CamSURF Progress Report

Daniel Guetta
Supervisor: Erik Winfree

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1 Introduction & Summary

The main focus of our research this summer is on DNA amplification circuits. The original plan was to focus on thresholding in such DNA circuits, and the efficiency and effectiveness of such thresholds. This is still a viable option, and some progress has been made in that respect, but the main focus of the project has somewhat shifted to extending the range of DNA amplifiers, and using such large-range amplifiers as a tool to quantify the amount of DNA present in solution – effectively, a DNA circuit alternative to quantitative PCR.

Our work so far has been mostly theoretical – we have looked at a number of DNA amplification circuits, and attempted to modify them to improve their performance in some way or other. The behaviour of circuits was examined on a computer using a computer program written by Dave Yu Zhang and a number of custom made MathCad macros. I have, however, also spent some time familiarising myself with the equipment in the lab, in anticipation of experiments we will be carrying out in the next few weeks.

This reports first gives a brief summary of the existing circuits considered, and then gives an account of the methods we have tried to improve them.

Please note that this report is still a work in progress and, as such, is still incomplete in a number of places. The reader will therefore find a number of sections in which we have not yet included all the material mentioned in the introduction of that section.

2 Existing Amplification Circuits

There exists a number of amplification circuits – so far, we have focused on the first:

- An exponential, autocatalytic amplifier
- A simple linear catalytic amplifier
- A quadratic feed-forward catalytic cascade

2.1 The exponential, autocatalytic amplifier

This was the main system we investigated, and is introuced by Zhang [1].

2.2 Function

At the crux of the autocatalytic amplifier is the following reaction:

$$\text{Catalyst} + \text{Gate} \rightarrow 2\ \text{Catalyst} + \text{Output}$$

As such, at every cycle of the system, more catalyst is produced, and the rate of output production increases exponentially.

2.3 Discussion

The main strengths of the autocatalytic amplifier are as follows:

- The system can amplify even the tiniest of signals, in a manageable amount of time.
- For low concentrations, the function of the amplifier is strongly dependent on the initial concentration of catalyst – it is therefore relatively easy to use the amplifier to accurately determine the amount of catalyst present in a given solution.

However, it also has a number of weaknesses:

- As the concentration increases, the amplifier quickly "saturates". It is then almost imposible, for higher concentration, to dertermine accurately the amount of catalyst that was originally present in the system.
- Due to inevitable leaks in the system, and the amplifier's formidable amplifying abilities, the amplifier responds

2.4 Aims for improvement

There were two aims we were hoping to achieve by improving our circuits, and we dealt with each separately:

- Increasing the range of our amplifiers to allow them to detect higher and higher concentrations while still retaining the ability to amplify low concentrations within reasonable time scales.
- Increasing the ability of our amplifiers to detect tiny signals on a constant background, where the magnitude of the noise may well be larger than that of our signal.

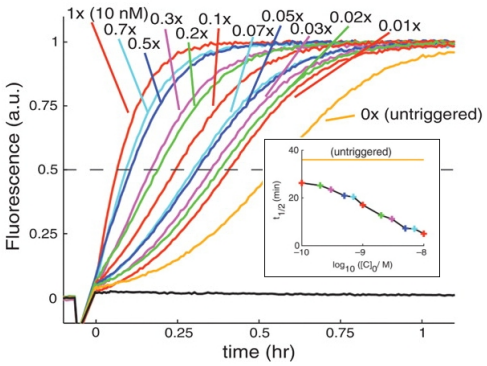

Figure 1: Performance of the exponential autocatalytic amplifier (reproduced from [1]). As is evident from the inset, it is possible to use the reaction (characterised by its half time) to determine the original amount of catalyst present.

3 Strategy I – reducing the "0" level

DNA circuit are not perfect – there is always a "leak" reaction going on which produces a constant background signal. This is especialy true of the autocatalytic amplifier circuit, which hugely amplifies the smallest of signals. This, in effects, "wastes" part of the range of the circuit, which it would be able to amplify and detect were it not for the constant background.

We reasoned that by somehow eliminating this background signal from the circuit, we could recover some wasted "wasted" range.

