Appendix B

Supplementary Material for A Synthetic Polymer that Grows Exponentially Fast[0]

[0]This work was coauthored by Nadine Dabby & Ho-Lin Chen*, and is in preparation [Dabby and Chen, 2013a] with the following contributions: experiments and analysis were performed by N.D. with supervision from H-L.C.; manuscript was written with input from both authors.

B.1 Exponential Growth System Experiments

B.1.1 DNA Sequences Final Version 6-3v1

Table B.1: 6-3 Toehold Design Version1

m6n3 Insertion Init1.v1	ACCGCACGTCCACGGTGTCGCACCCAC
m6n3 Insertion Init2.v1	AACGCGACACCGTGGACGTGCGGT
m6n3 Insertion Init2.ROX	AACGCGACACCGTGGACGTGCGGT /3Rox_N/
m6n3 Insertion_H1.v1	GTGGGTGCGACACCGTGGACGTGCCTCAGACCAAGAGCACGTCCACGGTGTCGCGTT
m6n3 Insertion_H2.v1	TCTGAGGCACGTCCACGGTGTCGCACCCACAACGCGACACCGTGGACGTGCGGT
m6n3 Insertion_H3.v1	ACCGCACGTCCACGGTGTCGCACCCACAACGCGACACCGTGGACGTGCTCTTGG
m6n3 Divide_H1-3'	CCAAGAGCACGTCCACGGTGTCGCGTT
m6n3 Divide_H1-5'	GTGGGTGCGACACCGTGGACGTGCCTCAGA
m6n3v1.H2-Rox-Quench	/5IAbRQ/TCTGAGGCACGTCCACGGTGTCGCACCCACAACGCGACACCGTGGACGTGCGGT/3Rox_N/
m6n3 Linear_H3.v1	ACCGCACGTCCACGGTGTCGCTTTTTTTTTGCGACACCG TGG ACG TGC TCT TGG
m6n3v1.H2RQ_DISPLACE	ACCGCACGTCCACGG TGT CGC GTT GTG GGT GCG ACA CCG TGG ACG TGC CTC AGA

B.1.2 Leakage in Various Designs

Leakage and rate trials of 3 other Hairpin Designs: With Sequences.

Table B.2: 4-4 Toehold Design Version1

m4n4 Insertion_init1	GAGAGCACGTCCACGGTGTCGCACCC
m4n4 Insertion_init2	ACAGGCGACACCGTGGACGTGCTCTC
m4n4 H1	GGGTGCGACACCGTGGACGTGCCAGCCTCGGCACGTCCACGGTGTCGCCTGT
m4n4 H2	GCTGGCACGTCCACGGTGTCGCACCCACAGGCGACACCGTGGACGTGCTCTC
m4n4 H3	GAGAGCACGTCCACGGTGTCGCACCCACAGGCGACACCGTGGACGTGCCGAG

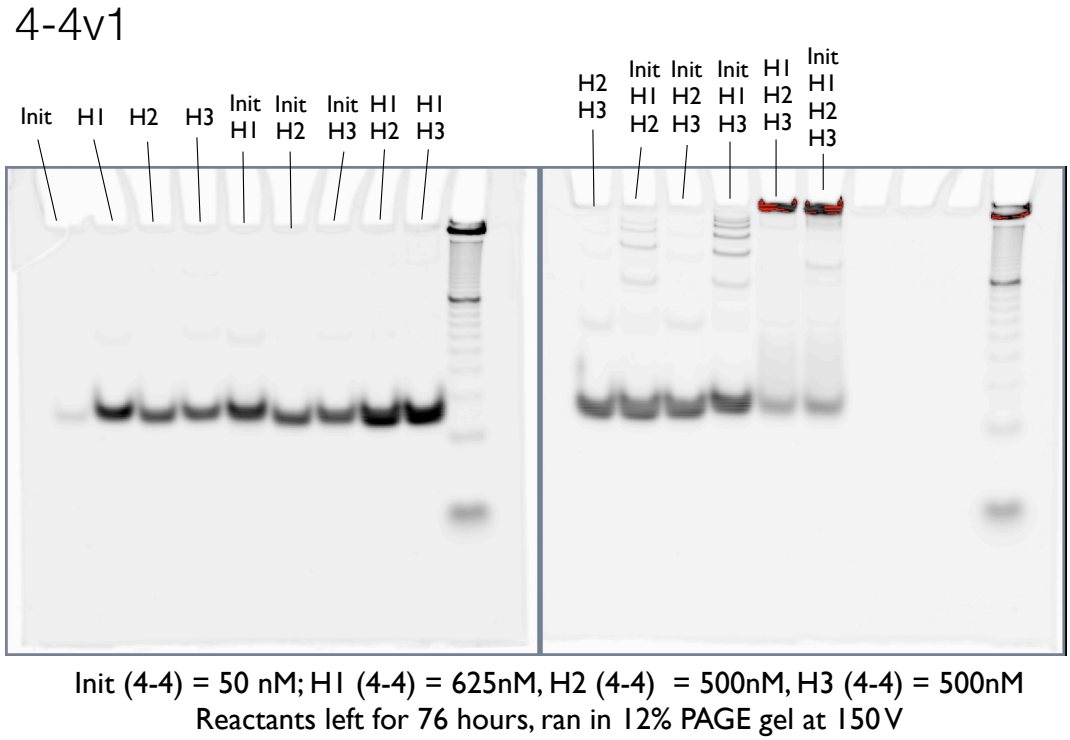

Figure B.1: Combinatorial gel for 4-4v1 design. The polyacrylamide gel above shows that a small leak occurs between reactants Hairpin 1 (H1) and Hairpin 2 (H2) and Hairpin 3 (H3) in the absence of Initiator. This set of strands reacts significantly more slowly than the other designs.

