Supplementary Info

Directed Enzymatic Activation of 1-D DNA Tiles

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Summary

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S1 DNA Sequences

S1.1 Mechanism 1: Simultaneous Activation of Tiles

Table T1: Sequence Design for Simultaneous Activation System

Name	**Domain**	**Sequence (Hyphenated by domain sequence)**
Tile A Top Strand	$a\ c\ b\ e$	GAGGTGAAATTGACTT-GCACACGCAGTTTCGCGCAACCCG-GCGCGGCATTAAATCG-TTT
Tile B Top Strand	$\bar{a}\ c\ \bar{b}\ e$	AAGTCAATTTCACCTC-GCACACGCAGTTTCGCGCAACCCG-CGATTTAATGCCGCGC-TTT
Middle Strand	$\bar{c}\ e$	CGGGTTGCGCGAAACTGCGTGTGC-TTT
Blunt Tile B Top Strand	$\bar{a}\ c$	AAGTCAATTTCACCTC-GCACACGCAGTTTCGCGCAACCCG
Protector Strand A	$\bar{a}\ g_1\ \bar{f}$	AAGTCAATTTCACCTC-TTTTT-CGTGTTTGCATCAGC
Protector Strand B	$b\ g_2\ \bar{f}$	GCGCGGCATTAAATCG-TTTTTTTTTTTTTTT-CGTGTTTGCATCAGC
Primer	f	GCTGATGCAAACACG

S1.2 Mechanism 2: Sequential Activation of Tiles

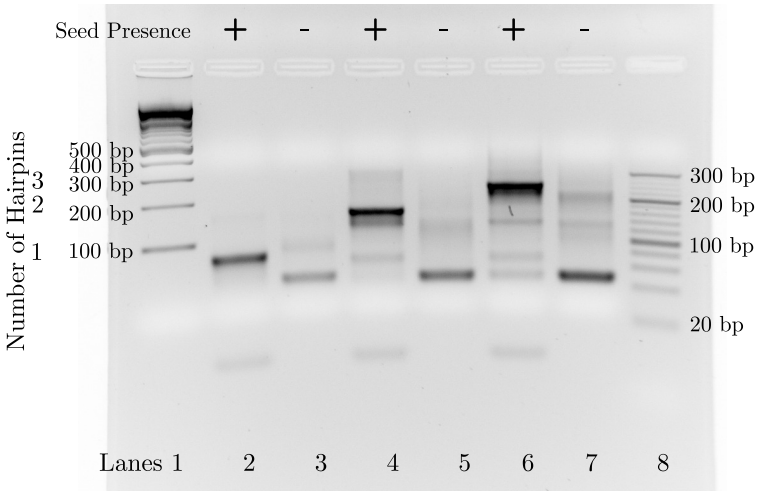
Table T2: Sequence Design for Sequential System

Name	**Domain**	**Sequence (Hyphenated by domain sequence)**
Hairpin A	$\bar{a}\ t_1\ f\ s\ a$	TACTGACATACTCTCTCCGCG- CAACCT-TGTCATCAGTGATGCCATAAA - TTTTTTTTTTTTTTT-CGCGGAGAGAGTATGTCAGTA
Hairpin B	$\bar{b}\ t_2\ \bar{a}\ s\ b$	ACAGTCTCTCACGGCAGTCAG- TTGGAA-TACTGACATACTCTCTCCGCG- TTTTTTTTTTTTTTT CTGACTGCCGTGAGAGACTGT
Hairpin C	$\bar{c}\ t_3\ \bar{b}\ s\ c$	TGATCGCTGACGCTACTTCAC- ATCGAT-ACAGTCTCTCACGGCAGTCAG- TTTTTTTTTTTTTTT-GTGAAGTAGCGTCAGCGATCA
Hairpin D	$\bar{d}\ t_4\ \bar{c}\ s\ d$	ATCTAAGTATCTCTCGGGTCC- GGTACT-TGATCGCTGACGCTACTTCAC- TTTTTTTTTTTTTTT GGACCCGAGAGATACTTAGAT
Hairpin E	$\bar{e}\ t_5\ \bar{d}\ s\ e$	CGATGATACTTTTCAGCCGGA- TGTCTG-ATCTAAGTATCTCTCGGGTCC- TTTTTTTTTTTTTTT-TCCGGCTGAAAAGTATCATCG
Seed F	f	TTTATGGCATCACTGATGACA
Seed A	a	CGCGGAGAGAGTATGTCAGTA
Seed B	b	CTGACTGCCGTGAGAGACTGT
Seed C	c	GTGAAGTAGCGTCAGCGATCA
Seed D	d	GGACCCGAGAGATACTTAGAT

S2 Gel Analysis, Control Experiments

S2.1 Experimental Data

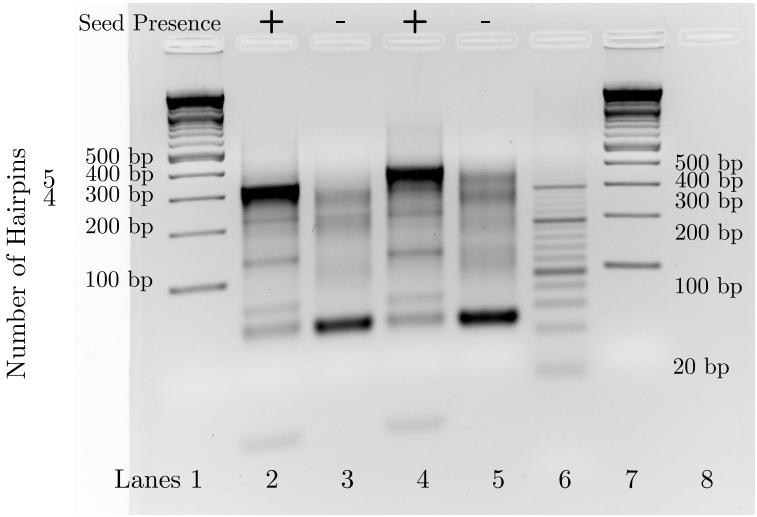
S2.1.1 Lower Number of Hairpins and Leaks



