Enzyme Free DNA Amplification

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Abstract

This paper examines a scheme for extending the quantitative range of an autocatalytic DNA amplifier and quantifier developed by Zhang et. al. [2], with a view to developing a useable enzyme-free, constant-temperature alternative to PCR for DNA detection. We found that the addition of a simple reaction to the scheme in [2] affected the saturation value of the circuit in a concentration-dependent way for high concentrations, and that this behaviour could be used to quantify larger concentrations of DNA. We designed and tested a part of the circuit, but were not able to obtain any significant results, due to a technical error in understanding the toehold exchange model we used.

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1 Introduction

DNA amplification is ubiquitous in today's world, from diagnostic medicine, where small amounts of DNA collected from a patient need to be amplified to diagnose a disease, to crime scene investigations, where small amounts of DNA need to be amplified to identify a suspect.

The most prevalent method of DNA Amplification is PCR - Polymerase Chain Reaction. The method was developed by Kary Mullis in 1983, and involves the addition of enzymes and primers to a small amount of DNA followed by thermal heating and cooling of the sample [1].

PCR is highly efficient and accurate. However, there are a number of problems. The process is reasonably expensive, for two reasons – first, the enzymes used cost a non-negligible amount, and second of all, the process is covered by a number of patents. The process is also somewhat inconvenient – samples need to be shipped back to a laboratory and thermally cycled to achieve amplification, and the process takes some time.

The aim of this project was to work towards a new method of DNA detection that is *enzyme free* and that operates at *constant temperature*. Specifically, we worked on extending the range of an autocatalytic DNA-based amplifier and quantifier developed by Zhang et. al. [1].

In this paper, we shall first examine the system in [1]. We will then explain our scheme for modifying the system. Finally, we will discuss the steps we took in implementing this scheme and our results.

2 Enzyme-free DNA Amplification

The enzyme-free DNA amplification system we will be studying was introduced in [1] as an application of an entropy-driven DNA catalyst.

At the crux of the mechanism lies an amplifier species capable of carrying out an autocatalytic reaction with the sample we wish to amplify. A simplified reaction scheme is

$$\text{Sample} + \text{Amplifier} \rightarrow 2 \times \text{Sample} + \text{Signal} \tag{1}$$

The signal molecule produced acts as an indicator of how far the reaction has gone.

Experimental results obtained using this system are reproduced in figure 1.

Evidently, the system performs as expected. Even starting with infinitesimal amounts of sample, a large amount of signal is produced. Furthermore, the rate at which signal is produced depends on the original amount of sample present. This allows us to make the method quantitative – by looking at the half-time

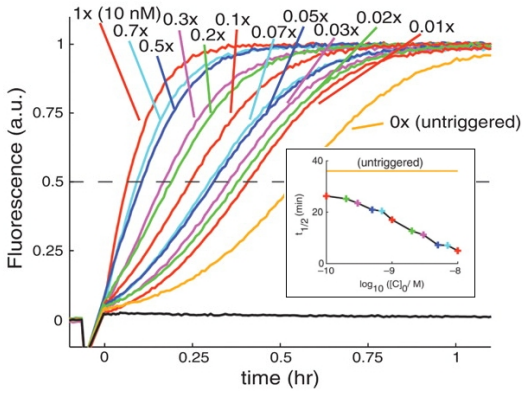

Figure 1: The current autocatalytic amplifier presented in [1]. Each curve was obtained by adding a different amount of sample to a fixed amount of amplifier species at time $t = 0$. The progress of the reaction was tracked using a fluorescence reporter.

of the reaction (a measure of how fast it goes) we can determine what original amount of sample was present.

There is, however, a problem – it is apparent from figure 1 that the quantitative range of the amplifier is very limited. It can amplify concentrations from 0.1nm to 10nm. This seriously limits its applications to practical situations.

3 Extending the range of amplification

The aim of this project, therefore, was to extend the range of the amplifier. A number of methods were investigated, and the one that was found most efficient was to add a second equation to the mechanism. In other words, as well as Equation 1, the amplifier needs to include the following reaction

$$\text{Signal} + \text{Sample} \rightarrow \text{Nothing} \tag{2}$$

By analogy to physical systems, this reaction 'damps' the system.

The simulated behaviour of the modified system is illustrated in Figure 2. The system behaves as before for low concentrations of sample, but at high concentration, saturation behaviour is observed, with the final fluorescence inversely proportional to the amount of sample added. This behaviour can be used to extend the range of the amplifier – at low concentration, the half-time can be used to determine initial sample concentration (as before) whereas at high concentrations, the final saturation value can be used.