Our investigations mostly concerned the autocatalytic amplifier, in which the background level is most problematic. We implemented two methods to attempt to reduce the background level. The first involved adding a certain amount of species that "scavenges" through the reaction mixture and eliminates any signal it finds. By making the reaction slow enough and the concentration of the scavenger low enough, we were hoping to eliminate noise, but not the signal itself. In the second method, we produced the scavenger species "on the fly".

3.1 Introduction of a "scavenger" species

We first experimented with the introduction of a scavenger species with the ability to carry out the following reaction:

$$\text{Catalyst} + \text{Scavenger} \rightarrow \textit{Inert species}$$

As is apparent in Figure 2, this system does work to some extent – the background level is significantly reduced.

Interestingly, it was found that the rate constant of the scavenger reaction is ciritical in determining the behaviour of the circuit. A high rate constant results in a "threshold" behaviour, whereas a low rate constant has no effect.

Though this system does work in reducing the background level, there is a major problems - the modified amplifier does not, as we would have hoped, increase the range of the amplifier. High concentrations are still "bunched up together". The only advantage we have gained is, possibly, a higher accuracy for the measurement of middling concentrations.

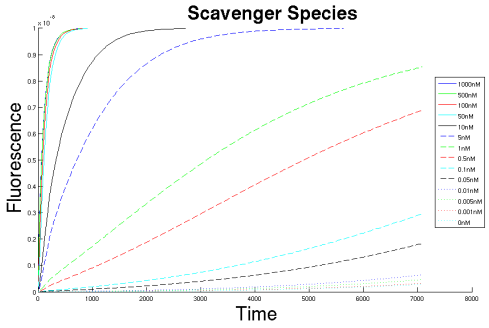

Figure 2: Autocatalytic system with 10 nM scavenger added at t = 0. Gate concentrations were 10 nM, and the rate constant for the scavenger reaction was 4×10^5.

3.2 "On the fly" production of scavenger

We suspected that the reason this system was somewhat inneficient was because the scavenger concentration never really matched the leak concentration – we had to add a large amount of scavenger at the start of the reaction to "swamp up" any future leak reactions instead of being able to provide scavenger as and when a leak occured. As a result, the scavenger present at the start of the reaction might also have absorbed some signal.

To remedy to this, we imagined a system in which a "Precusor scavenger" was present in the reaction and would slowly release scavenger into the reaction mixture. The idea was that we would make the Catalyst/Scavenger reaction very fast, but the scavenger release reaction very slow. This would ensure the scavenger was able to swamp up the leak at each stage of the reaction, but nothing else.

The results were somewhat encouraging (see Figure 3) in that the background level is reduced, but were rather unhelpful in terms of extending the range of the amplifier. The high and low concentrations were still "bunched together".

Much more interestingly, though, we noticed that the system was also behaving as a threshold (looking at the concentration of the threshold precursor

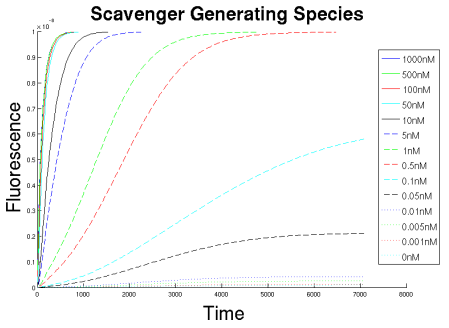

Figure 3: Using a "building up scavenger" system to reduce the background noise in the amplifier. This figure was obtained using a scavenger production rate constant of 1×10^{-6} and an original precursor-scavenger concentration of 1μM.

as the reaction proceeded confirmed that the effect was "real", and that we weren't just seeing a "trivial" threshold as the precursor was used up). Not only that, but we were able to control the position of the threshold by varying the rate of precursor threshold decay into the threshold species (see Figure 4).

This result opens up the possibility of analysing the concentration of a starting species in the time domain. Since the concentration of the precusor dynamically changes as the reaction proceeds, the position of the threshold is also constantly changing. There should, therefore, be a way to design a circuit that "catches" a given concentration at a given time, from which we can deduce the original concentration. The problem seems to be that the precursor does not decay fast enough to change the position of the threshold in any sensible amount of time. Using temperature as a variable might be an alternative (as temperature falls, the rate falls), but complications would ensue, because other rates would also change with temperature.