(a) **Ln 1:** 2-log ladder, **Ln 2:** Hairpin A + Seed F (0.5μM), **Ln 3:** Hairpin A (0.5μM), **Ln 4:** Hairpins A+B + Seed F (0.5μM), **Ln 5:** Hairpins A+B (0.5μM), **Ln 6:** Hairpins A+B+C + Seed F (0.5μM), **Ln 7:** Hairpins A+B+C (0.5μM), **Ln 8:** 20 bp ladder

Key	**Value**
Incubation	1 hr
Temp	52^{o}C
Loading Amount	1.5 pmol
Polymerase	BST 2.0, 2 units
Concentration	0.5 μM
Gel	Agarose, 3%
2-log ladder	165 ng
Lane	**Yield**
2	**45.33%**
4	**32%**
5	**2%**
6	**29.33%**
7	**6%**

Figure S1: Experiments for 1,2,3 Hairpins with Seed F at 52^{o}C with leaks.

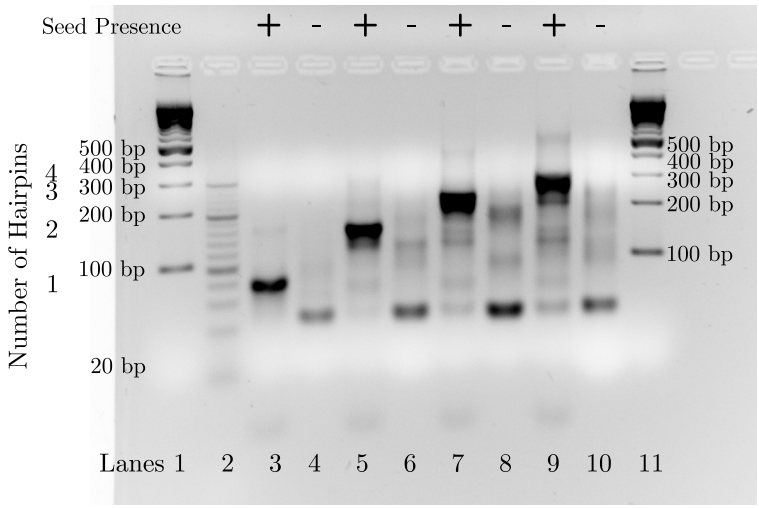


(a) **Ln 1:** 2-log ladder, **Ln 2:** Hairpins A+B+C+D + Seed F (0.5μM), **Ln 3:** Hairpins A+B+C+D (0.5μM), **Ln 4:** Hairpins A+B+C+D+E + Seed F (0.5μM), **Ln 5:** Hairpins A+B+C+D+E (0.5μM), **Ln 6:** 20 bp ladder, **Ln 7:** 2-log ladder

Key	**Value**
Incubation	1 hr
Temp	52^{o}C
Loading Amount	1.5 pmol
Polymerase	BST 2.0, 2 units
Concentration	0.5 μM
Gel	Agarose, 3%
2-log ladder	165 ng
Lane	**Yield**
2	**30%**
3	**8.67%**
4	**23.33%**
5	**6.67%**

Figure S2: Experiments for 4,5 hairpins with Seed F at 52^{o}C with leaks.

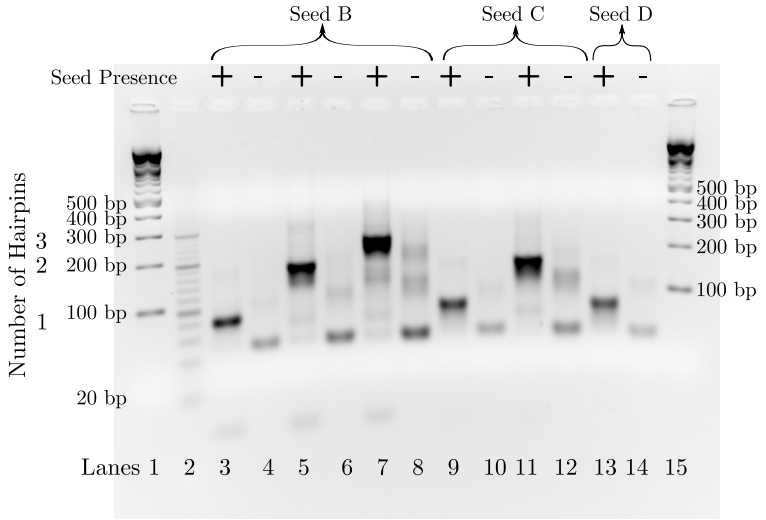
S2.1.2 Experiments with Seeds A,B,C,D



Key	Value
Incubation	1 hr
Temp	52^{o}C
Loading Amount	1.5 pmol
Polymerase	BST 2.0, 2 units
Concentration	0.5 μM
Gel	Agarose, 3%
2-log ladder	165 ng
Lane	**Yield**
3	**64.67%**
5	**48.67%**
6	**3.33%**
7	**49.33%**
8	**13.33%**
9	**33.33%**
10	**4.67%**

(a) **Ln 1:** 2-log ladder, **Ln 2:** 20 bp ladder, **Ln 3:** Hairpin B + Seed A (0.5μM), **Ln 4:** Hairpin B (0.5μM), **Ln 5:** Hairpins B+C + Seed A (0.5μM), **Ln 6:** Hairpins B+C (0.5μM), **Ln 7:** Hairpins B+C+D + Seed A (0.5μM), **Ln 8:** Hairpins B+C+D (0.5μM), **Ln 9:** Hairpins B+C+D+E + Seed A (0.5μM), **Ln 10:** Hairpins B+C+D+E (0.5μM), **Ln 11:** 2-log ladder

Figure S3: Experiments with Seed A at 52^{o}C with leaks.

