A domain-level DNA strand displacement reaction enumerator allowing arbitrary non-pseudoknotted secondary structures

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Supplemental Online Material

1 Reaction enumeration algorithm details

The reaction enumeration algorithm works by passing complexes through a progression of several mutable sets; as new complexes are enumerated, they are added to one of these sets, then eventually removed and transferred to a later set. All complexes accumulate in either $T$ (transient complexes) or $E$ (resting complexes). This progression enforces the requirement that each complex be classified as a resting complex or transient complex, that all fast reactions are enumerated before slow reactions, and that complexes are not enumerated more than once. For simplicity, we assume an operation $\text{Pop}(S)$ exists that removes and returns some element from the mutable set $S$. In Alg. 1 we provide pseudocode for the reaction enumeration algorithm.

- $B$ contains complexes that have had no reactions enumerated yet. Complexes are moved out of $B$ into $F$ when their neighborhood is considered. We define the neighborhood of a complex $c$ to be the set of complexes that can be produced by a series of zero or more fast reactions starting from complex $c$.
- $F$ contains complexes in the current neighborhood which have not yet had fast reactions enumerated. These complexes will be moved to $N$ once their fast reactions have been enumerated.
- $N$ contains complexes enumerated within the current neighborhood, but that have not yet been characterized as transient or resting complexes. Each of these complexes is classified, then moved into $S$ or $T$.
- $S$ contains resting complexes which have not yet had bimolecular reactions with set $E$ enumerated yet. All self-interactions for these complexes have been enumerated.
- $E$ contains enumerated resting complexes. Only cross-reactions with other end states need to be considered for these complexes. These complexes will remain in this list throughout function execution.
- $T$ contains transient complexes which have had their fast reactions enumerated. These complexes will remain in this list throughout function execution.

Additionally, two other sets are accumulated over the course of the enumeration:

- $R$ contains all reactions that have been enumerated.
- $Q$ contains all resting macrostates that have been enumerated.

1.1 Terminating conditions

Although our enumerator is designed to avoid enumerating implausible polymerization reactions, as described in Fig. 2, it is possible to enumerate systems which result in genuine polymerization, such as those described by $\text{[1,2]}$. To allow such enumerations to terminate, our enumerator places a soft limit on the maximum number of complexes and the maximum number of reactions that can be enumerated before the enumerator will exit. These limits are checked before the neighborhood of fast reactions is enumerated, for each complex in $B$. The limits are configurable by the user.

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If the number of complexes in $\mathcal{E} \cup \mathcal{S} \cup \mathcal{T}$ is greater than the maximum number of complexes, or the number of reactions in $\mathcal{R}$ is greater than the configured maximum, the partially-enumerated network is “cleaned up” by deleting all reactions in $\mathcal{R}$ that produce complex(es) leftover in $\mathcal{B}$. That is, no complex will be reported in the output that has not had its neighborhood of fast reactions enumerated exhaustively. Evaluating the limits only between consideration of each neighborhood (rather than during) prevents the pathological mis-classification of resting and transient complexes, but means the limit may be exceeded if the last neighborhood considered is large.
1.2 Pseudocode

Algorithm 1 Reaction enumeration

1: procedure Enumerate(A : {Complex})  \(\triangleright\) Complexes
2: \(E \leftarrow \{\} ; S \leftarrow \{\} ; T \leftarrow \{\}\)  \(\triangleright\) Reactions
3: \(Q \leftarrow \{\}\)  \(\triangleright\) Resting macrostates
4: \(B \leftarrow A\)
5: while \(B \neq \{\}\) do  \(\triangleright\) Enumerate fast reactions from \(A\)
6: \(b \leftarrow \text{pop}(B)\)
7: \((S', T', Q', R') \leftarrow \text{enumerateNeighborhood}(b)\)  \(\triangleright\) Find fast reactions from \(b\)
8: \(S \leftarrow S \cup S'; T \leftarrow T \cup T'; R \leftarrow R \cup R'; Q \leftarrow Q \cup Q'\)
9: end while
10: while \(S \neq \{\}\) do  \(\triangleright\) Enumerate slow reactions between resting complexes
11: \(s \leftarrow \text{pop}(S)\)
12: \((R', B') \leftarrow \text{getSlowReactions}(s, S \cup E)\)  \(\triangleright\) Find slow reactions from \(s\)
13: \(E \leftarrow E \cup \{s\}\)  \(\triangleright\) \(s\) moves to \(E\) once slow reactions are enumerated
14: \(B \leftarrow B \cup B' \setminus (E \cup S \cup T)\)  \(\triangleright\) Store new complexes
15: \(B \leftarrow B \cup B' \setminus (E \cup S \cup T)\)  \(\triangleright\) Store new complexes
16: \(S \leftarrow S \cup S'; T \leftarrow T \cup T'; R \leftarrow R \cup R'; Q \leftarrow Q \cup Q'\)
17: end while
18: end while
19: end procedure

procedure enumerateNeighborhood(c : Complex)  \(\triangleright\) Calculates fast reactions from \(c\), sorts complexes into resting/transient complexes
20: \(F \leftarrow \{c\}\)  \(\triangleright\) Complexes from fast reactions in neighborhood
21: \(N \leftarrow \{\}\)  \(\triangleright\) Complexes in neighborhood
22: \(R_N \leftarrow \{\}\)  \(\triangleright\) (Fast) Reactions in neighborhood
23: while \(F \neq \{\}\) do  \(\triangleright\) Enumerate fast reactions from each complex in \(F\)
24: \(f \leftarrow \text{pop}(F)\)
25: \((R', F') \leftarrow \text{getFastReactions}(f)\)  \(\triangleright\) Find fast reactions from \(f\)
26: \(F \leftarrow F \setminus F'\)
27: \(N \leftarrow N \cup F'\)
28: \(R_N \leftarrow R_N \cup F'\)
29: end while
30: \(N' \leftarrow \text{Apply Tarjan’s algorithm}^{[3]}\) to find strongly-connected components of the directed graph \(G = (N', R_N)\)  \(\triangleright\) Resting macrostates are SCCs of \(G\)
31: \(Q' \leftarrow \{\text{strongly-connected components of } G \text{ with no outgoing fast reactions}\}\)  \(\triangleright\) Resting complexes are in a resting macrostate
32: \(S' \leftarrow \{s : s \in q \text{ for any } q \in Q'\}\)  \(\triangleright\) Transient complexes are everything else
33: \(T' \leftarrow N \setminus S'\)
34: \(R_N \leftarrow R_N \cup R_N\)
35: return \((S', T', Q', R_N)\)
36: end procedure