Table B.3: 5-4 Toehold Design Version2

5-4 v1.init1	GTTA-GCCCTGTATTGGGCTCGC-TCTCG
5-4 v1.init2	GCCT-GCGAGCCCAATACAGGGC-TAAC
5-4 v1.H1	CGAGA-GCGAGCCCAATACAGGGC-ACTCA-ATCAC-GCCCTGTATTGGGCTCGC-AGGC
5-4 v1.H2	TGAGT-GCCCTGTATTGGGCTCGC-TCTCG-GCCT-GCGAGCCCAATACAGGGC-TAAC
5-4 v1.H3	GTTA-GCCCTGTATTGGGCTCGC-TCTCG-GCCT-GCGAGCCCAATACAGGGC-GTGAT

5-4v2

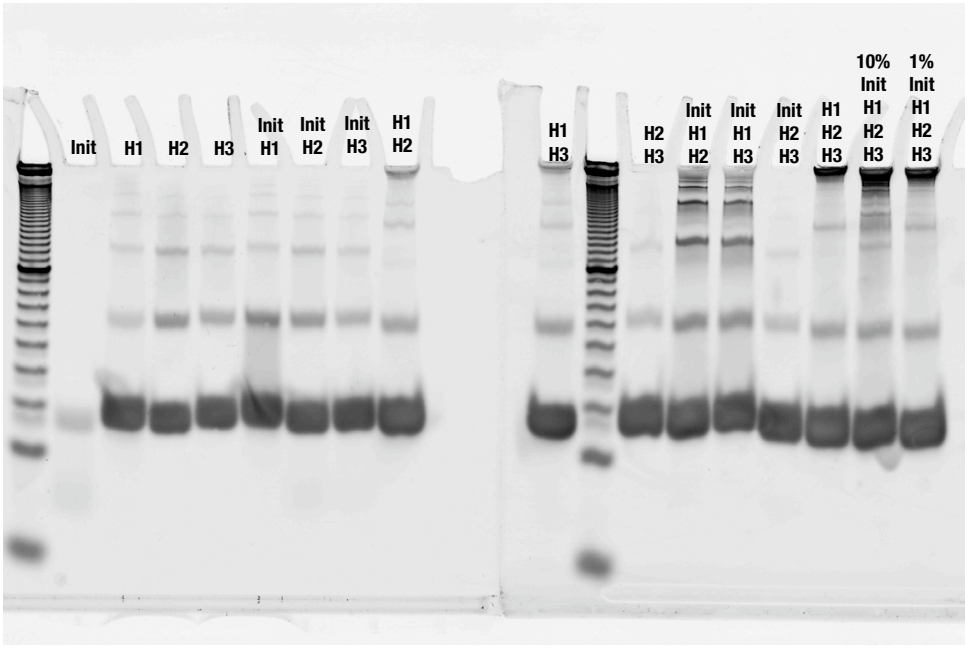

Figure B.2: Combinatorial gel for 5-4v2 design. The polyacrylamide gel above shows that a small leak occurs between reactants Hairpin 1 (H1) and Hairpin 2 (H2) and between Hairpin 1 (H1) and Hairpin 3 (H3) in the absence of Initiator.

Table B.4: 6-3 Toehold Design Version 2

6-3v2.init1	GTC-CGGGACGGACCCGTGCGC-CTTACG
6-3v2.init2	CTT-GCGCACGGGTCCGTCCCG-GAC
6-3v2.H1	CGTAAG-GCGCACGGGTCCGTCCCG-TGTCCA-AGCTAG-CGGGACGGACCCGTGCGC-AAG
6-3v2.H2	TGGACA-CGGGACGGACCCGTGCGC-CTTACG-CTT-GCGCACGGGTCCGTCCCG-GAC
6-3v2.H3	GTC-CGGGACGGACCCGTGCGC-CTTACG-CTT-GCGCACGGGTCCGTCCCG-CTAGCT