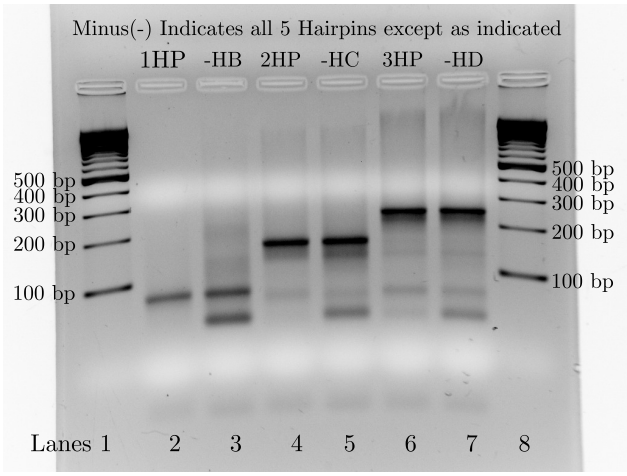


Key	Value
Incubation	1 hr
Temp	52^{o}C
Loading Amount	1.5 pmol
Polymerase	BST 2.0, 2 units
Concentration	0.5 μM
Gel	Agarose, 3%
2-log ladder	165 ng
Lane	**Yield**
3	**61.33%**
5	**50.67%**
7	**50%**
8	**8.67%**
9	**62%**
11	**62%**
12	**10%**
13	**55.33%**

(a) **Ln 1:** 2-log ladder, **Ln 2:** 20 bp ladder, **Ln 3:** Hairpin C + Seed B (0.5μM), **Ln 4:** Hairpin C (0.5μM), **Ln 5:** Hairpins C+D + Seed B (0.5μM), **Ln 6:** Hairpins C+D (0.5μM), **Ln 7:** Hairpins C+D+E + Seed B (0.5μM), **Ln 8:** Hairpins C+D+E (0.5μM), **Ln 9:** Hairpin D + Seed C (0.5μM), **Ln 10:** Hairpin D (0.5μM), **Ln 11:** Hairpins D+E + Seed C (0.5μM), **Ln 12:** Hairpins D+E (0.5μM), **Ln 13:** Hairpin E + Seed D (0.5μM), **Ln 14:** Hairpin E (0.5μM), **Ln 15:** 2-log ladder

Figure S4: Experiments with Other Seeds B,C,D at 52^{o}C with leaks.

S2.1.3 Experiments with Missing Link in the Chain

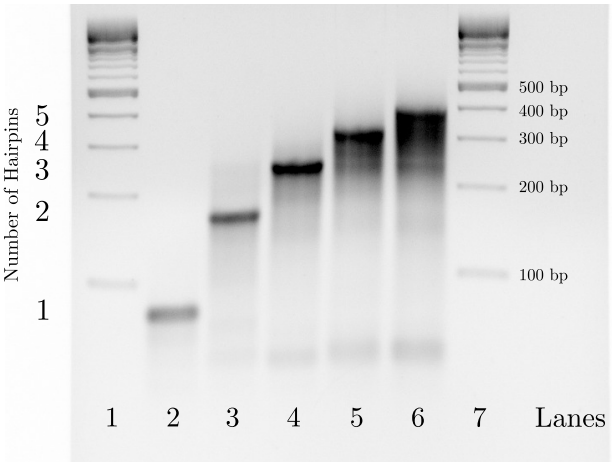


Key	Value
Incubation	1 hr
Temp	52^{o}C
Loading Amount	1 pmol
Polymerase	BST 2.0, 2 units
Concentration	0.1 μM
Gel	Agarose, 3%
2-log ladder	165 ng
Lane	**Yield**
2	**27%**
3	**29%**
4	**18%**
5	**24%**
6	**18%**
7	**19%**

(a) **Ln 1:** 2-log ladder, **Ln 2:** (1HP) Hairpin A + Seed F (0.5μM), **Ln 3:** (-HB) Hairpins ACDE + Seed F (0.5μM), **Ln 4:** (2HP) Hairpins A+B + Seed F (0.5μM), **Ln 5:** (-HC) Hairpins ABDE + Seed F (0.5μM), **Ln 6:** Hairpins A+B+C + Seed F (0.5μM), **Ln 7:** (-HD) Hairpins ABCE + Seed F (0.5μM), **Ln 8:** 2-log ladder

Figure S5: Control Experiment with 1 Hairpin Missing at 52^{o}C. Lanes 2,4,6 have been added to show expected behaviour when 1 hairpin is missing.