procedure getFastReactions(c : Complex)  \(\triangleright\) Calculates all fast (unimolecular) reactions that consume \(c\)
37: \(R \leftarrow \text{fast reactions consuming } c, C \leftarrow \text{union of products of reactions in } R\)
38: return \(R, C\)
39: end procedure

procedure getSlowReactions(c : Complex, S : {Complex})  \(\triangleright\) Calculates all slow (bimolecular) reactions that consume \(c\) and an element of \(S\)
40: \(R \leftarrow \text{slow reactions consuming } c \text{ and } s \in S, C \leftarrow \text{union of products of reactions in } R\)
41: return \(R, C\)
42: end procedure
**Algorithm 2** Reaction network condensation

1: \( F_x \leftarrow \text{undefined} \forall \text{complexes } x \) \( \triangleright \) The map \( F : \text{Complex} \rightarrow \{ \text{Fate} \} \) is global and begins empty

2: \( S \leftarrow \{ \} \) \( \triangleright \) The map \( S : \text{Complex} \rightarrow \{ \text{Complex} \} \) is global and begins empty

3: **procedure** CONDENSE\((C : \{ \text{Complex} \}, \mathcal{R} : \{ \text{Reaction} \})\) \( \triangleright \) Computes the fates for each complex, then generates a set of condensed reactions

4: \( R_s \leftarrow \{ r : r \in \mathcal{R}, r \text{ is slow} \} \) \( \triangleright \) Slow reactions

5: \( R_f \leftarrow \mathcal{R} \setminus R_s \) \( \triangleright \) Fast reactions

6: \( R_{f}^{(1,1)} \leftarrow \{ r \in R_f : \alpha(r) = (1, 1) \} \) \( \triangleright \) Fast \((1,1)\) reactions

7: Use Tarjan’s algorithm \([3]\) to compute the set of strongly-connected components from the graph \( \Gamma = (C, \mathcal{R}_{f}^{(1,1)}) \)

8: \( S \leftarrow \text{the set of strongly-connected components of } \Gamma \)

9: \( \mathcal{S}(x) \leftarrow \text{the strongly-connected component containing complex } x, \forall x \in C \) \( \triangleright \) For each SCC \( \mathcal{C}_c \) of \( \Gamma \)

10: for all \( C_c \in S \) do

11: computeFates\((C_c, \mathcal{R}_f)\)

12: end for

13: return condenseReactions\((R_s)\)

14: **end procedure**

15: **procedure** computeFates\((C : \{ \text{Complex} \}, \mathcal{R}_f : \{ \text{Reaction} \})\)

16: \( R_o \leftarrow \{ r = (A,B) \in \mathcal{R}_f : A \subseteq C, B \setminus C = \emptyset \} \) \( \triangleright \) Outgoing fast reactions

17: if \( |R_o| = 0 \) then

18: \( F(c) \leftarrow \{ |C| \} \forall c \in C \) \( \triangleright \) \( F(c) \) is the resting macrostate \( C \) containing the complex \( c \)

19: else

20: for all \( c \in C \) do

21: \( R_o(1,n) \leftarrow \{ r \in R_o : \alpha(r) = (1, n) \} \)

22: \( P_n \leftarrow \bigcup_{r=(A,B) \in R_{f}^{(1,1)}} B \)

23: for all \( x \in P_n \) do

24: If \( F(x) \) is undefined, computeFates\((\mathcal{S}(x), \mathcal{R}_f)\)

25: end for

26: \( F(c) \leftarrow \bigcup_{r=(A,B) \in R_o} (\bigoplus_{b \in B} F(b)) \) \( \triangleright \) \( F(c) \) are the possible fates from outgoing reactions

27: end for

28: end if

29: **end procedure**

30: **procedure** condenseReactions\((\mathcal{R}_s : \{ \text{Reaction} \})\) \( \triangleright \) Condensed reaction space from the set of slow reactions

31: \( \hat{\mathcal{R}} \leftarrow \{ \} \) \( \triangleright \) Condensed reactions

32: for all \( s = (A,b) \in \mathcal{R}_s \) do

33: \( A' \leftarrow \sum_{a \in A} F(a) \) \( \triangleright \) Fates of reactants are all trivial

34: for all \( B' \in F(b) \) do

35: \( r' \leftarrow (A', B') \) \( \triangleright \) For each fate of \( b \)

36: \( \hat{\mathcal{R}} \leftarrow \hat{\mathcal{R}} \cup r' \) \( \triangleright \) Generate new reaction

37: end for

38: end for

39: return \( \hat{\mathcal{R}} \)