4 Designing the damping reaction

The design of appropriate DNA species to carry out the reaction in reaction 2 was tricky, because they had to be compatible with the species involved in the main amplifying reaction (reaction 1).

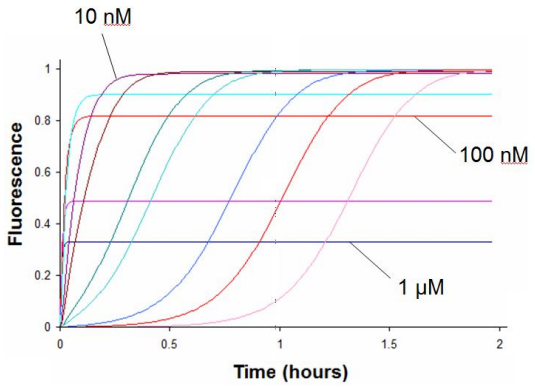

Figure 2: Mass-action kinetics simulation of the extended amplifier, with damping reaction 2. Each curve represents a different amount of sample added at $t = 0$. The system behaves as before for low concentrations of sample, but at high concentration, saturation behaviour is observed, with the final fluorescence inversely proportional to the amount of sample added.

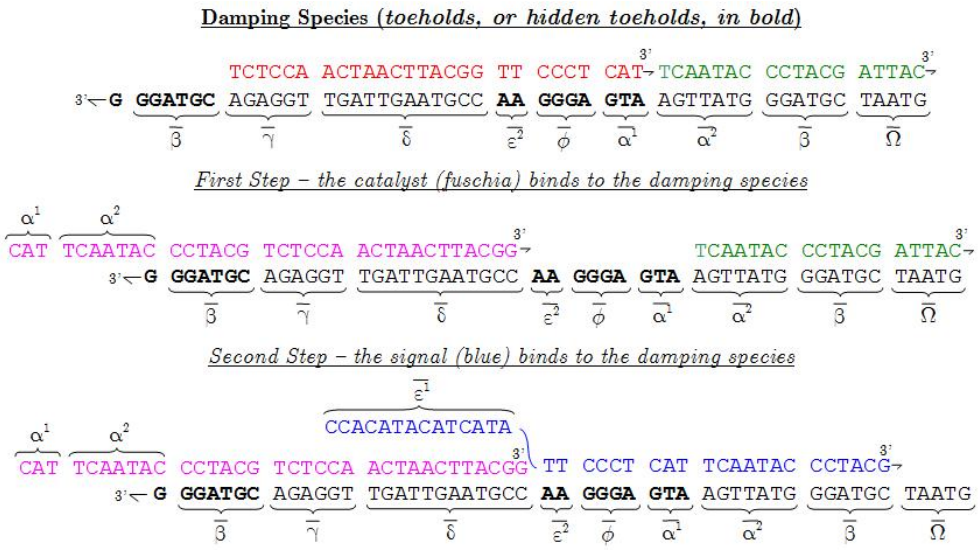

Figure 3: The design of the DNA strands used in the damping reaction. The mechanism of action of this gate is also illustrated.

	Step 1	Step 2	Step 3
Forward rate	1.3×10^5	2.9×10^6	608
Backward rate	2.8×10^6	0.05	0.99

Table 1: The predicted rate of each reaction in the damping system, in $s^{-1}M^{-2}$, at room temperature.

	Damping species (nm)	Signal (nm)	Catalyst (nm)
Experiment 1	200	200	200
Experiment 2	100	100	400

Table 2: Summary of reactions carried out.

The design we used is in Figure 3, and represents a trade-off between the various factors we had to take into considerations.

The function of the damping species involves three steps (for an explanation of the terminology and notation in this discussion see, for example, [2]). The catalyst first binds to the gate via a toehold sequence β. The strand $\gamma\delta\epsilon^2\phi\alpha^1$ then falls of by branch migration (Step 1). The signal then binds to the remaining complex via the toehold $\epsilon^2\phi\alpha^1$, and strand $\alpha^2\beta\Omega$ falls off by branch migration (Step 2). There is also a competing reaction, in which the catalyst binds in the second step instead of the signal (Step 3). Steps 1 and 2 are also illustrated in figure 3.

The result is, as desired, the 1:1 annihilation of a signal and a catalyst molecule.

The theoretical rate of operation of this gate was calculated using a two-state model of toehold exchange developed by Zhang in [3]. The binding energies of each of the toeholds were worked out using a model developed by Zucker and Turner [4]. The resulting forward and backward rates for each of the steps above are listed in table 1.