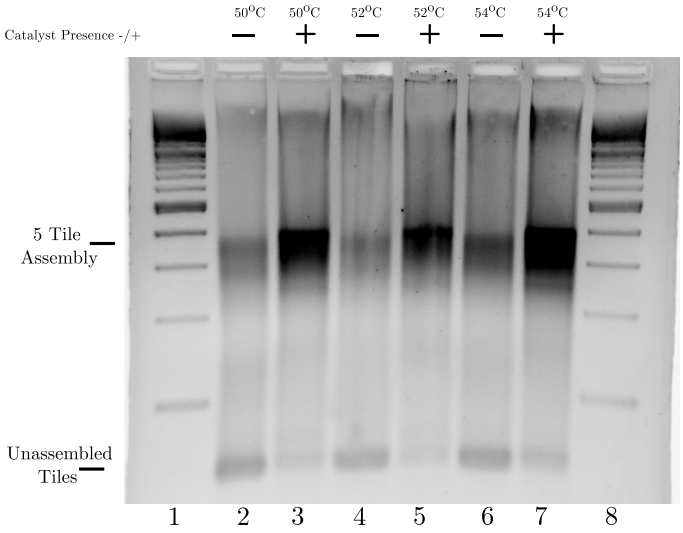
S2.1.4 Experiments with Low BST



Key	Value
Incubation	1 hr
Temp	52^{o}C
Loading Amount	27 pmol
Polymerase	BST, 0.1 units
Concentration	0.5 μM
Gel	Agarose, 3%
2-log ladder	990 ng
Lane	**Yield**
2	**21.79%**
3	**16.65%**
4	**17.78%**
5	**9.75%**
6	**8.74%**

(a) **Ln 1:** 20 bp ladder, **Ln 2:** Hairpin A + Seed F (0.5μM), **Ln 3:** Hairpins A+B + Seed F (0.5μM), **Ln 4:** Hairpins A+B+C + Seed F (0.5μM), **Ln 5:** Hairpins A+B+C+D + Seed F (0.5μM), **Ln 6:** Hairpins A+B+C+D+E + Seed F (0.5μM), **Ln 7:** (Leak) Hairpins A+B+C+D+E (0.5μM), **Ln 8:** 2-log ladder

Figure S6: Sequential assembly using 0.1 units of BST Polymerase at 52^{o}C.

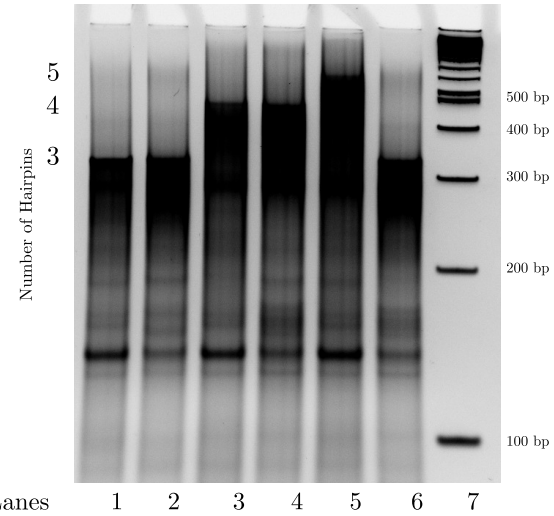


Key	Value
Incubation	1 hr
Temp	52^{o}C
Loading Amount	27 pmol
Polymerase	BST, 0.1 units
Concentration	0.5 μM
Gel	Agarose, 3%
2-log ladder	990 ng
Lane	**Yield**
2	**6.04%**
3	**13.35%**
4	**3.98%**
5	**10.88%**
6	**6.45%**
7	**20.55%**

(a) 3% Agar for 50^{o}C,52^{o}C,54^{o}C. **Ln 1,8:** 2-log ladder, **Ln 2:** (50^{o}C) - Hairpins A+B+C+D+E (0.5μM), **Ln 3:** (50^{o}C) - Hairpins A+B+C+D+E + Seed F (0.5μM), **Ln 4:** (52^{o}C) - Hairpins A+B+C+D+E (0.5μM), **Ln 5:** (52^{o}C) - Hairpins A+B+C+D+E + Seed F (0.5μM), **Ln 6:** (54^{o}C) - Hairpins A+B+C+D+E (0.5μM), **Ln 7:** (54^{o}C) - Hairpins A+B+C+D+E + Seed F (0.5μM)

Figure S7: Experiments using 0.1 units BST Polymerase at 50^{o}C, 52^{o}C, 54^{o}C

S2.1.5 Experiments at Lower Temperature (37^{o}C)

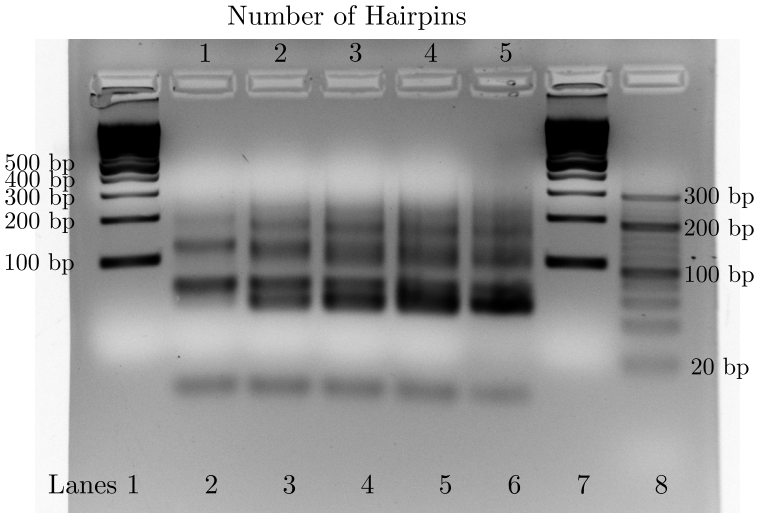


Key	Value
Incubation	3 hr
Temp	37^{o}C
Loading Amount	8 pmol
Polymerase	BST, 0.1 units
Concentration	0.5 μM
Gel	PAGE, 6%
2-log ladder	990 ng
Lane	**Yield**
1	**11.75%**
2	**8.88%**
3	**17.75%**
4	**11.75%**
5	**11.13%**
6	**12%**