40: **end procedure**
## 2 Case study raw data

| Input Filename | (n, m) | Reaction | Semantics | Rate (calculated) | Rate (experiment) |
|---------------|--------|----------|-----------|-------------------|-------------------|
| Zhang2009-3way-00 | (1, 14) | $S + X \rightarrow L + Y$ condensed | | 11.891 | 8.170 |
| Zhang2009-3way-01 | (2, 13) | $S + X \rightarrow L + Y$ condensed | | 209.564 | 144.000 |
| Zhang2009-3way-02 | (3, 12) | $S + X \rightarrow L + Y$ condensed | | 3680.666 | 1080.000 |
| Zhang2009-3way-03 | (4, 11) | $S + X \rightarrow L + Y$ condensed | | 61798.887 | 50500.000 |
| Zhang2009-3way-04 | (5, 10) | $S + X \rightarrow L + Y$ condensed | | 650503.785 | 964000.000 |
| Zhang2009-3way-05 | (6, 9) | $S + X \rightarrow L + Y$ condensed | | 1653058.538 | 2360000.000 |
| Zhang2009-3way-06 | (7, 8) | $S + X \rightarrow L + Y$ condensed | | 2087718.874 | 3220000.000 |
| Zhang2009-3way-07 | (8, 7) | $S + X \rightarrow L + Y$ condensed | | 2400000.000 | 3150000.000 |
| Zhang2009-3way-08 | (9, 6) | $S + X \rightarrow L + Y$ condensed | | 2700000.000 | 2770000.000 |
| Zhang2009-3way-09 | (10, 5) | $S + X \rightarrow L + Y$ condensed | | 3000000.000 | 2830000.000 |
| Zhang2009-3way-10 | (15, 0) | $S + X \rightarrow L + Y$ condensed | | 4500000.000 | 4780000.000 |

Table 1: Data for Fig. 7a. Zhang and Winfree (2009) – 3-way strand displacement
| Input Filename | (n, m) | Reaction | Semantics | Rate (calculated) | Rate (experiment) |
|----------------|--------|----------|------------|-------------------|-------------------|
| Zhang2009-3wayX-00 | (1, 4) | S + X → L + Y | condensed | 13.919 | 7.700 |
| Zhang2009-3wayX-01 | (1, 3) | S + X → L + Y | condensed | 13.922 | 5.480 |
| Zhang2009-3wayX-02 | (1, 2) | S + X → L + Y | condensed | 13.207 | 23.500 |
| Zhang2009-3wayX-03 | (1, 1) | S + X → L + Y | condensed | 12.516 | 18.900 |
| Zhang2009-3wayX-04 | (2, 5) | S + X → L + Y | condensed | 138.274 | 43.600 |
| Zhang2009-3wayX-05 | (2, 4) | S + X → L + Y | condensed | 245.292 | 214.050 |
| Zhang2009-3wayX-06 | (2, 3) | S + X → L + Y | condensed | 245.346 | 273.000 |
| Zhang2009-3wayX-07 | (2, 2) | S + X → L + Y | condensed | 232.750 | 249.000 |
| Zhang2009-3wayX-08 | (2, 1) | S + X → L + Y | condensed | 220.581 | 231.000 |
| Zhang2009-3wayX-09 | (3, 6) | S + X → L + Y | condensed | 309.170 | 66.900 |
| Zhang2009-3wayX-10 | (3, 5) | S + X → L + Y | condensed | 2431.660 | 215.000 |
| Zhang2009-3wayX-11 | (3, 4) | S + X → L + Y | condensed | 4305.421 | 939.000 |
| Zhang2009-3wayX-12 | (3, 3) | S + X → L + Y | condensed | 4306.368 | 974.000 |
| Zhang2009-3wayX-13 | (3, 2) | S + X → L + Y | condensed | 4086.192 | 846.000 |
| Zhang2009-3wayX-14 | (3, 1) | S + X → L + Y | condensed | 3873.391 | 231.000 |
| Zhang2009-3wayX-15 | (4, 7) | S + X → L + Y | condensed | 381.692 | 131.000 |
| Zhang2009-3wayX-16 | (4, 6) | S + X → L + Y | condensed | 5427.681 | 407.000 |
| Zhang2009-3wayX-17 | (4, 5) | S + X → L + Y | condensed | 41498.277 | 4250.000 |
| Zhang2009-3wayX-18 | (4, 4) | S + X → L + Y | condensed | 71709.175 | 21300.000 |
| Zhang2009-3wayX-19 | (4, 3) | S + X → L + Y | condensed | 71724.079 | 24100.000 |
| Zhang2009-3wayX-20 | (4, 2) | S + X → L + Y | condensed | 689410.828 | 1580000.000 |
| Zhang2009-3wayX-21 | (4, 1) | S + X → L + Y | condensed | 669445.127 | 1730000.000 |
| Zhang2009-3wayX-22 | (5, 7) | S + X → L + Y | condensed | 111326.440 | 161000.000 |
| Zhang2009-3wayX-23 | (5, 6) | S + X → L + Y | condensed | 90332.748 | 972000.000 |
| Zhang2009-3wayX-24 | (5, 5) | S + X → L + Y | condensed | 503452.200 | 3450000.000 |
| Zhang2009-3wayX-25 | (5, 4) | S + X → L + Y | condensed | 799090.167 | 1530000.000 |
| Zhang2009-3wayX-26 | (5, 3) | S + X → L + Y | condensed | 799091.806 | 1580000.000 |
| Zhang2009-3wayX-27 | (5, 2) | S + X → L + Y | condensed | 689410.828 | 1580000.000 |
| Zhang2009-3wayX-28 | (5, 1) | S + X → L + Y | condensed | 669445.127 | 1730000.000 |
| Zhang2009-3wayX-29 | (6, 7) | S + X → L + Y | condensed | 111326.440 | 161000.000 |
| Zhang2009-3wayX-30 | (6, 6) | S + X → L + Y | condensed | 872844.303 | 4050000.000 |
| Zhang2009-3wayX-31 | (6, 5) | S + X → L + Y | condensed | 1586271.727 | 1480000.000 |
| Zhang2009-3wayX-32 | (6, 4) | S + X → L + Y | condensed | 1672957.506 | 3040000.000 |
| Zhang2009-3wayX-33 | (6, 3) | S + X → L + Y | condensed | 1672983.602 | 2590000.000 |
| Zhang2009-3wayX-34 | (6, 2) | S + X → L + Y | condensed | 1666616.282 | 3000000.000 |
| Zhang2009-3wayX-35 | (6, 1) | S + X → L + Y | condensed | 1047996.408 | 4700000.000 |
| Zhang2009-3wayX-36 | (7, 7) | S + X → L + Y | condensed | 1962075.431 | 1110000.000 |
| Zhang2009-3wayX-37 | (7, 6) | S + X → L + Y | condensed | 2081440.832 | 2900000.000 |
| Zhang2009-3wayX-38 | (7, 5) | S + X → L + Y | condensed | 2089499.345 | 3570000.000 |
| Zhang2009-3wayX-39 | (8, 7) | S + X → L + Y | condensed | 2400000.000 | 1940000.000 |
| Zhang2009-3wayX-40 | (8, 6) | S + X → L + Y | condensed | 2400000.000 | 2680000.000 |
| Zhang2009-3wayX-41 | (8, 5) | S + X → L + Y | condensed | 2400000.000 | 3140000.000 |
| Zhang2009-3wayX-42 | (8, 4) | S + X → L + Y | condensed | 2400000.000 | 3370000.000 |