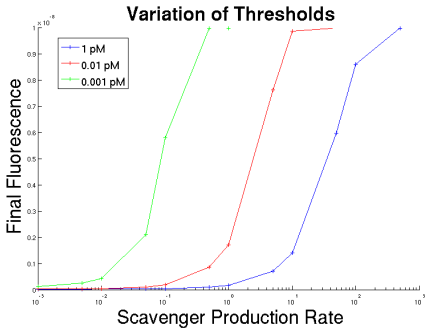

Figure 4: Variation of threshold position with rate constant of the threshold producing reaction.

Idea?

4 Strategy II – "Damping" the system

We attempted to draw analogies from damped oscillating systems in physics. To implement this behaviour in our chemical circuit, we added an additional reaction in our scheme, of the form

$$\text{Catalyst} + \text{Product} \rightarrow \textit{Inert Species}$$

The results are illustrated in Figure 5 for the autocatalytic system. At low concentrations, the system behaved as normal, but at high concentrations, the circuit saturates much earlier, with a much reduced "final fluorescence".

This means that each concentration now has two pieces of data associated with it. These two pieces of data are resolved at different concentrations, and one can be used where the other is not so well resolved. This considerably extends the working range of the amplifier.

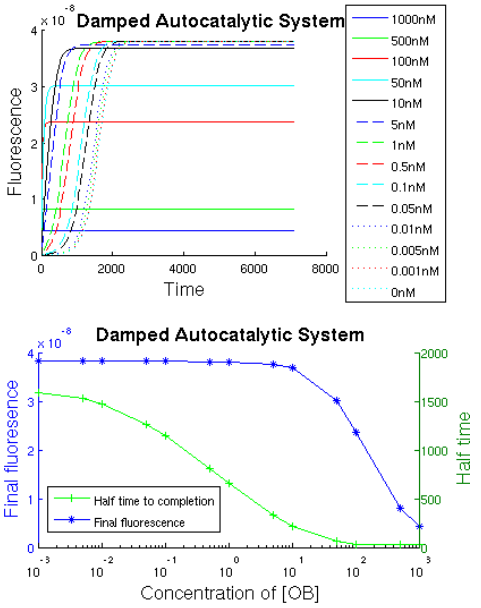

Figure 5: The "damped" autocatalytic amplifer. There are now *two* pieces of information for each concentration – the half-time and the final concentration. The former is resolved for low concentrations, and the latter is resolved for high concentrations. This considerably extends the working range of the amplifier. *Diagram calculated with gate concentrations of 10 nM and 13 nM, concentration of reporter species 30 nM, and rate constant for the annihilation reaction* 4×10^5.

4.1 Discussion

The characteristic behaviour of this system arises from the fact that the **output strand** can go two ways:

- It can either proceed to the downstream part of a circuit (in this case, the fluorescence reporter)
- Or it can react with catalyst, producing an inert species

The key point, however, is that the rate of first reaction is **independent of original catalyst concentration**, whereas the second reaction **increases exponentially with orignial catalyst concentration**. As such:

- At low catalyst concentrations, the second reaction is relatively slow throughout the reaction, and the sytsem behaves are previously expected.

- At high catalyst concentrations, the second reaction is extremely fast. Some signal escapes to produce some fluorescence, but most of it is quickly captured by the catalyst. The reaction also reaches plateau relatively fast, because the autocatalytic reaction is very fast.

This mechanism is consistent with various observations (these observations were all made for the autocatalytic amplifier sytsem):

- As more gate/reporter is added, the circuit shifts its sensitivity to higher concentrations – the concentration only matters by virtue of how big it is compared to the gate species. In other words, an initial catalyst concentration of 10 nM will give the same result in the original circuit as a 100 nM starting concentration will in a circuit in which tenfold concentrations of each species have been added.
- The behaviour of the circuit as the rate of the Catalyst + Product $\rightarrow$*Inert Species* reaction changes is as follows:
  - At low values of this rate constant, the circuit behaves as if the initial catalyst concentrations were much smaller (ie: just as if we'd increased the concentration of the gates). This is sensible – we determined above that the concentration of catalyst affects the circuit by affecting the rate of this particular reaction. If we decrease the rate manually, we will, of course, obtain the same effect than we would if we had decreased it by decreasing the catalyst concentration.
  - When this rate constant is roughly equal to the reporter system rate constant, the behaviour above is observed.
  - When this rate constant is significantly higher, one obtains, as expected, the reverse of the behaviour above – the concentrations behave as if they were much larger.