(a) 6% PAGE, sequential assembly using BST Polymerase at 37^{o}C. **Ln 1:** Hairpin A+B+C + Seed F (0.5μM) **Ln 2:** Hairpin C+D+E + Seed B (0.5μM), **Ln 3:** Hairpin A+B+C+D + Seed F (0.5μM), **Ln 4:** Hairpin B+C+D+E + Seed A (0.5μM), **Ln 5:** Hairpin A+B+C+D+E + Seed F (0.5μM), **Ln 6:** Hairpin B+C+D+ Seed A (0.5μM), **Ln 7:** 2-log ladder.

Figure S8: Experiments using 0.1 units BST Polymerase at 37^{o}C

S2.1.6 Control Experiments (Minus Polymerase)

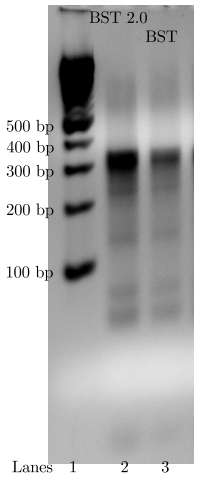


Key	Value
Incubation	1 hr
Temp	52^{o}C
Loading Amount	3.33 pmol
Concentration	0.5 μM
Gel	Agarose, 3%
2-log ladder	165 ng

(a) **Ln 1:** 2-log ladder **Ln 2:** Hairpin A + Seed F (0.5μM) **Ln 3:** Hairpin A+B + Seed F (0.5μM), **Ln 4:** Hairpin A+B+C + Seed F (0.5μM), **Ln 5:** Hairpin A+B+C+D + Seed F (0.5μM), **Ln 6:** Hairpin A+B+C+D+E + Seed F (0.5μM), **Ln 7:** 2-log ladder **Ln 8:** 20 bp ladder

Figure S9: Experiments in the absence of Polymerase at 52^{o}C. Secondary structures are seen between hairpins. This is expected, since multiple hairpins bind to each other and can form higher order structures. However, this does not classify as a leak, since there is no irreversible extension of the binding, and the hairpins have not been rendered useless, i.e., they can still be involved in reactions and perform computation.

S2.1.7 Control Experiments (BST vs BST 2.0)

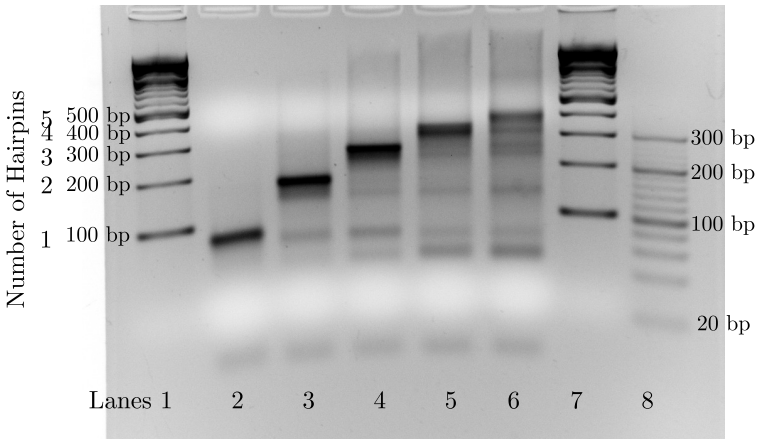


Key	Value
Incubation	1 hr
Temp	52^{o}C
Loading Amount	1 pmol
Concentration	0.1 μM
Gel	Agarose, 3%
2-log ladder	165 ng
Lane	**Yield**
2	**18.2%**
3	**12.8%**

(a) **Ln 1:** 2-log ladder **Ln 2:** Hairpin A+B+C+D + Seed F + BST 2.0(2 units), 0.1μM **Ln 3:** Hairpin A+B+C+D + Seed F + BST (4 units), 0.1μM

Figure S10: Experiments for 4 Hairpins - BST vs BST 2.0 at 52^{o}C. *BST Polymerase* yield 12.8% is consistently lower than the yield with *BST 2.0* Polymerase 18.2%.

S2.1.8 Experiments at lower concentration (100 nM vs 500 nM)



Key	Value
Incubation	1 hr
Temp	52^{o}C
Loading Amount	1.5 pmol
Concentration	0.1 μM
Gel	Agarose, 3%
2-log ladder	165 ng
Lane	**Yield**
2	**46%**
3	**27%**
4	**16%**
5	**16%**
6	**10%**

(a) **Ln 1:** 2-log ladder, **Ln 2:** Hairpin A + Seed F (0.5μM), **Ln 3:** Hairpins A+B + Seed F (0.5μM), **Ln 4:** Hairpins A+B+C + Seed F (0.5μM), **Ln 5:** Hairpins A+B+C+D + Seed F (0.5μM), **Ln 6:** Hairpins A+B+C+D+E + Seed F (0.5μM), **Ln 7:** 2-log ladder **Ln 8:** 20 bp ladder

Figure S11: Sequential assembly at 100 nM, at 52^{o}C. The yield of the 4 and 5 Hairpin complexes is 16% and 10%, compared to their yields 19.33% and 16% in figure 6.