Table 2: Data for Fig. 7 b. Zhang and Winfree (2009) – 3-way toehold exchange
## Table 3: Data for Fig. 7c. Dabby (2013) – 4-way strand displacement

| Input Filename | Simulation | Reporter Metric | Semantics | Concentration (simulation) | Time (simulation) | Concentration (experiment) | Time (experiment) |
|---------------|------------|-----------------|-----------|-----------------------------|------------------|-----------------------------|------------------|
| Dabby2013-4way-00 | S=6.1 I=660 | T | completion-time condensed, k_{fast}=0.01 | 1.58 | 51.8 | 1.98 | 42 |
| Dabby2013-4way-01 | S=6.1 I=660 | T | completion-time condensed, k_{fast}=0.01 | 1.58 | 248 | 1.98 | 49 |
| Dabby2013-4way-02 | S=6.1 I=660 | T | completion-time condensed, k_{fast}=0.01 | 1.58 | 474 | 1.98 | 91 |
| Dabby2013-4way-03 | S=6.1 I=660 | T | completion-time condensed, k_{fast}=0.01 | 1.58 | 5.2 | 1.98 | 2 |
| Dabby2013-4way-04 | S=6.1 I=660 | T | completion-time condensed, k_{fast}=0.01 | 1.58 | 6.4 | 1.98 | 15 |
| Dabby2013-4way-05 | S=6.1 I=660 | T | completion-time condensed, k_{fast}=0.01 | 1.58 | 5.2 | 1.98 | 15 |
| Dabby2013-4way-06 | S=6.1 I=660 | T | completion-time condensed, k_{fast}=0.01 | 1.58 | 6.4 | 1.98 | 75 |
| Dabby2013-4way-07 | S=6.1 I=660 | T | completion-time condensed, k_{fast}=0.01 | 1.58 | 5.7 | 1.98 | 25 |
| Dabby2013-4way-08 | S=6.1 I=660 | T | completion-time condensed, k_{fast}=0.01 | 1.58 | 1.6 | 1.98 | 75 |
| Dabby2013-4way-09 | S=6.1 I=660 | T | completion-time condensed, k_{fast}=0.01 | 1.58 | 3.4 | 1.98 | 75 |
| Dabby2013-4way-10 | S=6.1 I=660 | T | completion-time condensed, k_{fast}=0.01 | 1.58 | 249 | 1.98 | 51 |
| Dabby2013-4way-11 | S=6.1 I=660 | T | completion-time condensed, k_{fast}=0.01 | 1.58 | 240 | 1.98 | 75 |
| Dabby2013-4way-12 | S=6.1 I=660 | T | completion-time condensed, k_{fast}=0.01 | 1.58 | 252 | 1.98 | 60 |
| Dabby2013-4way-13 | S=6.1 I=660 | T | completion-time condensed, k_{fast}=0.01 | 1.58 | 736 | 1.98 | 91 |
| Dabby2013-4way-14 | S=6.1 I=660 | T | completion-time condensed, k_{fast}=0.01 | 1.58 | 734 | 1.98 | 91 |
| Dabby2013-4way-15 | S=6.1 I=660 | T | completion-time condensed, k_{fast}=0.01 | 1.58 | 732 | 1.98 | 91 |
| Dabby2013-4way-16 | S=6.1 I=660 | T | completion-time condensed, k_{fast}=0.01 | 1.58 | 730 | 1.98 | 91 |
| Dabby2013-4way-17 | S=6.1 I=660 | T | completion-time condensed, k_{fast}=0.01 | 1.58 | 728 | 1.98 | 91 |
| Dabby2013-4way-18 | S=6.1 I=660 | T | completion-time condensed, k_{fast}=0.01 | 1.58 | 727 | 1.98 | 91 |
| Dabby2013-4way-19 | S=6.1 I=660 | T | completion-time condensed, k_{fast}=0.01 | 1.58 | 726 | 1.98 | 91 |

## Table 4: Data for Fig. 7d. Genot et al. (2011) – remote-toehold strand displacement

| Input Filename | Simulation | Reporter Metric | Semantics | Concentration (simulation) | Time (simulation) | Concentration (experiment) | Time (experiment) |
|---------------|------------|-----------------|-----------|-----------------------------|------------------|-----------------------------|------------------|
| Genot2011-SF4A-02 | S=6.6 I=330 | T | completion-time condensed, k_{fast}=0.01 | 2.25 | 10577 | 1.98 | 25 |
| Genot2011-SF4A-01 | S=6.6 I=330 | T | completion-time condensed, k_{fast}=0.01 | 8.49 | 736 | 1.98 | 25 |
| Genot2011-SF4A-00 | S=6.6 I=330 | T | completion-time condensed, k_{fast}=0.01 | 1.98 | 10217 | 1.98 | 25 |
| Genot2011-F4D-00 | S=6.6 I=330 | T | completion-time condensed, k_{fast}=0.01 | 1.98 | 10217 | 1.98 | 25 |
| Genot2011-F4C-00 | S=6.6 I=330 | T | completion-time condensed, k_{fast}=0.01 | 1.98 | 10217 | 1.98 | 25 |
| Genot2011-F4B-00 | S=6.6 I=330 | T | completion-time condensed, k_{fast}=0.01 | 1.98 | 10217 | 1.98 | 25 |
| Genot2011-F4A-00 | S=6.6 I=330 | T | completion-time condensed, k_{fast}=0.01 | 1.98 | 10217 | 1.98 | 25 |