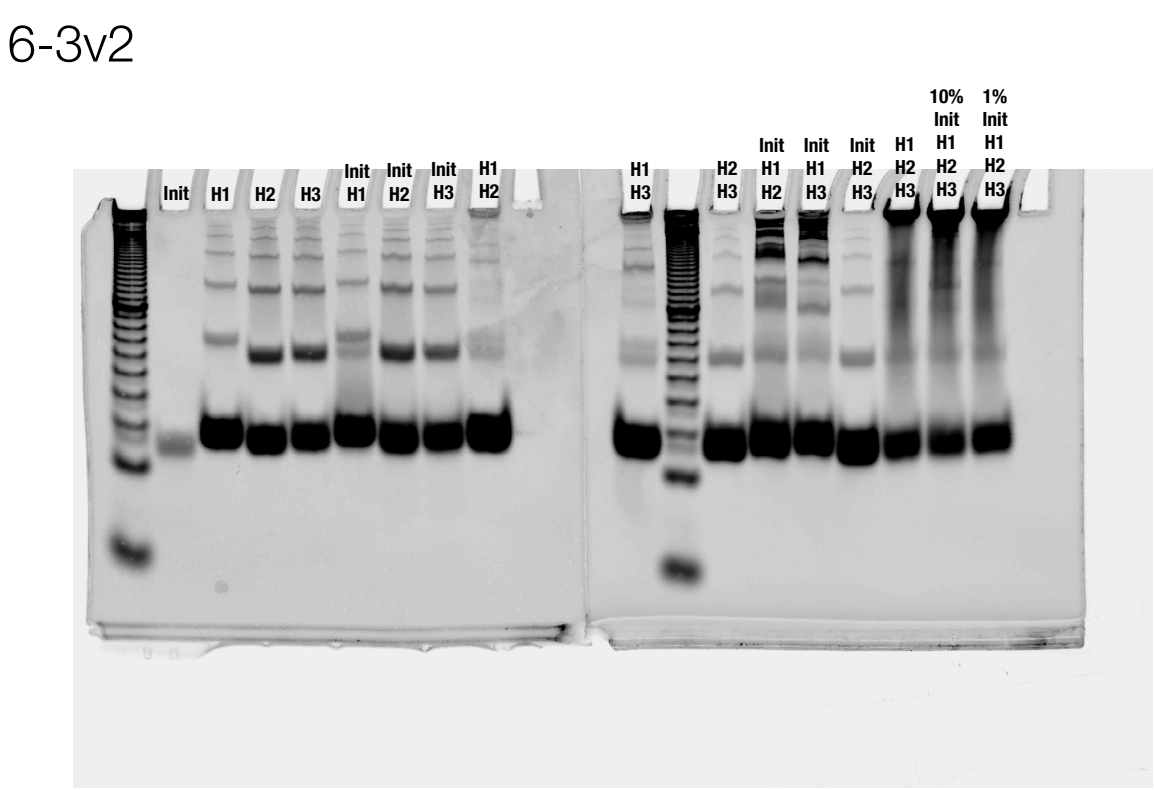

Figure B.3: Combinatorial gel for 6-3v2 design. The polyacrylamide gel above shows that a small leak occurs between reactants Hairpin 1 (H1) and Hairpin 2 (H2) and between Hairpin 1 (H1) and Hairpin 3 (H3) in the absence of Initiator.

B.1.3 Joining

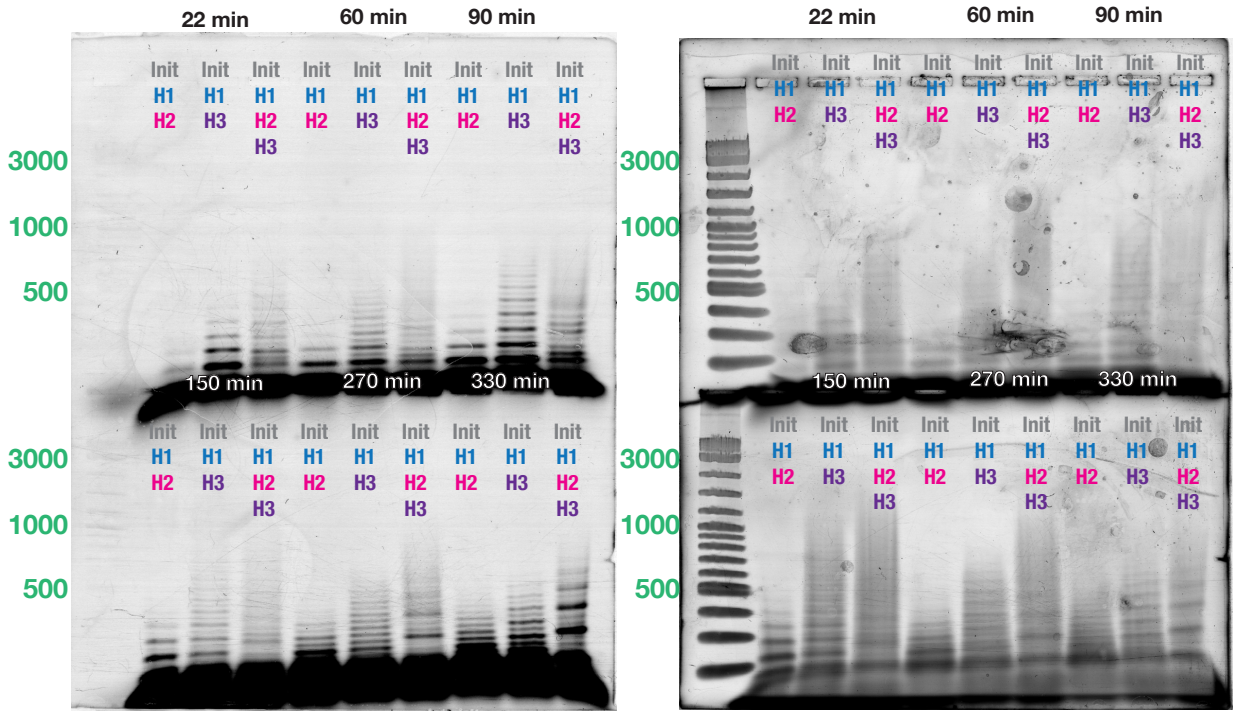

Figure B.4: The time lapse reaction of the experimental system with [Initiator] equivalent to hairpin concentrations. The gel on the left and right are the same but imaged under different conditions (left no stain, imaged at fluorophore emission wavelength; right same gel stained with SYBR Gold and imaged at SYBR Gold emission wavelength). If the polymers are randomly joining we would see an upward shift in the gel bands over time. This data shows that there is minimal joining.

