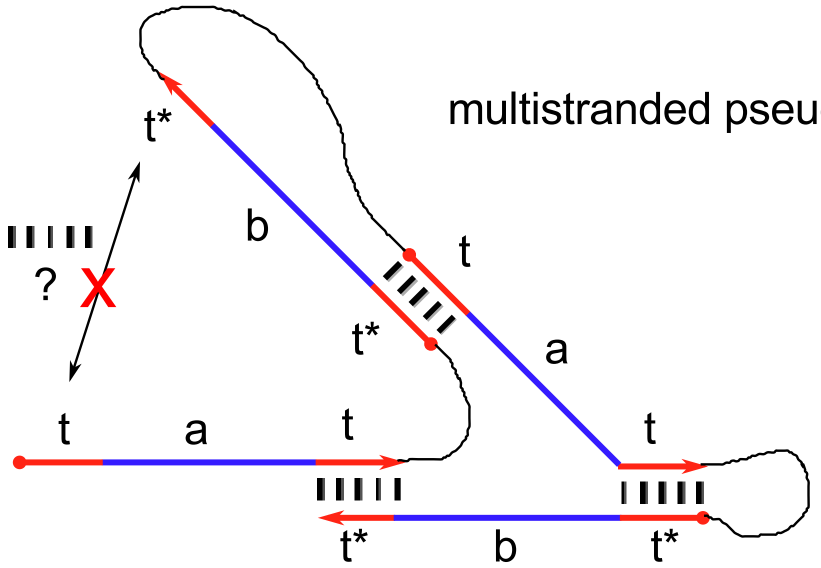


`t a t + t* b t* + t a t + t* b t*`

`. . ( + (  . .  + ) . ( + )  . )`

multistranded pseudoknot

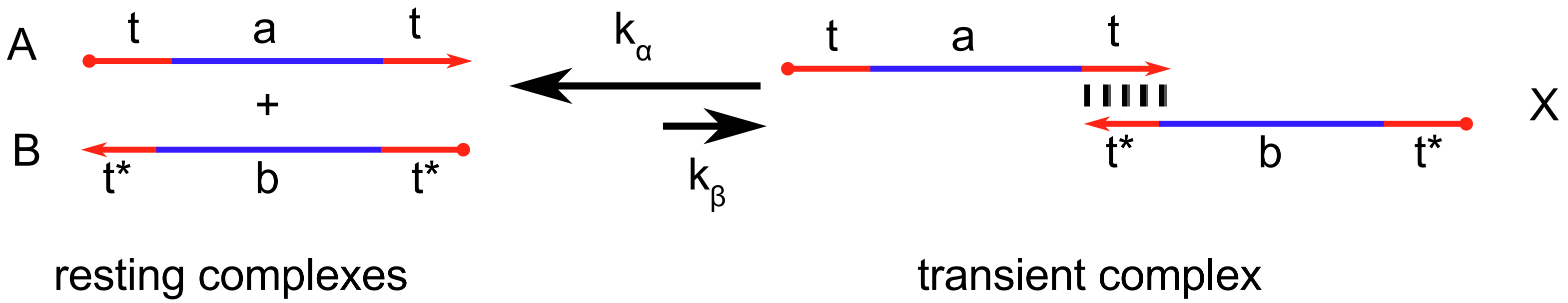





```
t a t + t* b t* + t a t + t* b t*
. . ( + (  . .  + ) . ( + )  . )
```

SEPARATION OF TIMESCALES

unimolecular reactions are fast
bimolecular reactions are slow



$$\{X \xrightarrow{k_\alpha} A + B;\ A + B \xrightarrow{k_\beta} X\}$$

at low concentrations:

$$k_\beta[A][B] << k_\alpha[X]$$

MODEL PARAMETERS

rate-independent model

open reactions where domain-length $> L$ are negligible

unimolecular reactions are fast

bimolecular reactions are slow

rate-dependent model

assume typical rate constant for every reaction:

k = rate(rtype, dlength)

unimolecular reactions with $k < k_{\text{slow}}$ are negligible

unimolecular reactions with $k < k_{\text{fast}}$ are slow

unimolecular reactions with $k \geq k_{\text{fast}}$ are fast

bimolecular reactions are slow

REACTION ENUMERATION

- every complex has all valid fast reactions enumerated
- transient complexes have no slow reactions enumerated
- resting complexes have all valid slow reactions enumerated
- all initial complexes are included

valid according to enumeration semantics:

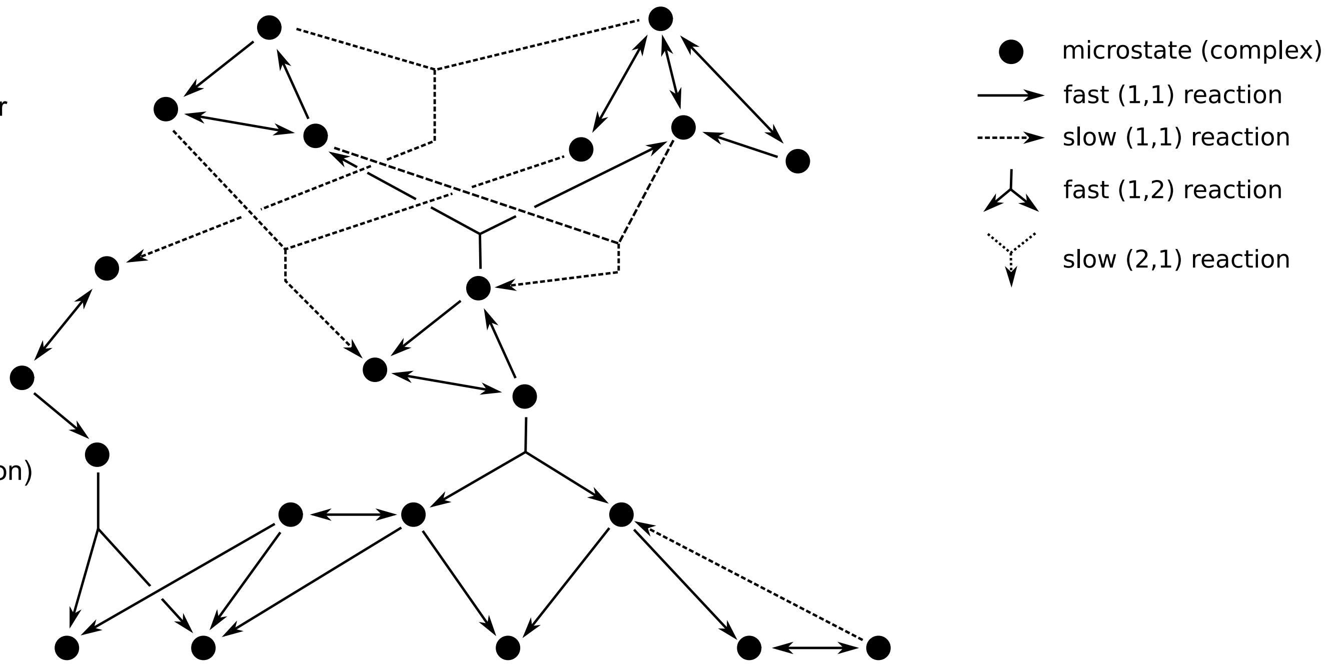
- all valid, except open $> L$
- max-helix semantics: reaction types are greedy
- probability threshold for reactants of bimolecular reactions.
- probability threshold for products of unimolecular reactions.

CRN CONDENSATION

Goal: represent CRN in terms of overall slow reactions

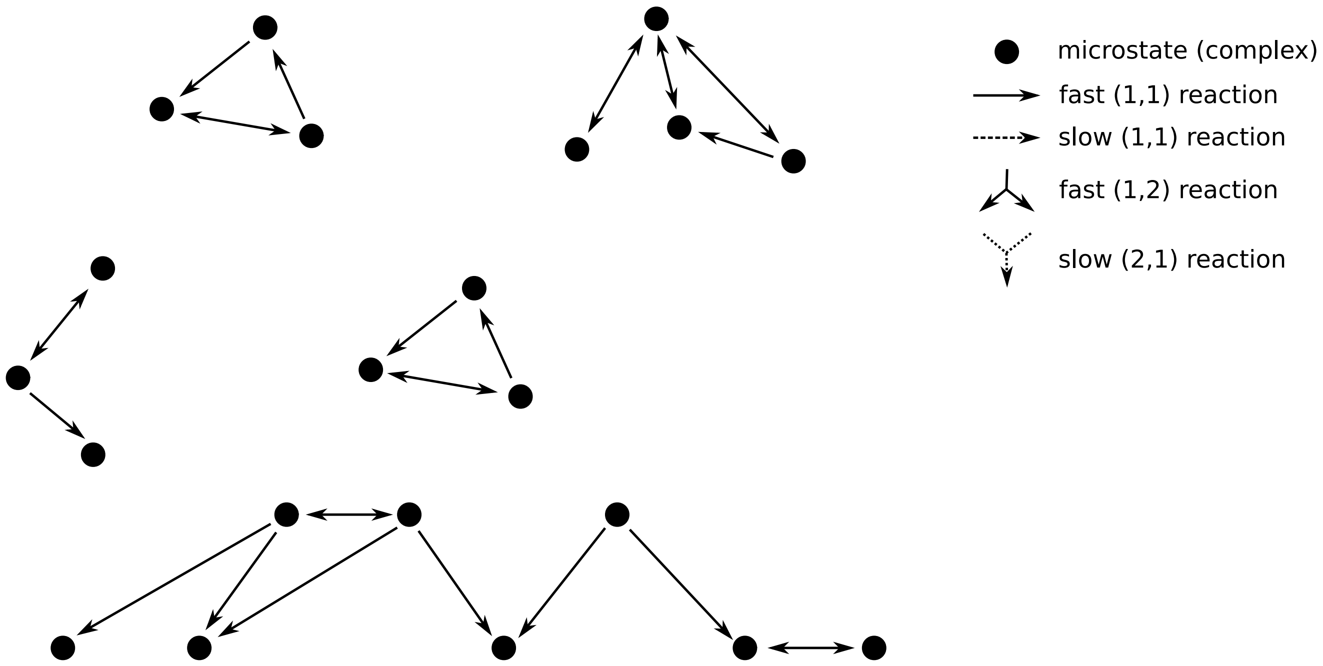
properties / requirements:

- all fast reactions are unimolecular
- reactions have arity (n,m) with n > 0 and m > 0
- reactants of slow reactions must be resting states
- reactants and products of fast (1-2) reactions are in different SCCs (mass conservation)



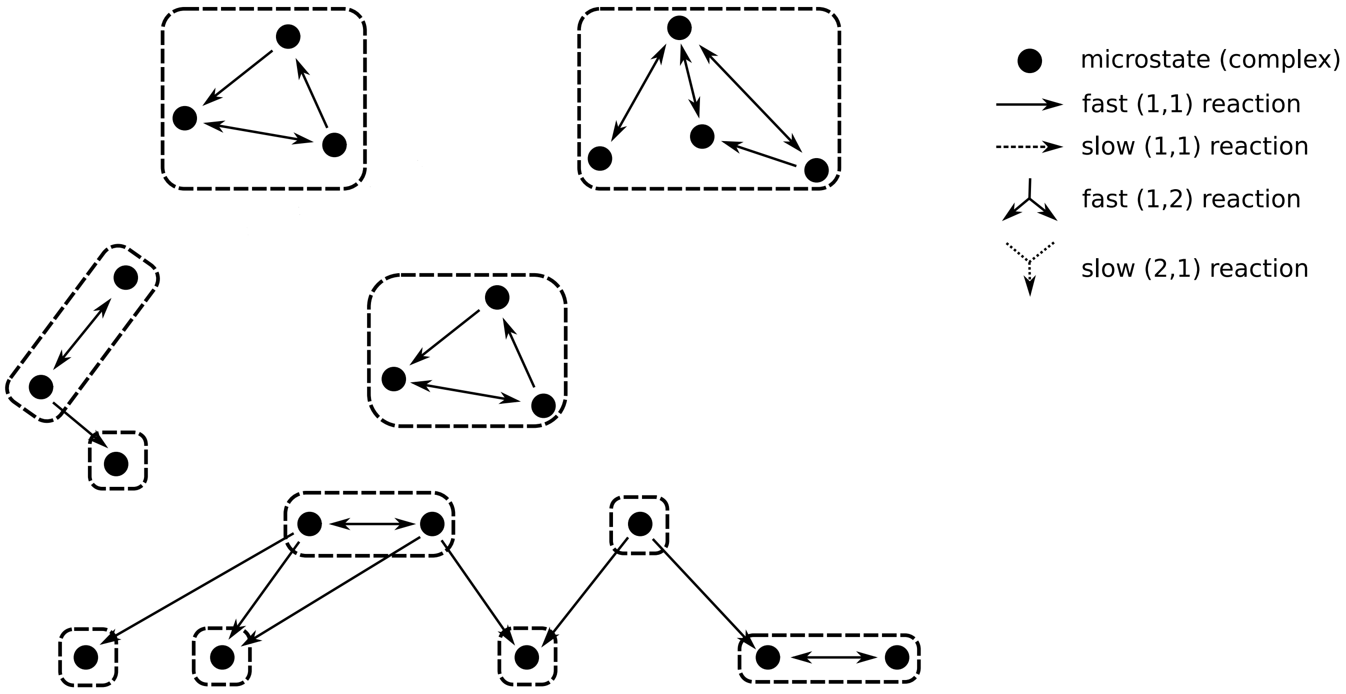
CRN CONDENSATION

Step 1: Make a graph that contains only fast (1,1) reactions