## Table 5: Data for Fig. 8. Kotani & Hughes (2017) – Autocatalytic system

| Input Filename | Simulation | Reporter Metric | Semantics | Concentration (simulation) | Time (simulation) | Concentration (experiment) | Time (experiment) |
|---------------|------------|-----------------|-----------|-----------------------------|------------------|-----------------------------|------------------|
| Kotani2017-F4-00 | S=6.6 I=330 | T | completion-time condensed, k_{fast}=0.01 | 2.25 | 736 | 1.98 | 25 |
| Kotani2017-F4-01 | S=6.6 I=330 | T | completion-time condensed, k_{fast}=0.01 | 8.49 | 736 | 1.98 | 25 |
| Kotani2017-F4-02 | S=6.6 I=330 | T | completion-time condensed, k_{fast}=0.01 | 1.98 | 10217 | 1.98 | 25 |
| Kotani2017-F4-03 | S=6.6 I=330 | T | completion-time condensed, k_{fast}=0.01 | 1.98 | 10217 | 1.98 | 25 |
| Kotani2017-F4-04 | S=6.6 I=330 | T | completion-time condensed, k_{fast}=0.01 | 1.98 | 10217 | 1.98 | 25 |
| Kotani2017-F4-05 | S=6.6 I=330 | T | completion-time condensed, k_{fast}=0.01 | 1.98 | 10217 | 1.98 | 25 |
| Kotani2017-F4-06 | S=6.6 I=330 | T | completion-time condensed, k_{fast}=0.01 | 1.98 | 10217 | 1.98 | 25 |
| Kotani2017-F4-07 | S=6.6 I=330 | T | completion-time condensed, k_{fast}=0.01 | 1.98 | 10217 | 1.98 | 25 |
| Kotani2017-F4-08 | S=6.6 I=330 | T | completion-time condensed, k_{fast}=0.01 | 1.98 | 10217 | 1.98 | 25 |
| Kotani2017-F4-09 | S=6.6 I=330 | T | completion-time condensed, k_{fast}=0.01 | 1.98 | 10217 | 1.98 | 25 |
| Kotani2017-F4-10 | S=6.6 I=330 | T | completion-time condensed, k_{fast}=0.01 | 1.98 | 10217 | 1.98 | 25 |
| Kotani2017-F4-11 | S=6.6 I=330 | T | completion-time condensed, k_{fast}=0.01 | 1.98 | 10217 | 1.98 | 25 |
| Kotani2017-F4-12 | S=6.6 I=330 | T | completion-time condensed, k_{fast}=0.01 | 1.98 | 10217 | 1.98 | 25 |
| Kotani2017-F4-13 | S=6.6 I=330 | T | completion-time condensed, k_{fast}=0.01 | 1.98 | 10217 | 1.98 | 25 |
| Kotani2017-F4-14 | S=6.6 I=330 | T | completion-time condensed, k_{fast}=0.01 | 1.98 | 10217 | 1.98 | 25 |
| Kotani2017-F4-15 | S=6.6 I=330 | T | completion-time condensed, k_{fast}=0.01 | 1.98 | 10217 | 1.98 | 25 |

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A domain-level reaction enumerator. 9
1 diagonal-crossing-time:36000:50 condensed, seesaw-conc=5e-08 47.7 1.66e+03 25 1.44e+04
1 diagonal-crossing-time:36000:50 condensed, seesaw-conc=5e-08 47.7 1.66e+03 25 1.44e+04
1 diagonal-crossing-time:36000:50 condensed, seesaw-conc=5e-08 47.7 1.66e+03 25 1.44e+04
1 diagonal-crossing-time:36000:50 condensed, seesaw-conc=5e-08 47.7 1.66e+03 25 1.44e+04
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0 diagonal-crossing-time:36000:50 condensed, dG
0 diagonal-crossing-time:36000:50 condensed, dG
bp=-1.3 28.7 1.53e+04 30 1.26e+04
1 diagonal-crossing-time:36000:50 condensed, dG
1 diagonal-crossing-time:36000:50 condensed, dG
bp=-1.3 1.14 3.52e+04 5 2.63e+04
1 diagonal-crossing-time:36000:50 see
1 diagonal-crossing-time:36000:50 see
1 diagonal-crossing-time:36000:50 see
1 diagonal-crossing-time:36000:50 see
1 diagonal-crossing-time:36000:50 see
0 diagonal-crossing-time:36000:50 condensed, dG
1 diagonal-crossing-time:36000:50 see
| Y10 | Y11 | Y20 | Y21 |
|-----|-----|-----|-----|
| y20 | y20 | y20 | y20 |
| diagonal-crossing-time:36000:50 | diagonal-crossing-time:36000:50 | diagonal-crossing-time:36000:50 | diagonal-crossing-time:36000:50 |
| seesaw-conc=5e-08 | seesaw-conc=5e-08 | seesaw-conc=5e-08 | seesaw-conc=5e-08 |
| condensed | condensed | condensed | condensed |
| dG_Jp=p-1.3 | dG_Jp=p-1.3 | dG_Jp=p-1.3 | dG_Jp=p-1.3 |
| 38.2 | 8.47e+03 | 30 | 1.08e+04 |
| 1.17 | 3.52e+04 | 5 | 2.45e+04 |
| 1.17 | 3.52e+04 | 5 | 2.45e+04 |
| 1.17 | 3.52e+04 | 5 | 2.45e+04 |
| 1.17 | 3.52e+04 | 5.5 | 2.52e+04 |
| 1.17 | 3.52e+04 | 5.5 | 2.52e+04 |
| 1.17 | 3.52e+04 | 5.5 | 2.52e+04 |
| 0.0122 | 3.6e+04 | 4 | 2.59e+04 |
| 0.0122 | 3.6e+04 | 4 | 2.59e+04 |
| 0.0122 | 3.6e+04 | 4 | 2.59e+04 |
| 0.0246 | 3.6e+04 | 5 | 2.52e+04 |
| 0.0246 | 3.6e+04 | 5 | 2.52e+04 |
| 0.0246 | 3.6e+04 | 5 | 2.52e+04 |
| 0.0246 | 3.6e+04 | 5 | 2.52e+04 |
| 39 | 7.94e+03 | 37.5 | 7.2e+03 |
| 39 | 7.94e+03 | 37.5 | 7.2e+03 |
| 39 | 7.94e+03 | 37.5 | 7.2e+03 |
| 39 | 7.94e+03 | 37.5 | 7.2e+03 |
| 39 | 7.92e+03 | 40 | 6.48e+03 |
| 39 | 7.92e+03 | 40 | 6.48e+03 |
| 39 | 7.92e+03 | 40 | 6.48e+03 |
| 39 | 7.92e+03 | 40 | 6.48e+03 |
| 43.5 | 4.68e+03 | 40 | 7.2e+03 |
| 43.5 | 4.68e+03 | 40 | 7.2e+03 |
| 43.5 | 4.68e+03 | 40 | 7.2e+03 |
| 43.5 | 4.68e+03 | 40 | 7.2e+03 |