S2.2 Experimental Details

The main document gives a detailed account of the experimental methods. Experiments were done with either *BST Polymerase* or *BST 2.0 Polymerase*, and *BST 2.0 Polymerase* performed superior for the reaction conditions used. For *BST Polymerase*, the reaction buffer used was 1x Thermopol Buffer ((20 mM Tris-HCl 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$ and 0.1 % Triton X-100)), and for *BST 2.0 Polymerase*, the reaction buffer was 1x Isothermal Amplification Buffer (20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 50 mM KCl, 2 mM $MgSO_4$ and 0.1 % Tween® 20). The polymerases and buffers were purchased from NEB. All the agarose gels were run for 20 mins at 250V in an icebath.

S3 Error Rate Analysis

S3.1 Simultaneous Activation of Tiles

S3.1.1 Figure 5a) Polymer

Lane 12 shows the leak (polymer). Image analysis reveals:

Lane	Length (by molecular ladder)	Intensity
12	155 nt dsDNA	7.3%
12	100 nt dsDNA	32.3%
12	75 nt dsDNA	34.9%
12	30 nt dsDNA	5.4%

Polymer lengths are $24n + 16(n-1)$, where n is the number of tiles in the polymer (can be seen from figure 3). Thus, according to the 10 bp ladder, $n \approx 4$. Hence, there are 144 bp in the complex. ITA has 55 bp and ITB also has 55 bp as can be seen from figure 3. The relative amount of the polymer is $7.3/144 \approx 5.07\%$, while the relative amount of ITA is $32.3/55 \approx 58.73\%$ and ITB is $34.9/55 \approx 63.45\%$. Assuming that 2 ITA tiles and 2 ITB tiles each form 1 polymer tile, the relative quantity of the leak is $\frac{2*5.07}{2*5.07+58.73} = 14.72\%$.

S3.1.2 Figure 5b) Dimer

Lane 8 shows some leak (highest band). Image analysis using an AlphaImager revealed the following relative intensity.

Lane	Length (by molecular ladder)	Intensity
8	80 nt dsDNA	7.7%
8	78 nt dsDNA	50.9%
8	35 nt dsDNA	15.9%

We make an assumption that the error lane (dimer) must be generated entirely from Inactive Tile A (ITA) and Blunt Tile B (BTB) (although it is possible that stray strands because of incorrect stoichiometry while annealing ITA and BTB each caused some amount of leak.)

Since the intensity of a DNA band is proportional to the quantity of Ethidium Bromide, which in turn is proportional to the size (molecular weight) of the DNA complex, the intensity of each band was normalized by the number of duplex bases in each complex. The dimer contains 64 dsDNA while ITA contains 55 nt of dsDNA. Hence, the relative amount of dimer is $7.7/64 \approx 12.03\%$, while the relative amount of ITA is $50.9/55 \approx 92.54\%$. Hence, the relative quantity of dimer is $\frac{12.03}{12.03+92.54} = 11.5\%$.

S3.2 Sequential Activation of Tiles

Quantitation of DNA fragments for sequential activation was done *via* DNA mass ladders. Here, intensity comparison was done against a molecular ladder of known relative DNA fragment quantity. The 2-log ladder available from NEB was used for this purpose. 165 ng or 990 ng of the ladder was loaded in the gels as indicated. The 100 bp band or the 400 bp band was chosen as the reference bands in each image, and the intensity of each band was normalized based on its molecular weight. The molecular weights of a nanostructure were

calculated based on what strands were present in it. Table T3 summarizes the molecular weights used for the purposes of quantitation.

Table T3: Molecular weights of DNA complexes

Strand	**Weight gm/mol**
Extended F	14926.7
Extended A	34104.1
Extended B	34161.1
Extended C	34163.1
Extended D	34104.1
Hairpin A	25766.7
Hairpin B	25750.7
Hairpin C	25783.7
Hairpin D	25764.7
Hairpin E	25770.7

Nanostructure	**Weight gm/mol**	**Strands present**
1HP Complex	40693.4	Extended F, Hairpin A
2HP Complex	74781.5	Extended F, Extended A, Hairpin B
3HP Complex	108975.6	Extended F, Extended A,B, Hairpin C
4HP Complex	143119.7	Extended F, Extended A,B,C Hairpin D
5HP Complex	177229.8	Extended F, Extended A,B,C,D Hairpin E

S3.2.1 Error Rate Calculation

The error rates for sequential assembly have been calculated relative to the first hairpin assembly complex. Thus, in figure 6, the error rate of attachment of Tile B is $\frac{33.33-27.33}{33.33}$, i.e. 18.001%. The yield of the 3 hairpin complex 31.33%, and it is greater than that of the 2 hairpin complex. However, there is an overlap of two bands, which is the cause of this error. To get around this, we disregard the yield of the 3 hairpin complex, and we calculate the error rate of two tiles attachments, i.e. $\frac{27.33-19.33}{27.33}$ i.e. about 29% across two tiles, so $1-\sqrt{0.71}$, i.e. 15.7% per tile attachment for tiles C and D. The error rate of attachment of tile E is $\frac{19.33-16}{19.33} \approx 17.3\%$.

S4 Other Phenomenon: Strand Slippage

Prior to arriving at the current set of design for the hairpin based systems, a single hairpin was designed as shown in figure S12. Here, there is no spacer domain s and the t domain is 21 nt instead of 6 nt as in figure 4. A set of experiments were performed that showed the activity of a single hairpin and a single seed A in the presence of polymerase. As can be seen in figure S13, in lane 4, the polymerase succeeds in opening the stem, activating tile B in the process. However, the activated tile B shows some extra lanes, which shouldn't be present in the system.