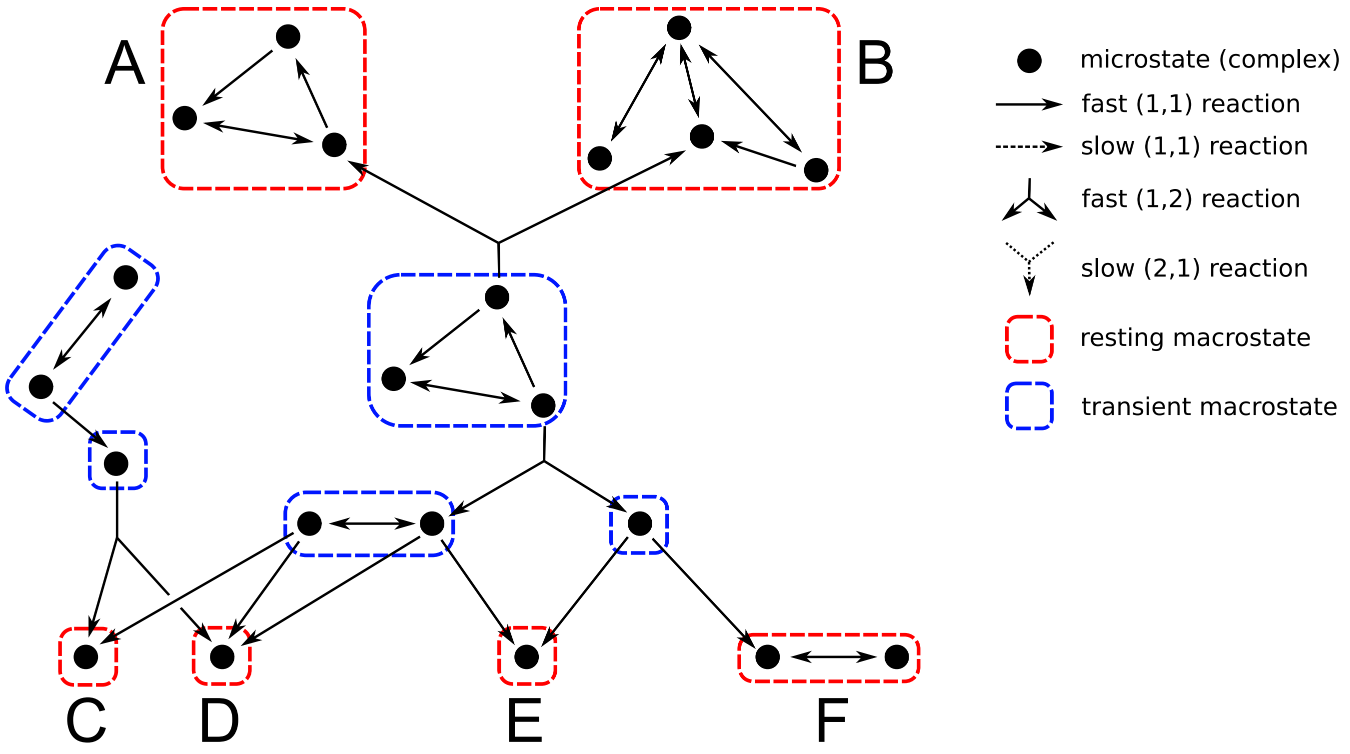
CRN CONDENSATION

Step 2: Identify strongly connected components (SCCs)



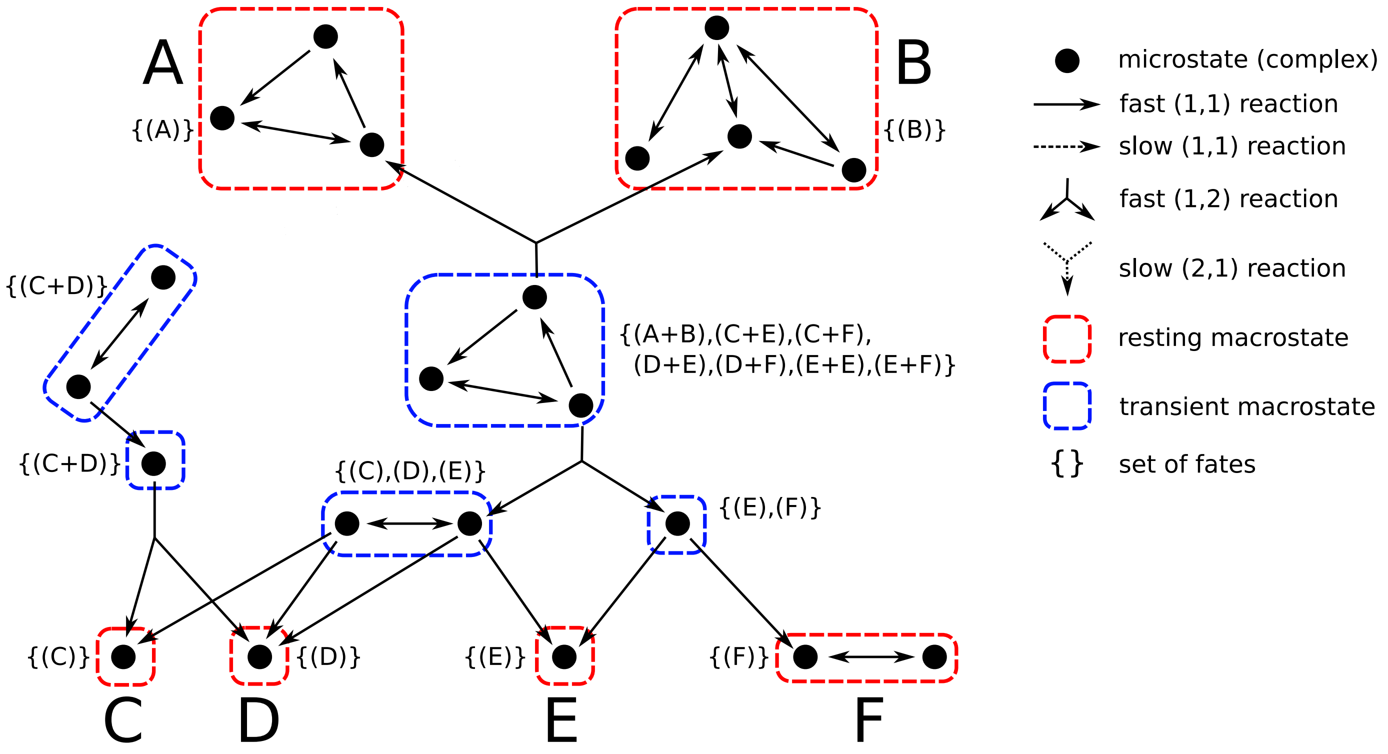
CRN CONDENSATION

Step 3: Define transient and resting macrostates



CRN CONDENSATION

Step 4: Assign fates to complexes (or macrostates)