Table 6: Data for Fig. 10. Qian & Winfree (2011) – Seesaw systems
| Input Filename | Simulation Reporter | Metric | Metric-values | Semantics | Concentration (simulation) | Time (simulation) | Concentration (experiment) | Time (experiment) |
|---------------|---------------------|--------|---------------|-----------|---------------------------|------------------|-----------------------------|------------------|
| Zhang2007-F1-00 | ROX diagonal-crossing-time | T:800 | C=10 | ROX diagonal-crossing-time | 7.18 | 2.08e+03 | 7.44 | 1.2e+03 |
| Zhang2007-F1-00 | ROX diagonal-crossing-time | T:800 | C=5 | ROX diagonal-crossing-time | 3.29 | 6.3e+03 | 2.45 | 4.1e+03 |
| Zhang2007-F1-00 | ROX diagonal-crossing-time | T:800 | C=1 | ROX diagonal-crossing-time | 1.18 | 1.23e+03 | 1.18 | 2.23e+03 |
| Zhang2007-F1-00 | ROX completion-time | T:800 | C=10 | ROX completion-time | 0.655 | 6.73e+03 | 1.03 | 6.07e+03 |
| Zhang2007-F1-00 | ROX completion-time | T:800 | C=5 | ROX completion-time | 0.211 | 6.05e+03 | 0.17 | 6.38e+03 |
| Zhang2007-F1-00 | ROX completion-time | T:800 | C=1 | ROX completion-time | 0.141 | 7.1e+03 | 0.32 | 4.75e+03 |
| Zhang2007-F1-00 | ROX completion-time | T:800 | C=0.6 | ROX completion-time | 0.5 | 1.47e+03 | 0.95 | 8.95e+03 |
| Zhang2007-F1-00 | ROX completion-time | T:800 | C=0.4 | ROX completion-time | 0.5 | 1.47e+03 | 0.95 | 8.95e+03 |
| Zhang2007-F1-00 | ROX completion-time | T:800 | C=0.2 | ROX completion-time | 0.5 | 1.47e+03 | 0.95 | 8.95e+03 |
| Zhang2007-F1-00 | ROX completion-time | T:800 | C=0.1 | ROX completion-time | 0.5 | 1.47e+03 | 0.95 | 8.95e+03 |
| Zhang2007-F1-00 | ROX completion-time | T:800 | I=20 | ROX completion-time | 0.5 | 1.47e+03 | 0.95 | 8.95e+03 |
| Zhang2007-F1-00 | ROX completion-time | T:800 | I=6 | ROX completion-time | 0.5 | 1.47e+03 | 0.95 | 8.95e+03 |
| Zhang2007-F1-00 | ROX completion-time | T:800 | I=2 | ROX completion-time | 0.5 | 1.47e+03 | 0.95 | 8.95e+03 |
| Zhang2007-F1-00 | ROX completion-time | T:800 | I=1 | ROX completion-time | 0.5 | 1.47e+03 | 0.95 | 8.95e+03 |
| Yin2008-F3-00 | I=20 | TET diagonal-crossing-time | 18000:2;-20 | condensed, k
slow=1e-05, k
fast=0.1 | 2.08 | 89 | 3.3 | 297 |
| Yin2008-F3-00 | I=6 | TET diagonal-crossing-time | 18000:2;-20 | condensed, k
slow=1e-05, k
fast=0.1 | 2.19 | 199 | 5.23 | 2.26e+03 |
| Yin2008-F3-00 | I=2 | TET diagonal-crossing-time | 18000:2;-20 | condensed, k
slow=1e-05, k
fast=0.1 | 2.29 | 297 | 7.37 | 4.41e+03 |
| Yin2008-F3-00 | I=1 | TET diagonal-crossing-time | 18000:2;-20 | condensed, k
slow=1e-05, k
fast=0.1 | 2.35 | 353 | 8.72 | 5.81e+03 |
| Yin2008-F3-00 | I=0.6 | TET diagonal-crossing-time | 18000:2;-20 | condensed, k
slow=1e-05, k
fast=0.1 | 2.36 | 393 | 9.83 | 6.93e+03 |
| Yin2008-F3-00 | I=0.4 | TET diagonal-crossing-time | 18000:2;-20 | condensed, k
slow=1e-05, k
fast=0.1 | 2.4 | 423 | 10.4 | 7.55e+03 |
| Yin2008-F3-00 | I=0.2 | TET diagonal-crossing-time | 18000:2;-20 | condensed, k
slow=1e-05, k
fast=0.1 | 2.47 | 473 | 11.2 | 8.3e+03 |
| Yin2008-F3-00 | I=0.1 | TET diagonal-crossing-time | 18000:2;-20 | condensed, k
slow=1e-05, k
fast=0.1 | 2.49 | 523 | 11.8 | 8.97e+03 |
| Yin2008-F3-00 | I=0.06 | TET diagonal-crossing-time | 18000:2;-20 | condensed, k
slow=1e-05, k
fast=0.1 | 2.53 | 559 | 12.1 | 9.28e+03 |
| Yin2008-F3-00 | I=0.02 | TET diagonal-crossing-time | 18000:2;-20 | condensed, k
slow=1e-05, k
fast=0.1 | 2.6 | 636 | 12.4 | 9.56e+03 |
| Yin2008-F3-00 | I=0.01 | TET diagonal-crossing-time | 18000:2;-20 | condensed, k
slow=1e-05, k
fast=0.1 | 2.66 | 684 | 12.5 | 9.7e+03 |
| Yin2008-F3-00 | I=20 | TET completion-time | -10 | condensed, k
slow=1e-05, k
fast=0.1 | 1.63 | 8.1e+03 | 2.54 | 4.95e+03 |
| Yin2008-F3-00 | I=6 | TET completion-time | -10 | condensed, k
slow=1e-05, k
fast=0.1 | 0.982 | 6.4e+03 | 1.43 | 5.61e+03 |
| Yin2008-F3-00 | I=2 | TET completion-time | -10 | condensed, k
slow=1e-05, k
fast=0.1 | 0.454 | 6.07e+03 | 1.23 | 5.9e+03 |
| Yin2008-F3-00 | I=1 | TET completion-time | -10 | condensed, k
slow=1e-05, k
fast=0.1 | 0.5 | 1.64e+03 | 0.7 | 4.07e+03 |
| Yin2008-F3-00 | I=0.6 | TET completion-time | -10 | condensed, k
slow=1e-05, k
fast=0.1 | 0.5 | 1.64e+03 | 0.7 | 4.07e+03 |
| Yin2008-F3-00 | I=0.4 | TET completion-time | -10 | condensed, k
slow=1e-05, k
fast=0.1 | 0.5 | 1.64e+03 | 0.7 | 4.07e+03 |
| Yin2008-F3-00 | I=0.2 | TET completion-time | -10 | condensed, k
slow=1e-05, k
fast=0.1 | 0.5 | 1.64e+03 | 0.7 | 4.07e+03 |
| Yin2008-F3-00 | I=0.1 | TET completion-time | -10 | condensed, k
slow=1e-05, k
fast=0.1 | 0.5 | 1.64e+03 | 0.7 | 4.07e+03 |
| Yin2008-F3-00 | I=0.06 | TET completion-time | -10 | condensed, k
slow=1e-05, k
fast=0.1 | 0.655 | 6.73e+03 | 1.03 | 6.07e+03 |
| Yin2008-F3-00 | I=0.02 | TET completion-time | -10 | condensed, k
slow=1e-05, k
fast=0.1 | 0.454 | 6.07e+03 | 1.23 | 5.9e+03 |
| Yin2008-F3-00 | I=0.01 | TET completion-time | -10 | condensed, k
slow=1e-05, k
fast=0.1 | 0.5 | 1.64e+03 | 0.7 | 4.07e+03 |