The rates for each of the steps result in the desired amplification behaviour, and the rate of the leak reaction is small enough not to make a difference.

5 Experimental results

Experiments were carried out to analyse the operation of the damping species, using time-lapse PAGE gels to monitor the progress of the reactions over time. Table 2 summarises the reactions that were carried out.

The concentration of each species at each time point was determined by quantifying the brightness of the relevant band on the gel. The gels used in this experiment were somewhat inaccurate, and the results were therefore somewhat erratic.

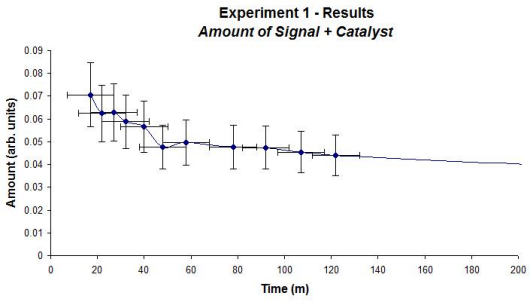

Figure 4: The graph of Signal + Catalyst concentration against time for experiment 1 (200nm of each of the damping species, catalyst and signal).

The most accurate result was obtained by looking at the amount of signal and catalyst in the gel at various time points. The results for experiments 1 are shown in figure 4, and those for experiment 2 in figure 5.

6 Analysis

The results of both experiments clearly indicate a problem. Observation of the relevant graph seems to indicate that the half time for the reaction in experiment 1 was roughly 40 min. From the rates calculated above, this half time should be roughly 53 seconds. Clearly, the reaction proceeds much too slowly.

This mistake arises from a misinterpretation of the model we used for toehold exchange. The model holds for very low concentrations of reagents, but fails for higher reagents. The reason lies in what we called 'step 1' – the $\gamma\delta\epsilon^2\phi\alpha^1$ part of the strand falling off. The assumption in the model is that this happens relatively fast compared to other reactions in the system. However, the strand is long, and it in fact falls off very slowly, and this seriously reduces the speed of our system.

Another important observation is that experiment 2 reaches equilibrium fairly quickly, and more or less remains there. This is by design; had the reaction continued, we might have concluded that the catalyst 'leak' reaction was playing a significant role. We prevented this from happening in two ways. First, the toehold for the catalyst in the second stage of the reaction was shorter than for the signal. Second, when the catalyst bound to the gate, a toehold remained for the reverse reaction to happen. The reaction was therefore reversible. When the signal binds, however, it completely displaces occluding strands and leaves no toehold for the reverse reaction to happen. This makes the signal-binding

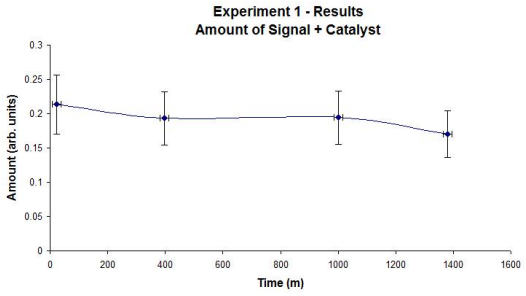

Figure 5: The graph of Signal + Catalyst concentration against time for experiment 2 (100nm of each of the damping species and signal, and 400nm of catalyst. Due to lack of tiem, the resolution was much lower than for the graph in experiment 1.

reaction much more likely.

7 Conclusions

We investigated the DNA detection, quantisation and amplification model developed by Zhang, et. al. [2]. We noted the fact that the range of concentrations the amplifier was able to detect as seriously limited, and we looked for a way to extend this range. We tried a damping reaction, that led to encouraging results when simulated, and we designed DNA strands for that reaction. Unfortunately, due to a misunderstanding in the model our design was based on, the added reaction did not work as expected, but ran much too slowly.

Opportunities for further work abound. The damping reaction should be redesigned to make the second toehold shorter, and to make the reaction proceed faster. This might involve re-designing the original system in [2]. Work could also be done to investigate the temperature dependence of these systems – preliminary data was collected to that effect, but the resolution was too low to lead to any significant conclusions.

8 References

1. something about PCR
2. Zhang, Tuberfield, Yurke & Winfree. Science 318, 1121 (2007)

3. Zhang & Winfree. "Control of DNA Strand Displacement Kinetics using Toehold Exchange" (2008)

4. Applet by Zucker and Turner, available at http://mfold.bioinfo.rpi.edu/cgi-bin/dna-form1.cgi