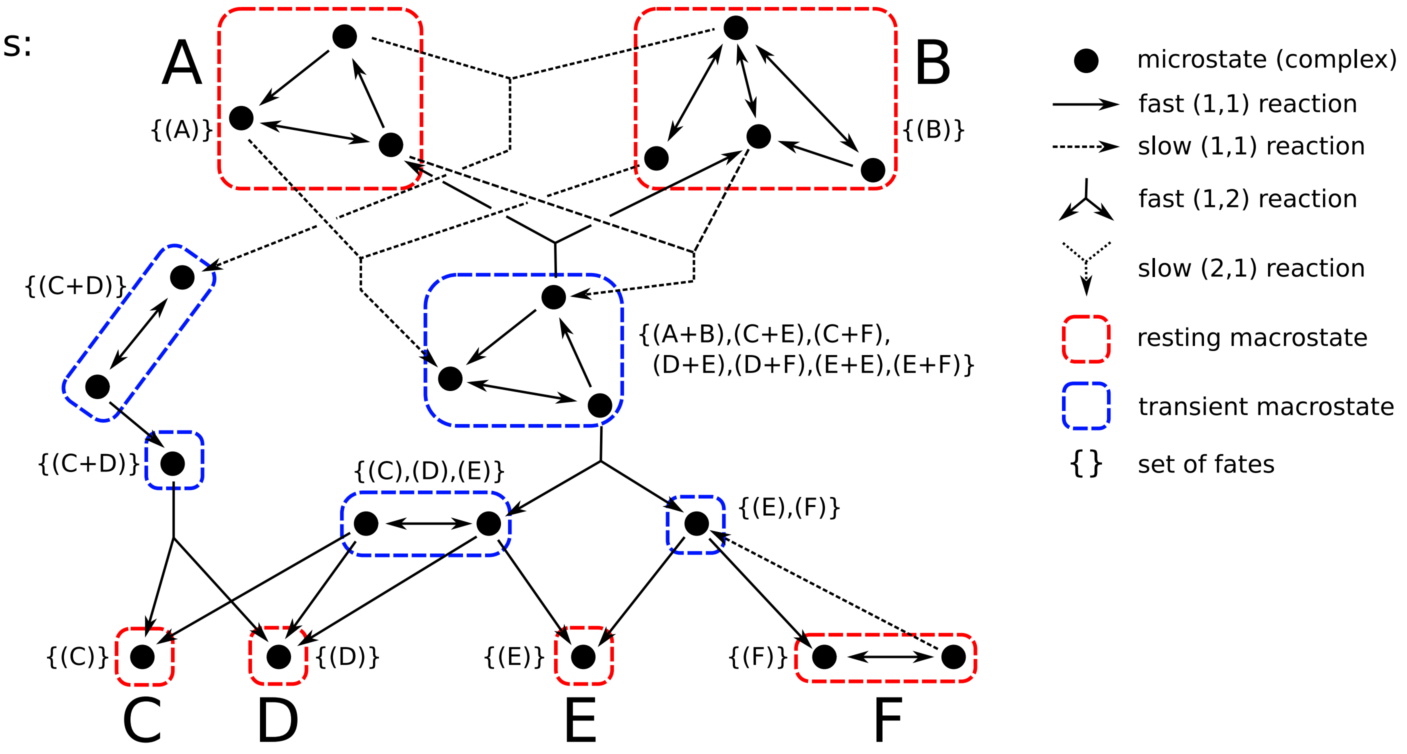


CRN CONDENSATION

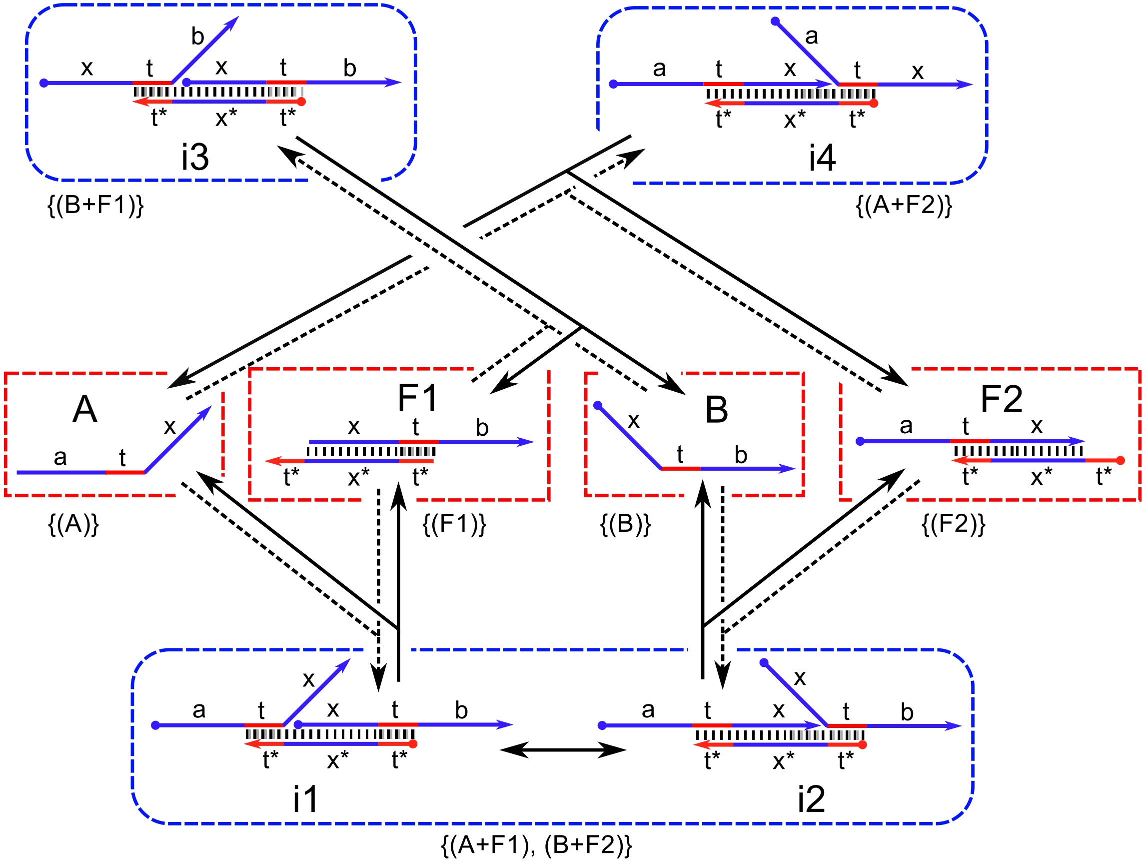
Step 5: Insert slow reactions & derive condensed reactions