Table 7: Data for Fig. 11 Part 1/2.
| Input Filename | Simulation | Reporter | Metric | Metric-values | Semantics | Concentration (simulation) | Time (simulation) | Concentration (experiment) | Time (experiment) |
|---------------|------------|----------|--------|---------------|-----------|---------------------------|------------------|---------------------------|------------------|
| Zhang2009-F3-00 | X=0.6     | F        | diagonal-crossing-time | 1200 : 0.4 | condensed | 0.372                  | 2.5e+03          | 0.3                   | 1.0e+03          |
| Zhang2009-F3-00 | X=0.3     | F        | diagonal-crossing-time | 1200 : 0.4 | condensed | 0.372                  | 2.5e+03          | 0.3                   | 1.0e+03          |
| Zhang2009-F3-00 | X=0.6     | F        | completion-time | 0.3 | condensed | 0.3                   | 1.0e+03          | 0.3                   | 1.0e+03          |
| Zhang2009-F3-00 | X=0.4     | F        | completion-time | 0.4 | condensed | 0.2                   | 5.0e+03          | 0.2                   | 5.0e+03          |
| Zhang2011-F3A-00 | T1=18     | T2=18    | F      | diagonal-crossing-time | 1800 : 12 | condensed, $k_{slow}=0.001$, $k_{fast}=0.1$ | 11.3            | 117             | 9.15            | 409             |
| Zhang2011-F3A-00 | T1=12     | T2=12    | F      | diagonal-crossing-time | 1800 : 8 | condensed, $k_{slow}=0.001$, $k_{fast}=0.1$ | 7.26            | 169             | 6.14            | 426             |
| Zhang2011-F3A-00 | T1=6      | T2=6     | F      | diagonal-crossing-time | 1800 : 4 | condensed, $k_{slow}=0.001$, $k_{fast}=0.1$ | 3.12            | 394             | 3.25            | 300             |
| Zhang2011-F3A-00 | T1=18     | T2=18    | F      | completion-time | 5.01 | condensed, $k_{slow}=0.001$, $k_{fast}=0.1$ | 5.01            | 5.01            | 5.01            | 5.01            |
| Zhang2011-F3A-00 | T1=12     | T2=12    | F      | completion-time | 5.01 | condensed, $k_{slow}=0.001$, $k_{fast}=0.1$ | 5.01            | 5.01            | 5.01            | 5.01            |
| Zhang2011-F3A-00 | T1=6      | T2=6     | F      | completion-time | 5.01 | condensed, $k_{slow}=0.001$, $k_{fast}=0.1$ | 5.01            | 5.01            | 5.01            | 5.01            |
| Kotani2017-F2-00 | C1=1      | D        | diagonal-crossing-time | 32400 : 10 | condensed, release cutoff=7 | 8.36            | 5.32e+03          | 7.49            | 4.89e+03          |
| Kotani2017-F2-00 | C1=0.5    | D        | diagonal-crossing-time | 32400 : 10 | condensed, release cutoff=7 | 7.55            | 7.95e+03          | 6.29            | 9.39e+03          |
| Kotani2017-F2-00 | C1=0.05   | D        | diagonal-crossing-time | 32400 : 10 | condensed, release cutoff=7 | 2.37            | 3.96e+03          | 2.3            | 1.0e+04          |
| Kotani2017-F2-00 | C1=0.1    | D        | completion-time | 0.5 | condensed, release cutoff=7 | 1.5            | 4.8e+03           | 1.5            | 4.8e+03           |
| Kotani2017-F2-00 | C1=0.01   | D        | completion-time | 0.5 | condensed, release cutoff=7 | 0.5            | 4.8e+03           | 0.5            | 4.8e+03           |
| Kotani2017-F2-00 | C1=0.001  | D        | completion-time | 0.5 | condensed, release cutoff=7 | 0.5            | 4.8e+03           | 0.5            | 4.8e+03           |
| Kotani2017-F4-00 | C1=0.1    | D        | diagonal-crossing-time | 18000 : 10 | condensed, $k_{slow}=0.0001$, $k_{fast}=0.001$ | 6.55            | 6.22e+03          | 6.11            | 6.8e+03           |
| Kotani2017-F4-00 | C1=0.01   | D        | diagonal-crossing-time | 18000 : 10 | condensed, $k_{slow}=0.0001$, $k_{fast}=0.001$ | 6.01            | 7.08e+03          | 4.81            | 1.03e+04          |
| Kotani2017-F4-00 | C1=0.001  | D        | diagonal-crossing-time | 18000 : 10 | condensed, $k_{slow}=0.0001$, $k_{fast}=0.001$ | 5.56            | 7.99e+03          | 5.56            | 1.03e+04          |
| Kotani2017-F4-00 | C1=0      | D        | diagonal-crossing-time | 18000 : 10 | condensed, $k_{slow}=0.0001$, $k_{fast}=0.001$ | 0              | 1.09e+04          | 3.85            | 1.09e+04          |
| Kotani2017-F4-00 | C1=0.1    | D        | completion-time | 5.01 | condensed, $k_{slow}=0.0001$, $k_{fast}=0.001$ | 5.01            | 5.01            | 5.01            | 5.01            |
| Kotani2017-F4-00 | C1=0.01   | D        | completion-time | 5.01 | condensed, $k_{slow}=0.0001$, $k_{fast}=0.001$ | 5.01            | 5.01            | 5.01            | 5.01            |
| Kotani2017-F4-00 | C1=0.001  | D        | completion-time | 5.01 | condensed, $k_{slow}=0.0001$, $k_{fast}=0.001$ | 5.01            | 5.01            | 5.01            | 5.01            |
| Kotani2017-F4-00 | C1=0      | D        | completion-time | 5.01 | condensed, $k_{slow}=0.0001$, $k_{fast}=0.001$ | 5.01            | 5.01            | 5.01            | 5.01            |