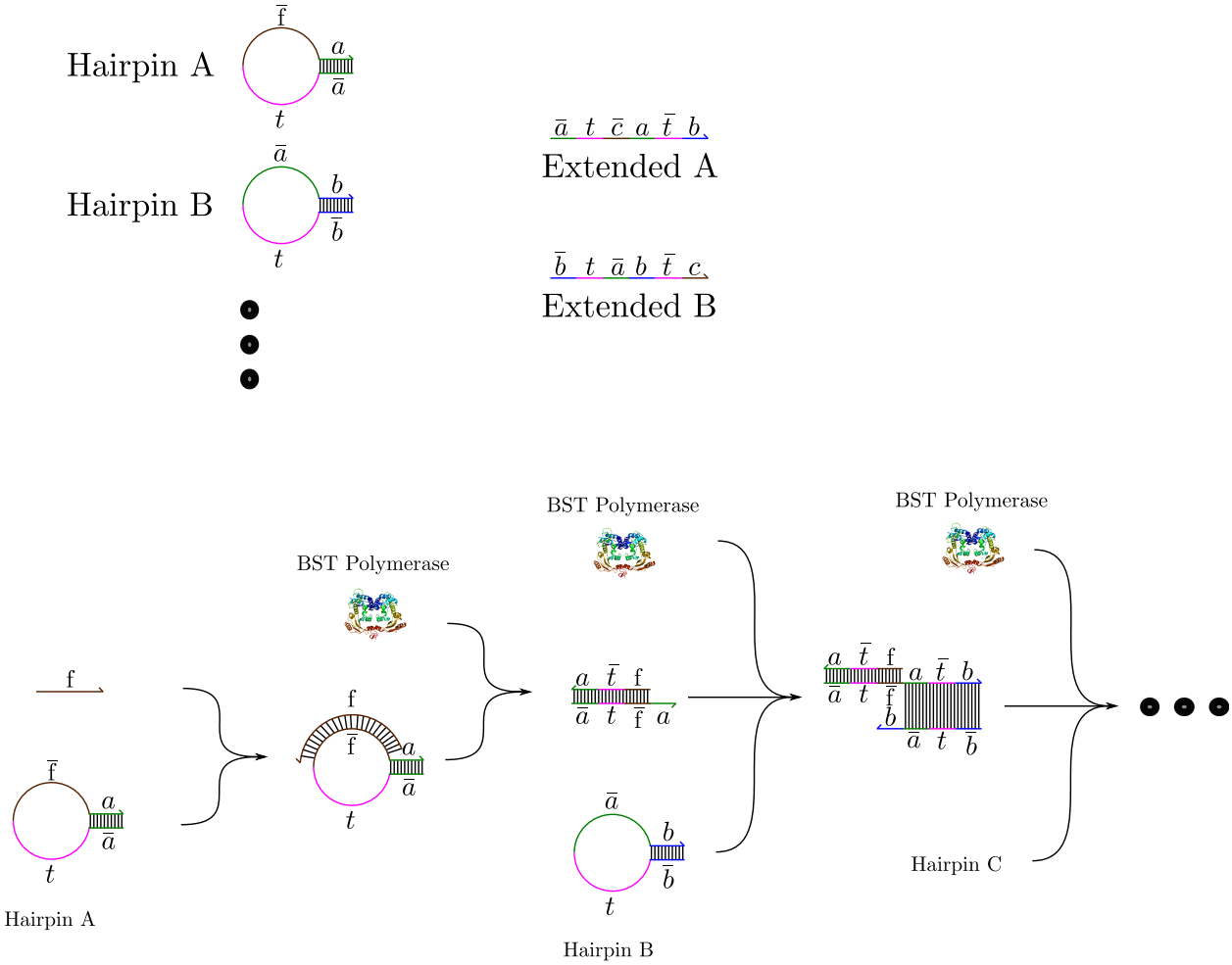
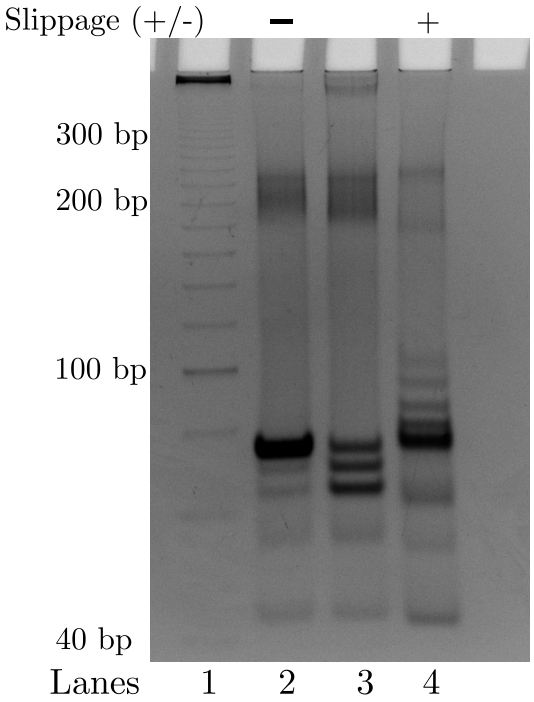


Figure S12: Directed Assembly of Tiles (Old Design)

On further investigation, it was found that the extra lanes were due to the repeating sequence of poly T's present in the t domain. Could this be because of a phenomenon called strand slippage [1]. By replacing the t domain with a 21 nt domain having no repeats (see table T4 for sequences), the problem was solved, as is seen in S13, lane 2.