condensed reactions:

```
A+B  -> A+B
A+B  -> C+D
A+B  -> C+E
A+B  -> C+F
A+B  -> D+E
A+B  -> D+F
A+B  -> E+E
A+B  -> E+F
F  -> F
F  -> E
```



DSD CONDENSATION



- fast (1,1) reaction
- fast (1,2) reaction
- slow (2,1) reaction
- resting macrostate
- transient macrostate
- {} set of fates

detailed reactions:

```
A + F1 -> i1
i1 -> i2
i2 -> B + F2
B + F2 -> i2
i2 -> i1
i1 -> A + F1
A + F2 -> i4
i4 -> A + F2
B + F1 -> i3
i3 -> B + F1
```

condensed reactions:

```
A + F1 -> B + F2
B + F2 -> A + F1
```

REACTION RATE CONDENSATION

Consider a condensed reaction:

$$P + Q \to K + L + M$$

It is composed of all detailed slow reactions:

$$p + q \to I$$

weighted by the decay probability over all pathways:

$$I \to \cdots \to k + l + m$$

where $p \in P, q \in Q, k \in K, l \in L, m \in M$
and I is a multiset of intermediate species

REACTION RATE CONDENSATION

Notation:

detailed reaction: $r = (A, B) \qquad A = \{|a_i|\}$

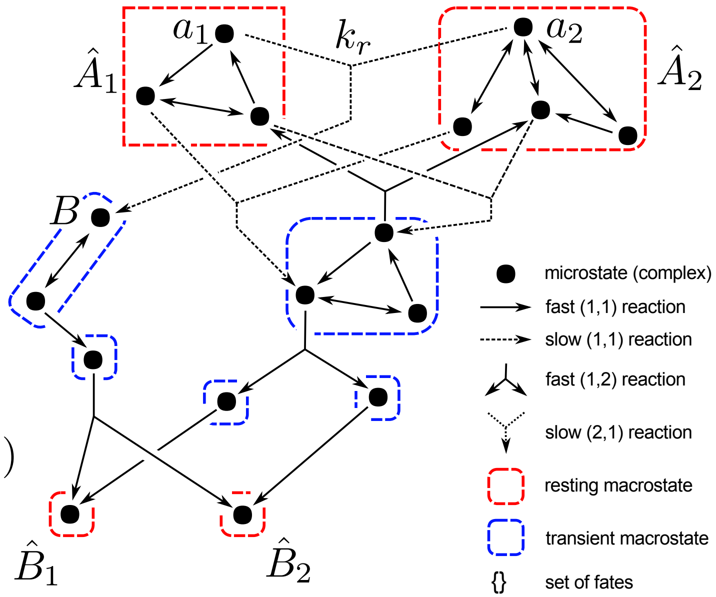
condensed reaction: $\hat{r} = (\hat{A}, \hat{B}) \qquad \hat{A} = \{|\hat{A}_i|\}$

given: $\hat{A} = (\hat{A}_1, \hat{A}_2) \quad \hat{B} = (\hat{B}_1, \hat{B}_2)$

define: $R_{\hat{A}} = \{r = ((a_1, a_2), B) : a_1 \in \hat{A}_1, a_2 \in \hat{A}_2\}$

then the condensed rate is:

$$k_{\hat{r}} = \sum_{r=((a_1,a_2),B)\in R_{\hat{A}}} P(a_1|\hat{A}_1) \cdot P(a_2|\hat{A}_2) \cdot k_r \cdot P(T_{B\to\hat{B}})$$