B.1.4 Kinetics

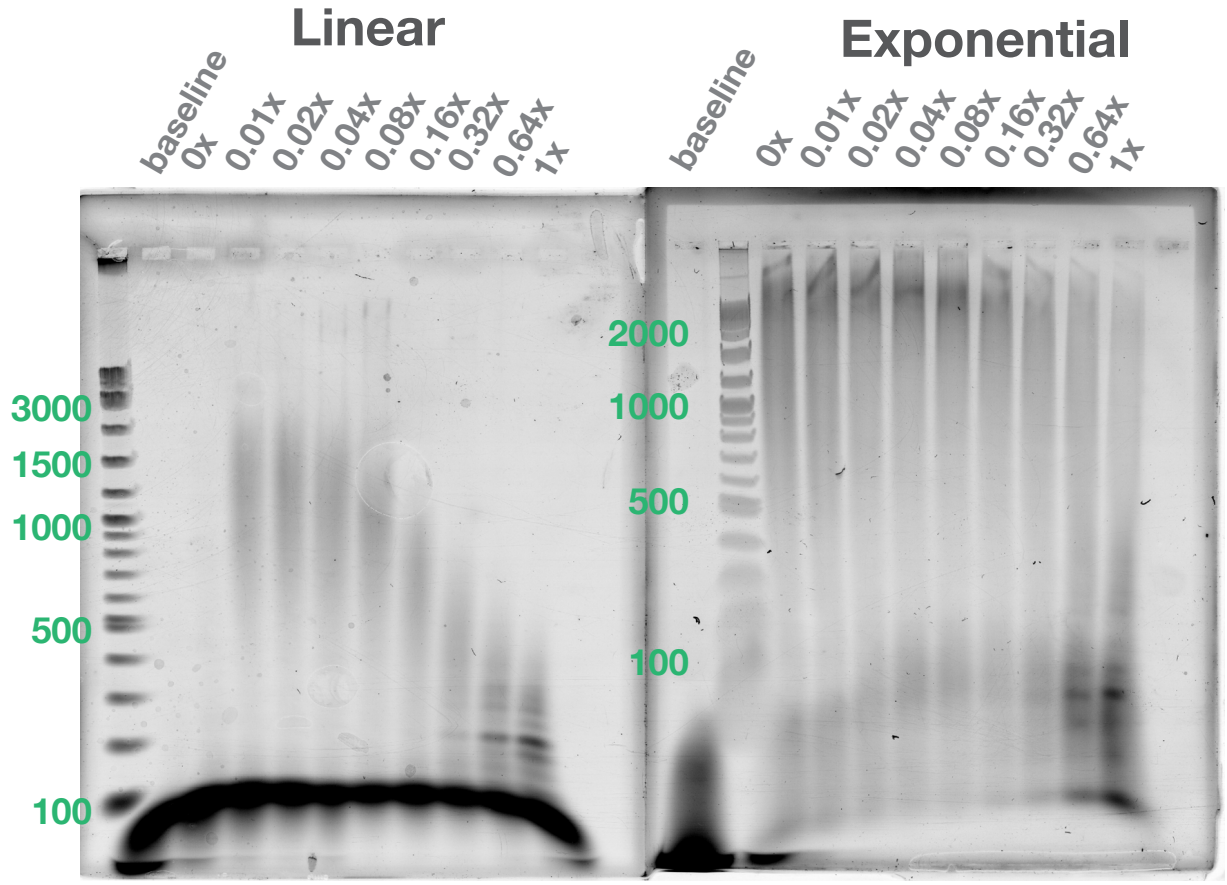

Figure B.5: The Spectrofluorimetry linear and exponential final values gels from Figure 4.9 are shown here after being stained with SYBR Gold. Post-staining makes the DNA ladder visible, allowing for the proper size classification of the polymers.

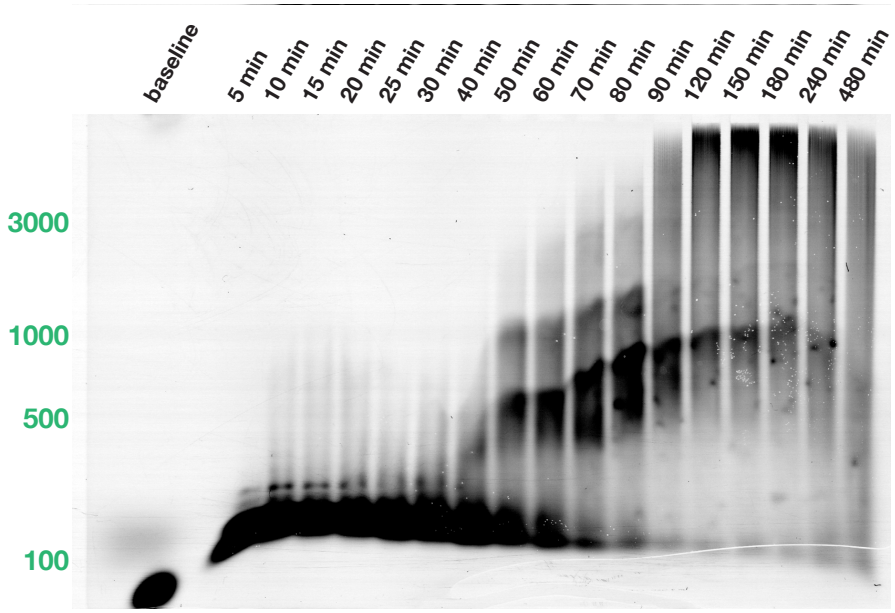

Figure B.6: Gel time-lapse studies of exponential polymer growth. Super Fine Resolution Agarose non-denaturing gels of the product of a polymerization reaction with 80 nM ROX-labeled Initiator, 1.5 μM Hairpin 1, and 1 μM of Hairpin 2 and Hairpin 3. ROX fluorescence was imaged prior to staining with SYBR Gold. Two additional experimental runs of this experiment can be found in Figures 4.13 and B.8.