  There is a further rather subtle effect arising from changing this rate constant. At very high rate constants, the rate constant first *increases slightly* as the concentration increases, and only then does it start to decrease (Figure X). We hoped we would be able to use this behaviour to detect even lower concentration, but simulations at lower concentrations revealed that this would not be possible (Figure 6).

  We suspect that the origin of this behaviour comes from a "trade-off" between the effect discussed above and the fact that the autocatalytic amplifier is an extremely efficient amplification system. As concentration is increased, the amplification properties "win out". It is very unclear to us, however, why this process is only apparent at high rate constants.

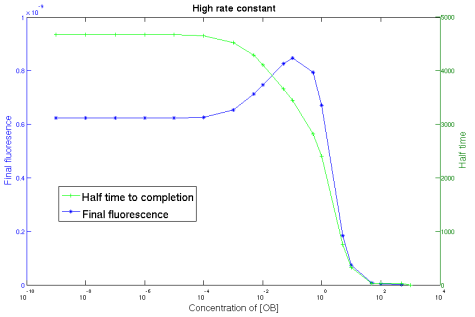

Figure 6: At high values of the rate constant for the damping reaction, the system shows interesting behaviours at low concentrations. This, however, quickly dissapears for *very* low concentrations.

Question!!

- Increasing the reporter complex concentration once again "shifts" the behaviour of input concentrations, and makes it look like concentrations are lower than they really are. Once again, this can be understood in terms of the scheme above – increasing the reporter complex concentration increases the rate of the fluorescence reaction, and makes the rate of the reaction with the catalyst considerably smaller.

In retrospect, it seems that we have found three different ways to shift the concentrations the amplifier "sees", regardless of what they actually. This cuold be useful in fine-tuning our circuits below.

4.2 Practical issues

The main practical isue with this "damped circuit" idea is the difficulty of implementing the Catalyst + Product →*Inert Species* reaction. We explore a number of possibilities:

1. Designing the signal and catalyst strands to be complementary to each other.
2. Using an AND gate of sorts, that is activated by *both* the signals we want to destroy. The reactions involved are

   $$\begin{gathered}\text{Catalyst} + \text{AND gate} \rightleftharpoons [\text{AND Catalyst complex}] \\ \text{Catalyst} + [\text{AND Signal complex}] \rightarrow \textit{Inert species} \\ \textit{as well as} \\ \text{Signal} + \text{AND gate} \rightleftharpoons [\text{AND Signal complex}] \\ \text{Signal} + [\text{AND Catalyst complex}] \rightarrow \textit{Inert species}\end{gathered}$$

   The two pairs of reactions grouped together \emph{must} have similar rates (because they involve the same types of reactions), but the gate design could be such that they are drastically different.
3. Using a modified AND gate, in which it is *not* the case that the pair of reactions grouped above must have common rates. For example, the gate might only display a toe-hold sequence for *one* of the two species.
4. Using a translator gate, to translate one of the signals into the complement of the other. The reactions involved would then be:

   $$\begin{gathered}\text{Signal} + \text{Translator} \rightleftharpoons \text{Complement of Catalyst} \\ \text{Catalyst} + \text{Complement of Catalyst} \rightarrow \textit{Inert species}\end{gathered}$$

4.3 The Exponential Autocatalytic Amplifier

We applied this method to the autocatalytic amplifier, using as many of the strategies proposed above. The first strategy proved to be impossible for the autocatalytic amplifier – the gate would never form if the catalyst strand was complementary to the signal strand. The other three methods were implemented.

4.3.1 Using "normal" AND gates

We first attempted to implement the reaction using a "normal" AND gate. There were several variables we investigated:

- Concentration of AND gate
- Concentration of autocatalytic gate
- The rate of each of the AND gate reactions
- The concentration of the reporter species

We investigated each of the factors in turn

Concentration of AND gate The concentration of the AND gate seemed to affect the behaviour of the circuit in many wierd and wonderful ways. At high concentrations, the variation with concentration looked more like a "hump", and, as the concentrations was decreased, started looking more like a "waterfall" (see Figure 7). For our purposes, a concentration of 20 nM gave the largest range over which either the half time or final concentration changed fast enough to make useful measurements.