REACTION RATE CONDENSATION

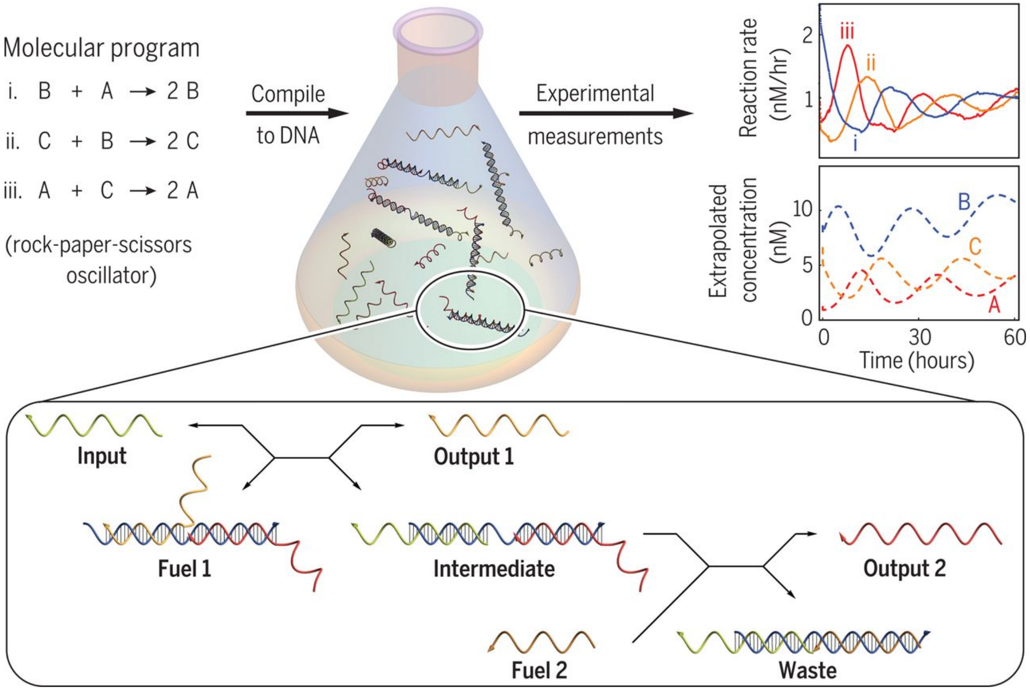
general form:

$$k_{\hat{r}} = \sum_{r=(A,B)\in R_{\hat{A}}} k_r \cdot \mathbb{P}[T_{B\to\hat{B}}] \cdot \prod_{a_i\in A} \mathbb{P}[a_i : \hat{A}_i]$$

where

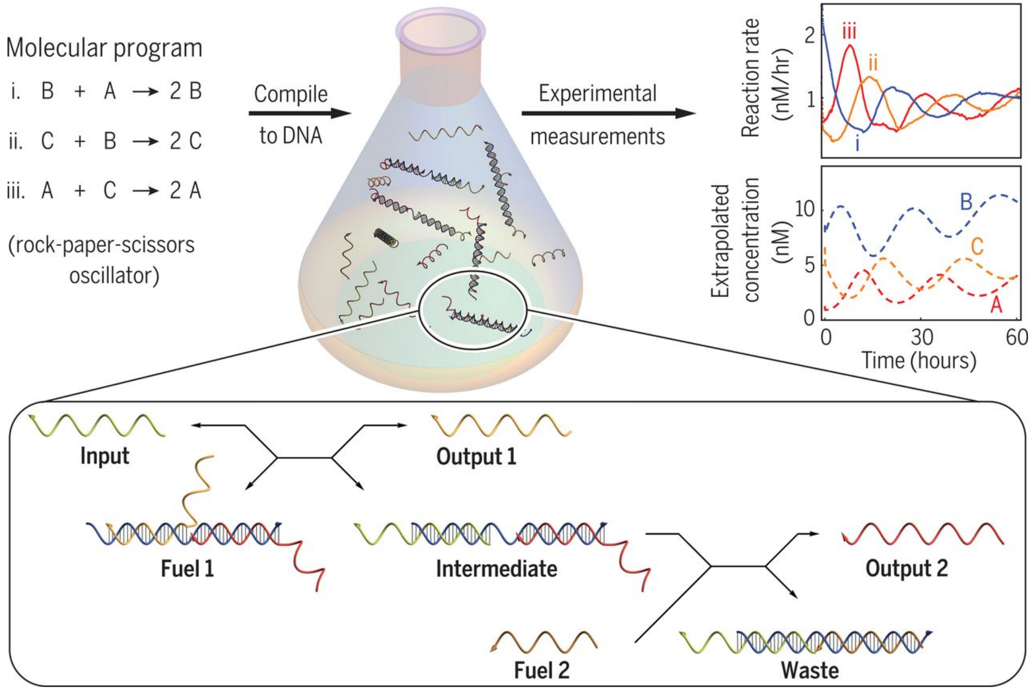
$\mathbb{P}[a_i : \hat{A}_i] =$ stationary distribution
$\mathbb{P}[T_{B\to\hat{B}}] =$ reaction decay probability

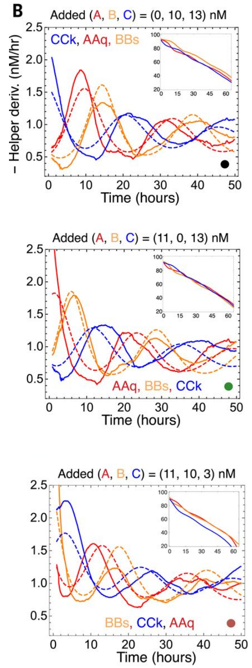
A DNA OSCIALLATOR



Srinivas, Parkin, Seelig, Winfree, Soloveichik:
Enzyme-free nucleic acid dynamical systems. Science (2017)