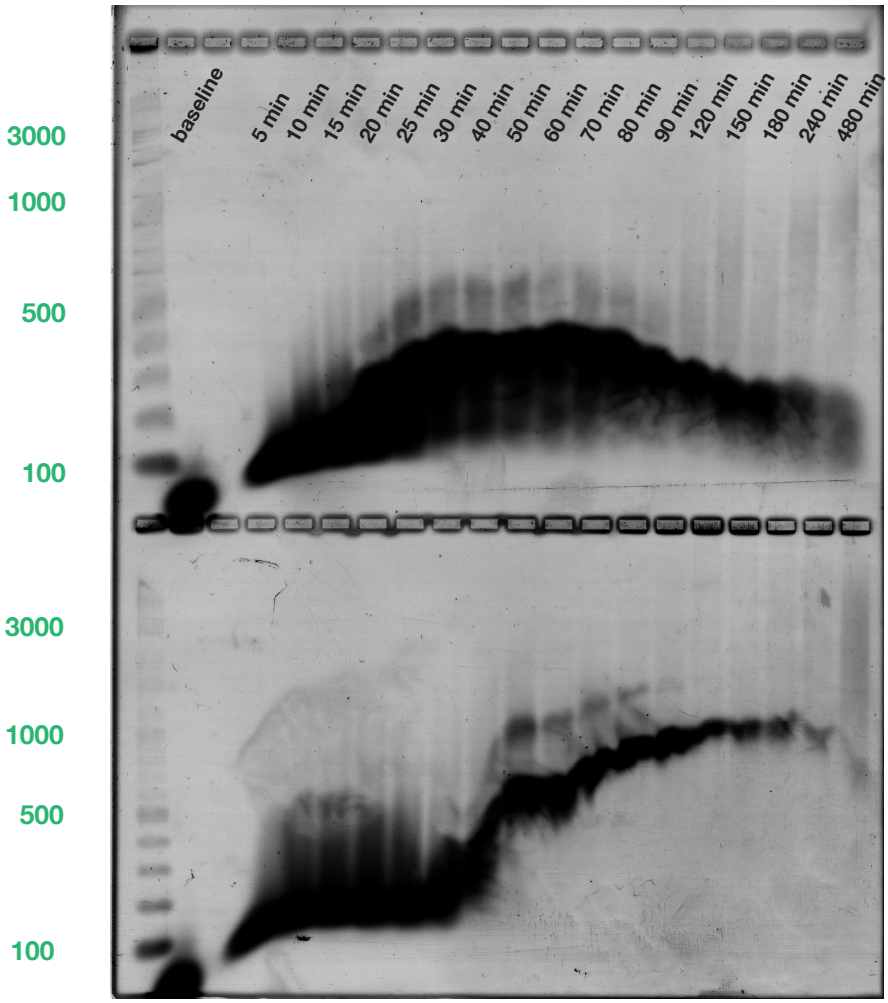

Figure B.7: The time lapse gels from Figure 4.12 (top) and Figure B.6 (bottom) are shown here after being stained with SYBR Gold. Post-staining makes the DNA ladder visible, allowing for the proper size classification of the polymers.

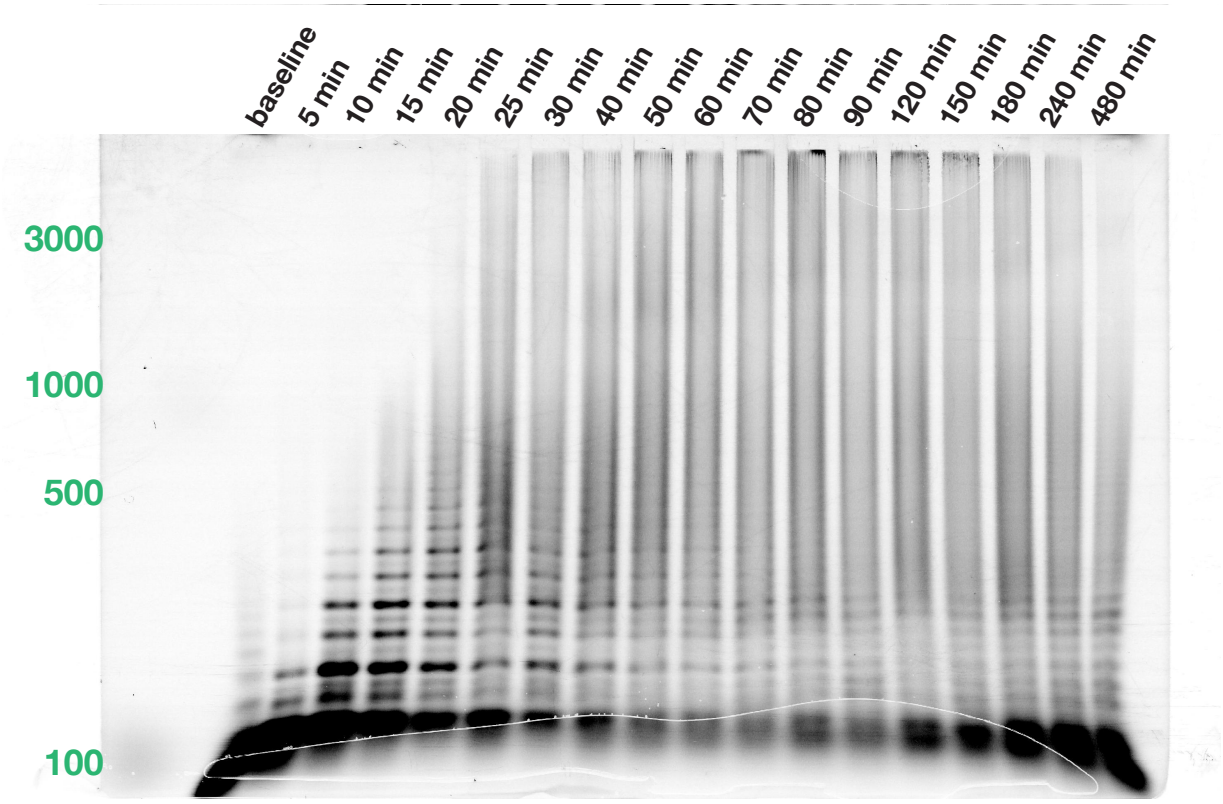

Figure B.8: Gel time-lapse studies of exponential polymer growth. Super Fine Resolution Agarose non-denaturing gels of the product of a polymerization reaction with 80 nM ROX-labeled Initiator, 1.5 μM Hairpin 1, and 1 μM of Hairpin 2 and Hairpin 3. ROX fluorescence was imaged prior to staining with SYBR Gold. Two additional experimental runs of this experiment can be found in Figures 4.13 and B.6.