Strand slippage is a phenomenon common in genomic studies, and is frequently cited as a reason for causing mutations[1]. It has been credited with causing both insertion and deletion mutations. Slippage occurs during DNA polymerization, in regions where there are repeats on the template strand. The repeats are usually from 1-4 nt in length, and longer repeats have been shown to have a lower slippage rate. Slippage can occur on either the template or the primer, leading to a shortening or lengthening of the synthesis strand respectively. Shortening refers to a deletion, while lengthening refers to an insertion mutation. The strand that slips forms a bulge loop, and this bulge loop is not part of the polymerization process.

A possible reason for this phenomenon, is that it is not uncommon for the polymerase molecule to dissociate from the template during the synthesis of the DNA backbone[2]. On resuming the synthesis (with either the same polymerase molecule or a different molecule), slippage occurs. The polymerase can dissociate from the template primer, due to different



(a) 10% PAGE, sequential assembly using 0.1 units BST Polymerase. **Ln 1:** 20 bp ladder **Ln 2:** Hairpin BwoT + Seed A (0.5μM, 37^{o}C), **Ln 3:** Hairpin BwoT + Seed A (0.5μM, 32^{o}C), **Ln 4:** Hairpin BwT + Seed A (0.5μM, 32^{o}C)

Key	**Value**
Incubation	70 mins
Loading Amount	20 pmol
Concentration	0.5 μM
Gel	PAGE, 10%

Figure S13: Strand slippage demonstration

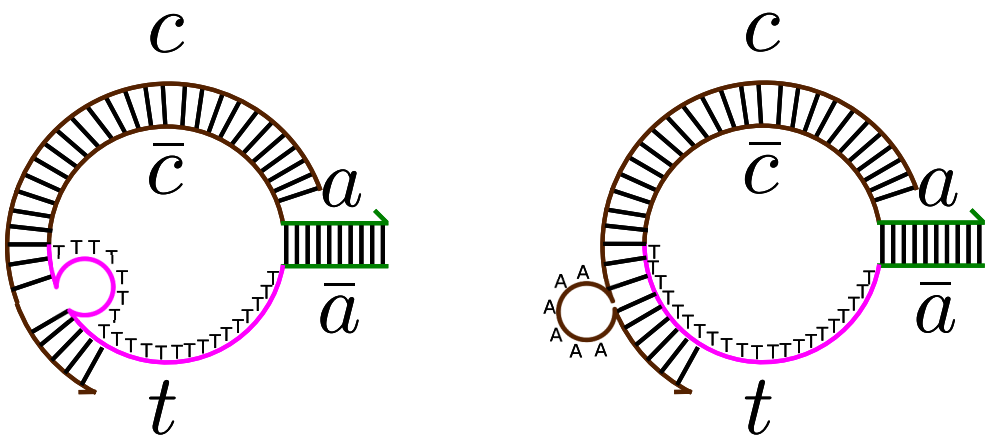
reasons, with the primary one being due to impurities present in the DNA. Another is due to breathing in the DNA duplex, which involves strands frequently dissociating and associating from each other, especially at the edges. After a dissociation step, when the two strands of DNA again attach with one another, there are multiple kinetic traps that the primer can get into, each of them a few nucleotides away from each other. Hence, the primer may temporarily re-attach at a different site, during which a polymerase molecule can restart its synthesis.

The *processivity* of polymerases plays an important role in this mechanism. Processivity is defined as the average time for which a polymerase stays on the primer-template duplex before dissociation. The better the processivity of the polymerase, the lesser the errors. Different types of polymerases have different processivity[3]. Two examples of strand slippage are as in figure S14:

An important observation however, is inconsistent with the theory of strand slippage in our experiments. Our region of repeats, is a poly T, of 21 nt in length. The polymerase slipping should occur at any of these 21 positions, randomly with equal probability, and the gel image hence obtained should be a smear (or 21 distinct bands). However, we get 6 distinct bands in our gel. The same set of 6 bands is consistently seen across multiple experiments, which leads us to conclude that the phenomenon is not random, and is in fact

Table T4: Sequences that displayed Strand Slippage

Name	**Domain**	**Sequence (Hyphenated by domain sequence)**
Hairpin BwT	$b\ t_{21T}\ \bar{a}\ b$	ACAGTCTCTCACGGCAGTCAG-TTTTTTTTTTTTTTTTTTTTT-TACTGACATACTCTCTCCGCG-CTGACTGCCGTGAGAGACTGT
Hairpin BwoT	$\bar{b}\ t_{random}\ \bar{a}\ b$	ACAGTCTCTCACGGCAGTCAG-GGCAACTAAACTCCACTCTAA-TACTGACATACTCTCTCCGCG-CTGACTGCCGTGAGAGACTGT
Seed A	a	CGCGGAGAGAGTATGTCAGTA



(a) Slipping of the template (b) Slipping of the primer

Figure S14: Example of Strand slippage

directed. This observation has not been investigated further, since our objective of achieving a single complex with the primer extended was achieved. Another reasoning consistent with the strand slippage theory, is that out of the various kinetic traps on the template strand, these 6 are more likely to occur. The base pair mobilities of these 6 bands are approximately 76bp, 83bp, 89bp, 101bp, 111bp and 116bp.

References

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3. Bloom, L. B.; Goodman, M. F. *eLS*; John Wiley & Sons, Ltd, 2001.