A DNA OSCIALLATOR





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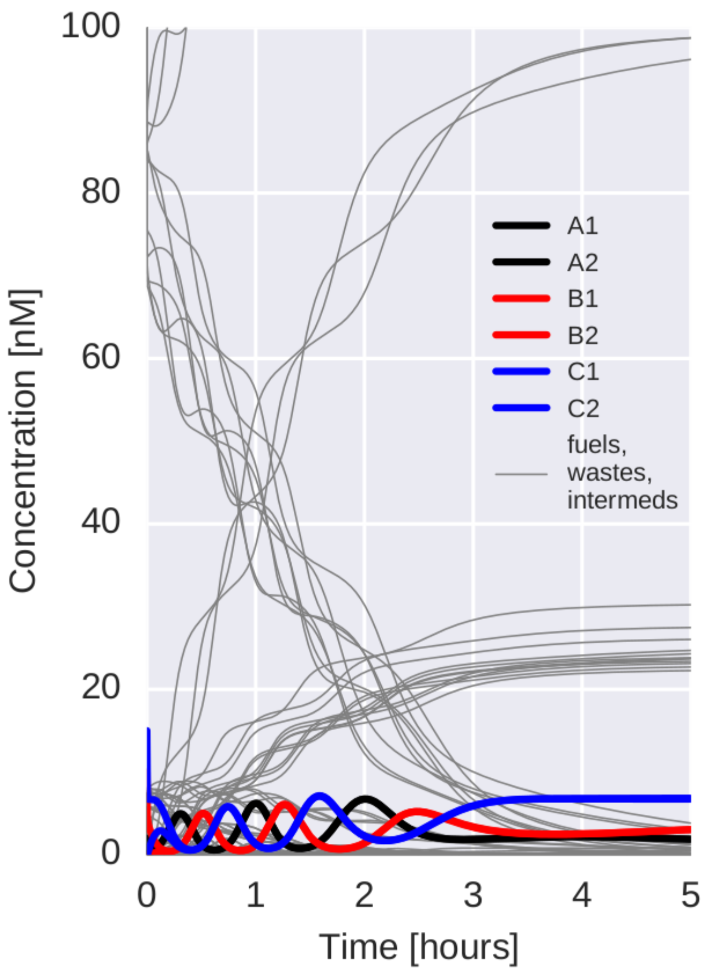
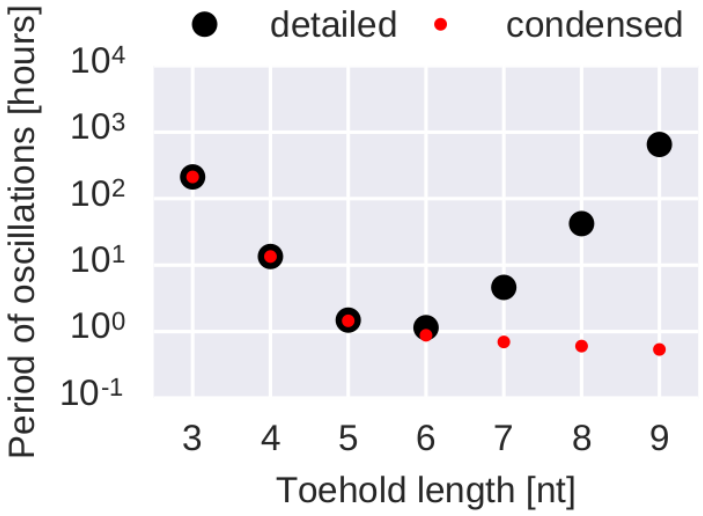
DETAILED VS. CONDENSED SIMULATION



$$A + B \to B + B$$
$$B + C \to C + C$$
$$C + A \to A + A$$



translation scheme: srinivas2017.ts

REACTION ENUMERATOR

model limitations

- no multistranded pseudoknots
- assumption of low concentrations
  - assumption of "typical" reaction rate constants

model parameters

- multiple layers of reaction-semantics
  - reaction types
  - max-helix notion (representation-independent)
  - reaction rate dependent enumeration

What the domain level can do:

- enumerate intended reaction pathways
- detect unintended reaction pathways
- **very fast** assessment of overall dynamics
- define a CRN for sequence-level simulations

What the domain level cannot do:

- include sequence-level variations within the domains

What the domain level could do:

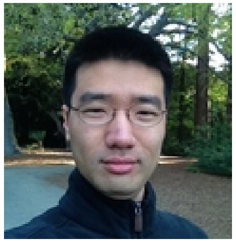
- detect and quantify particular leak reactions
- provide a coarse-graining for stochastic simulations

THANKS TO

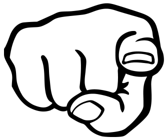
Erik Winfree

Casey Grun

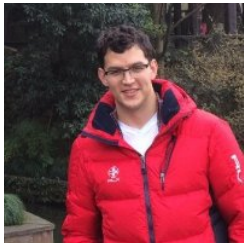
Karthik Sarma



Seung Woo Shin



you



Brian Wolfe

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

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