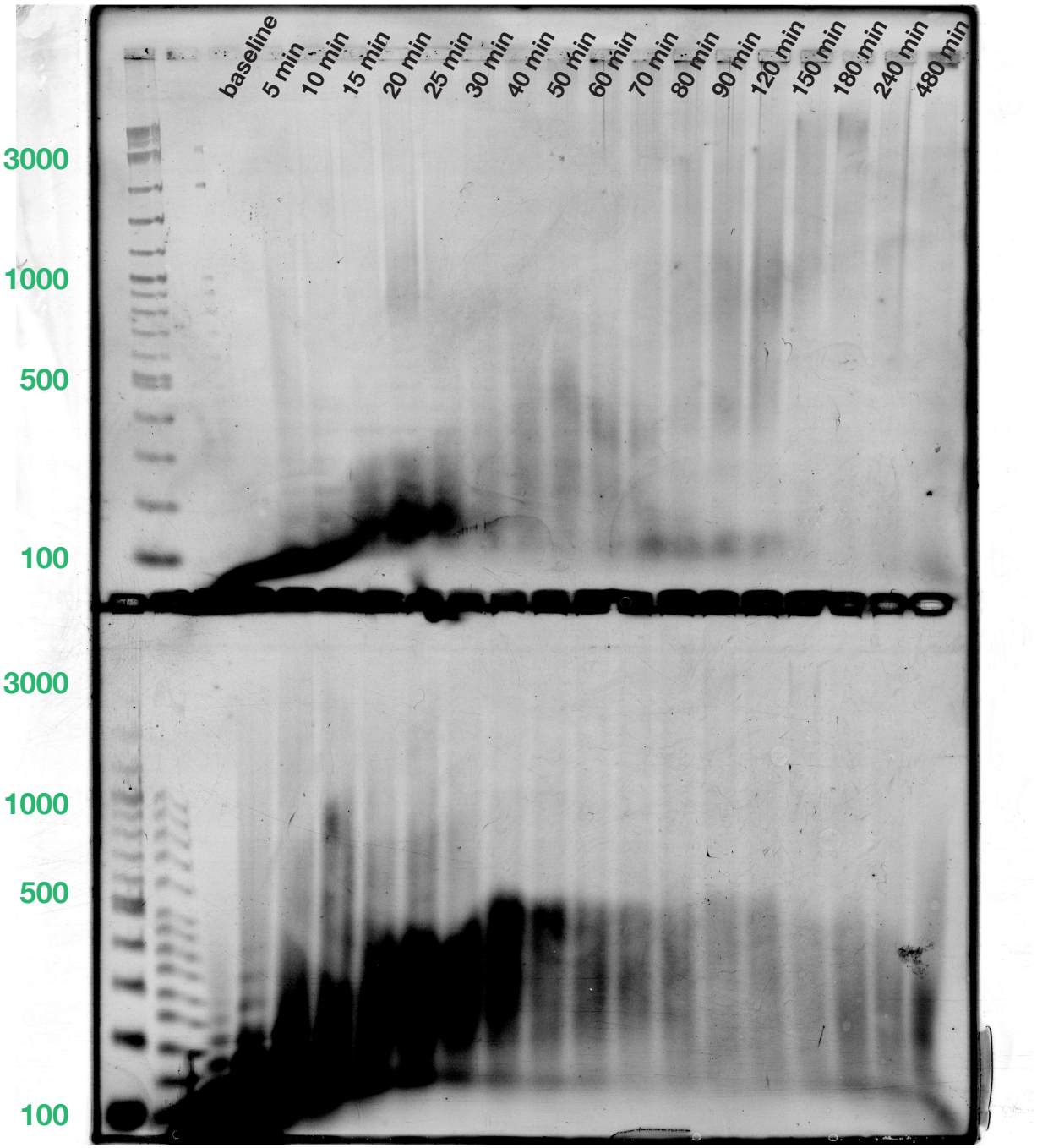

Figure B.9: The time lapse gels from Figure 4.13 (top) and Figure B.8 (bottom) are shown here after being stained with SYBR Gold. Post-staining makes the DNA ladder visible, allowing for the proper size classification of the polymers.

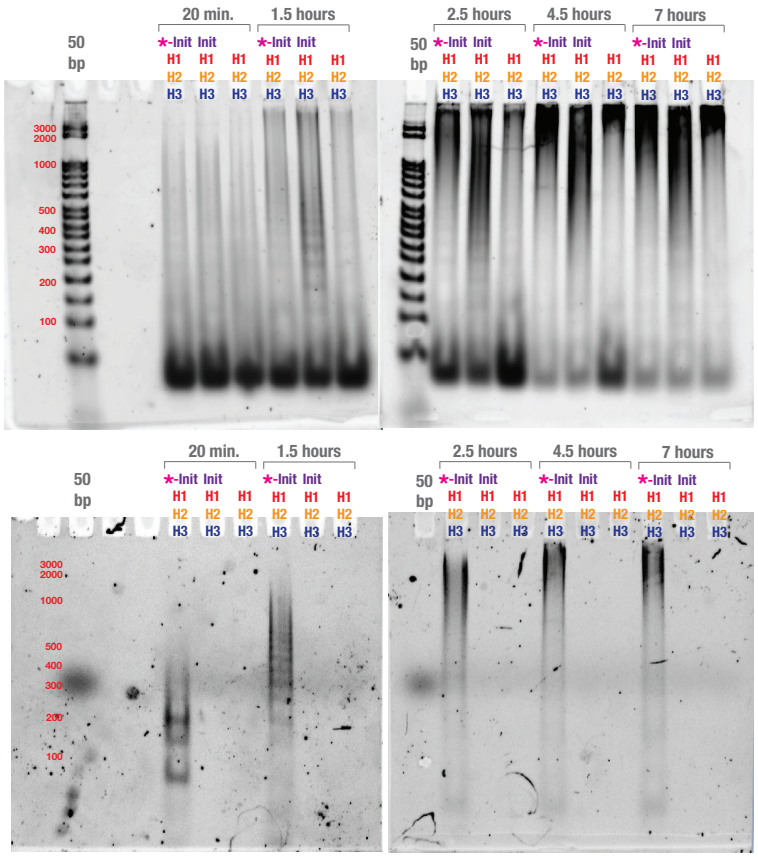

Figure B.10: Gel time-lapse studies of polymer growth. a) Polyacrylamide gels of the product of a reaction with 50 nM Initiator, 625 nM Hairpin 1, and 500nM of Hairpin2 and Hairpin 3. b) Agarose gels of the same samples.