Table 8: Data for Fig. 11 Part 2/2.
## 3 Seesaw semantics background

| Reaction Type                      | Reaction Description                                                                 |
|-----------------------------------|--------------------------------------------------------------------------------------|
| **Seesawing reactions**           | ![Seesawing reaction diagram](image1)                                                 |
| **Thresholding reactions**        | ![Thresholding reaction diagram](image2)                                             |
| **Reporting reactions**           | ![Reporting reaction diagram](image3)                                                |
| **Universal toehold binding reactions** | ![Universal toehold binding reaction diagram](image4)                              |
| **Side reactions**                | ![Side reaction diagram](image5)                                                     |
| **Leak reactions**                | ![Leak reaction diagram](image6)                                                     |

Fig. 1: Seesaw compiler reaction semantics. Note the reaction rate $k_{rs}$ is indistinguishable from $k_{rf}$ in Peppercorn’s semantics. Figure taken from Qian & Winfree (2011) [4] supporting online material.
Temperature 25°C

| Constant | Value |
|----------|-------|
| $k_s$    | $5 \times 10^4$ M$^{-1}$s$^{-1}$ |
| $k_f$    | $2 \times 10^6$ M$^{-1}$s$^{-1}$ |
| $k_{rs}$ | 1.3 s$^{-1}$ |
| $k_{rf}$ | 26 s$^{-1}$ |
| $k_l$    | 10 M$^{-1}$s$^{-1}$ |

Fig. 2: Comparison of superimposed squareroot circuit simulations. (top left) Peppercorn model with adjusted toehold binding strength. (top right) Seesaw compiler model. (bottom) Original plots taken from Qian & Winfree (2011) [4], supporting online material.
Bibliography

[1] Robert M Dirks and Niles A Pierce. Triggered amplification by hybridization chain reaction. Proceedings of the National Academy of Sciences, 101:15275–15278, 2004.

[2] Suvir Venkataraman, Robert M Dirks, Paul Wilhelm Karl Rothemund, Erik Winfree, and Niles A Pierce. An autonomous polymerization motor powered by DNA hybridization. Nature Nanotechnology, 2:490–494, 2007.

[3] Robert Tarjan. Depth-first search and linear graph algorithms. SIAM Journal on Computing, 1:146–160, 1972.

[4] Lulu Qian and Erik Winfree. Scaling up digital circuit computation with DNA strand displacement cascades. Science, 332:1196–1201, 2011.