A (very rough and somewhat dodgy) mechanistic explanation for these observations can be proposed:

- At *high* AND gate concentrations
  - At low catalyst concentrations, only a small amount of signal is produced. This is immediately "trapped" by the very fast AND gate, and so it can no longer cause fluorescence.
  - At higher concentrations, the autocatalytic circuit starts producing lots of more signal (more than can be trapped by the AND gate fast enough), and so fluorescence is produced. Furthermore, the signal is produced over a significant period of time, which gives the reporter an advantage over the fast AND gate.
  - At even higher concentrations, all output is produced instantaneously at the start of the reaction, and so the AND gate has an advantage over the reporter species, and the fluorescence decreases.

  It is interesting to note that the point at which the final fluorescence has, once again, decreased to its final value is roughly equal to the gate concentration, though I can't think of any particularly simple explanation for this.
- At *low* AND gate concentrations – we effectively observe the same process as above, but since the AND gate concentration is small, there isn't enough to trap all of the signal molecules, so the response is not 0 even for low catalyst concentrations.

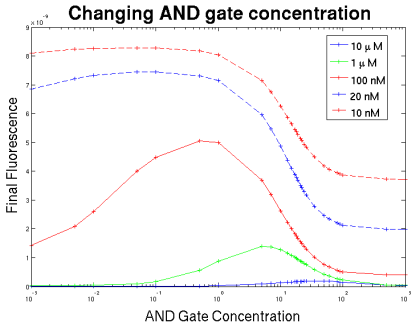

Figure 7: Effect of changing the AND gate concentration on the resulting amplification. These were taken with an autocatalytic gate concentration of 10 nM, a reporter species concentration of 30 nM, and all AND gate reactions at a rate of 3×10^6.

Rate of each AND gate reaction We tried reducing the AND gate reaction rates individually, one after the other.

Reducing the rate at which the signal strand bound to the AND gate had no particularly useful effect (see Figure 8).

However, reducing the rate at which the signal strand bound to the AND gate gave us *just* the trace we wanted (Figure 9). We managed to recover our original pattern, and in fact, even extend it slightly further to high concentrations.

This is, therefore, clearly a viable mechanism for the implementation of this circuit.

If I were to choose this system for implementation, I would investigate the effect of gate concentration again given this particular rate constant...

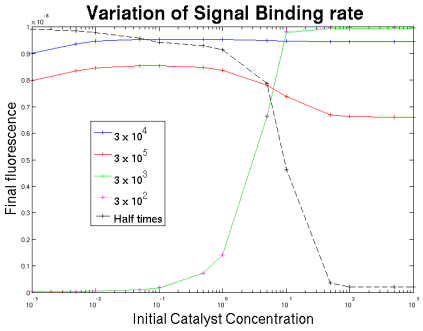

Figure 8: Variation of circuit behaviour with signal binding rate (to the AND gate or catalyst-AND complex). No useful behaviour is observed. *Note that the half-time curve is only approximate and is included for comparisson only. The scale in no way reflects the actual half time, and there will, in fact, be small variations of half-time between rates.*

Success!

4.3.2 Using "modified" AND gates

The "modified" AND gates we will be using are:

- AND gates to which only the signal can initially bind
- AND gates to which only the catalyst can initially bind

In each case, factors we can play with are:

- The concentration of the gate
- The rate of the original binding step
- The rate of the subsequent binding step

This leads to so many possible combinations that we will only report pertinent results.