B.2 Division Experiments

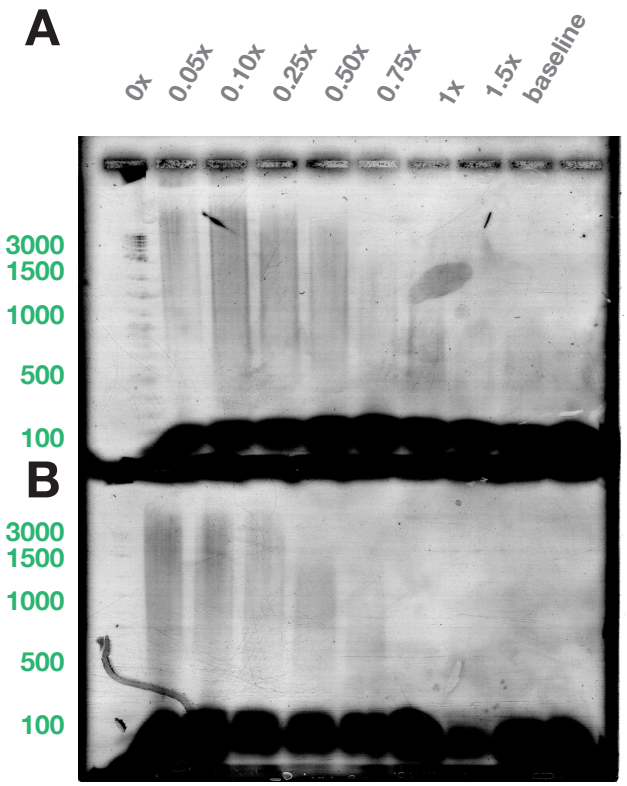

Figure B.11: The polymer division gels from Figure 4.16 are shown here after being stained with SYBR Gold. Post-staining makes the DNA ladder visible, allowing for the proper size classification of the polymers.

B.2.1 Division Kinetics

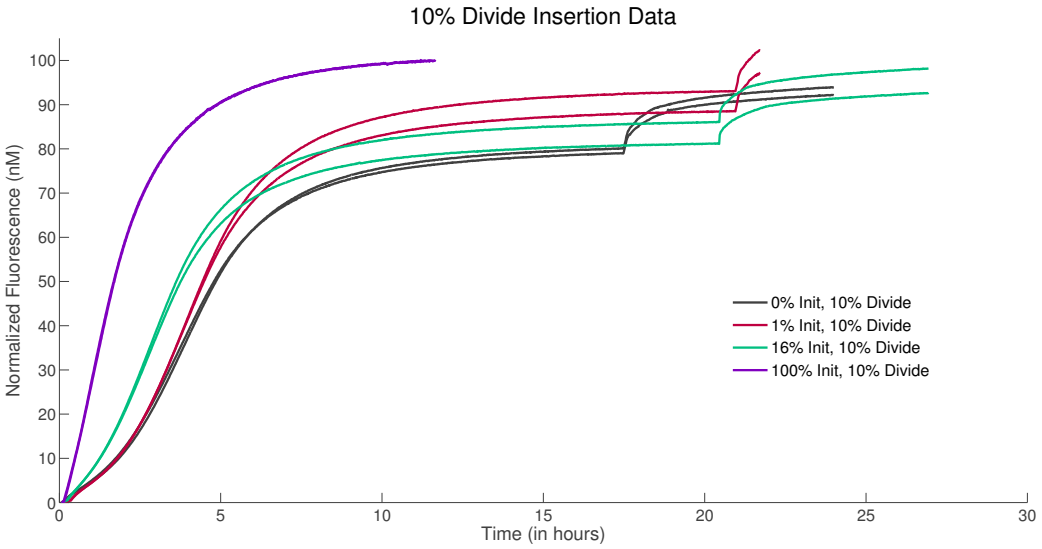

Figure B.12: Polymer division kinetics examined via fluorescence. As Hairpin 2 is incorporated into the growing and dividing polymer, the system's fluorescence increases. Plotted above are the kinetic traces of Hairpin 2 (all hairpins are present at 100 nM, the Divide complex is present at 10 nM) with varying amounts of Initiator.

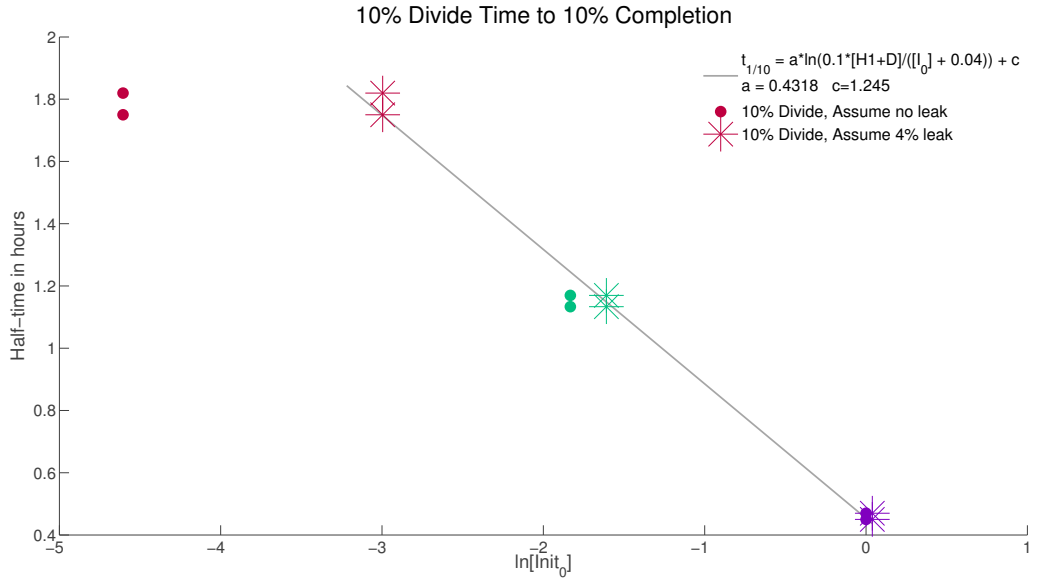

Figure B.13: Linear fit of the 10% completion time as a function of the relative concentration of Initiator to Hairpins. Filled circles correspond to a system where we assume no leak. Asterisks indicated the same points but assuming a leak equivalent to 4% of the Initiator concentration.

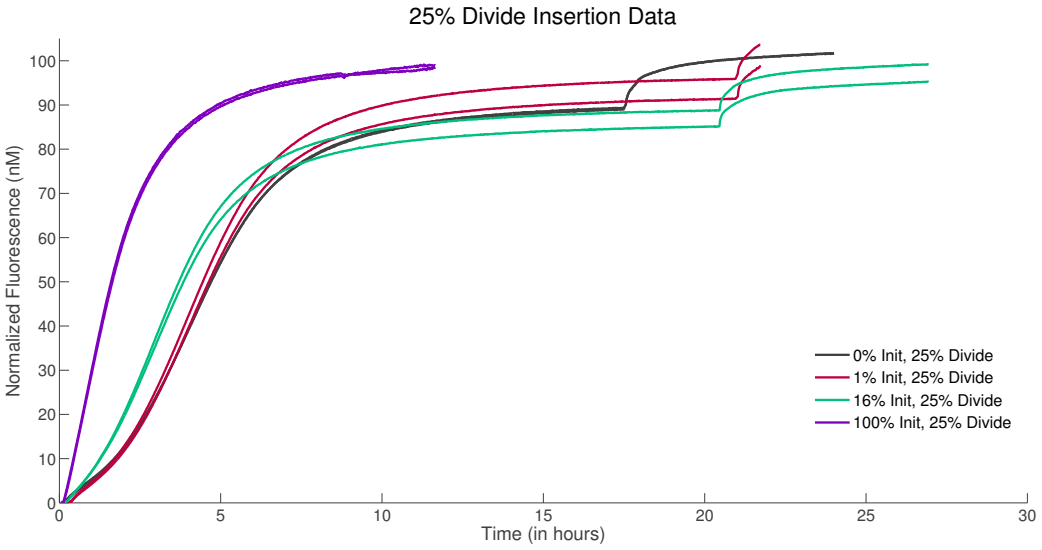

Figure B.14: Polymer division kinetics examined via fluorescence. As Hairpin 2 is incorporated into the growing and dividing polymer, the system's fluorescence increases. Plotted above are the kinetic traces of Hairpin 2 (all hairpins are present at 100 nM, the Divide complex is present at 25 nM) with varying amounts of Initiator.

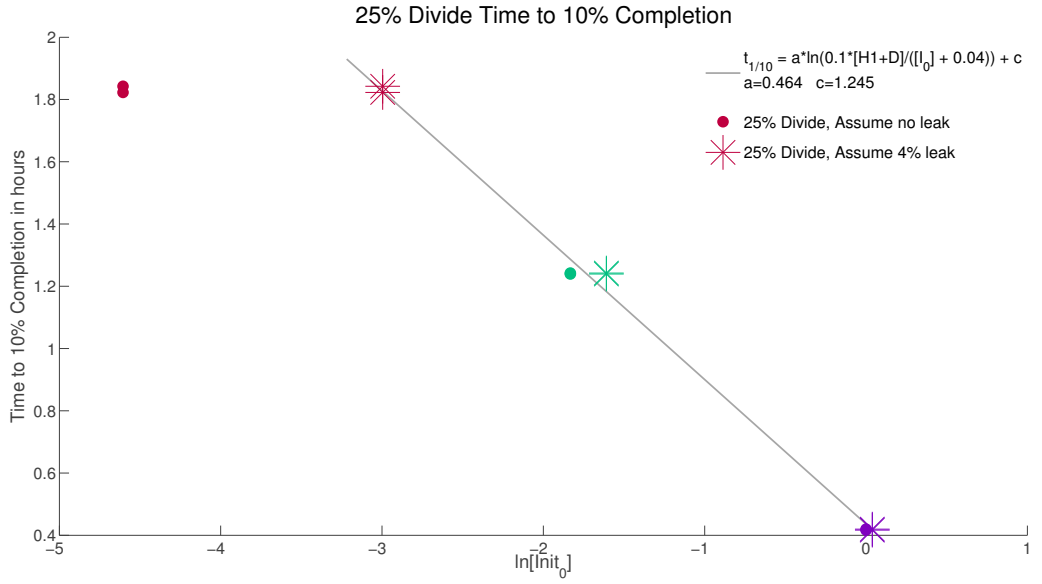

Figure B.15: Linear fit of the 10% completion time as a function of the relative concentration of Initiator to Hairpins. Filled circles correspond to a system where we assume no leak. Asterisks indicated the same points but assuming a leak equivalent to 4% of the Initiator concentration.