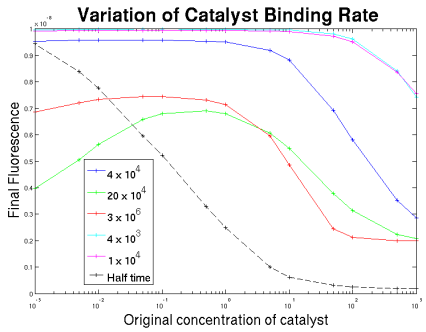

Figure 9: Variation of circuit behaviour with catalyst binding rate (to the AND gate or signal-AND complex). The behaviour at 4×10^4 is exactly what we need. *Note that the half-time curve is only approximate and is included for comparisson only. The scale in no way reflects the actual half time, and there will, in fact, be small variations of half-time between rates.*

AND Gate with initial Catalyst Binding We only briefly investigated the large number of ways such a circuit could be configured, but we found two particularly interesting configurations:

- With the initial gate concentration at 10 nM and the rate of the two reactions 3×10^4 and 3×10^6 respectively, we obtained an almost perfect replica of the system we want (Figure 10).
- With the same initial rate concentration, but which rates 3×10^6 and 3×10^2 respectively, we obtained a reasonably good threshold (Figure 11).

If time allows, this circuit will be investigated further.

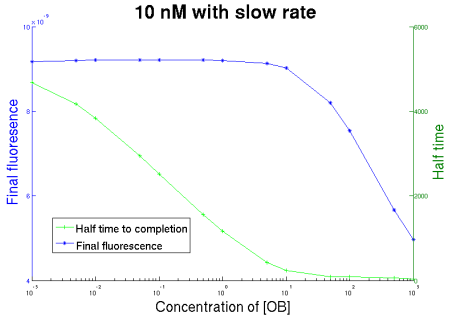

Figure 10: Required behaviour.

AND Gate with initial signal binding No results were particularly worth mentioning. All reduced the range of the amplifier considerably.

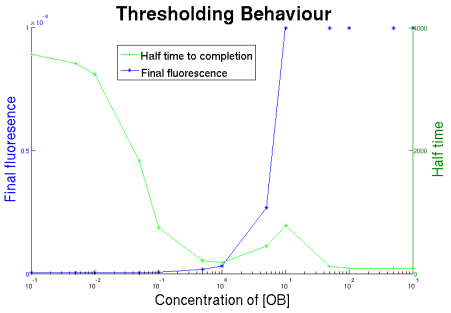

Figure 11: Thresholding behaviour

4.3.3 Using a "translator" gate

We also experimented with a translator gate, which translated the signal to the complement of the catalyst. The reactions involved were:

$$\text{Signal} + \text{Translator} \rightleftharpoons \text{Complement of Catalyst}$$
$$\text{Catalyst} + \text{Complement of Catalyst} \rightarrow \textit{Inert species}$$

There were two variables worth changing in this case:

- The concentration of the gate
- The rate of the translator reaction

Theoretically, the rate of the catalyst + complement catalyst annihilation reaction could also be controlled by making the "complementary" sequence not quite complementary, but this is difficult and bound to give rise to complications, so we did not investigate this effect further.

We first set the rate of the translator reaction to 3×10^6 and varied the original concentration of the gate species. The result, however, looked entirely hopeless. We therfeore decided to investigate the effect of varying the rate first.

Sadly, the result was no better – when we decreased the gate concentration, we effectively got rid of all resolution in our circuit, whereas when the concentration was high, the circuit was resolved in the low-concentration regions, were the half-time is already well resolved (Figure 12).

This method, therefore, seems impracticable for use in our amplification circuit.

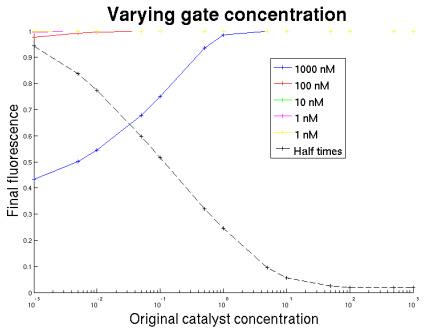

Figure 12: Using a threshold gate. All plots taken with a translator rate of 3×10^4. It is clear, from the plot, that no trace offers good resolution at high initial catalyst concentration. *Note that the half-time curve is only approximate and is included for comparisson only. The scale in no way reflects the actual half time, and there will, in fact, be small variations of half-time between concentrations.*

5 Tiny Signal Detection

We also investigated the possibility of having our system detect tiny signals on a significantly noisy background, due to spontaneous gate dissociation (ie: gates being activated despite the abscence of input).

Our idea was to implement a system containing *two* gates as well as an allosteric DNA catalyst, as described in [2]:

- Gate 1
  - Activated by the signal
  - Acts to *activate* the allosteric catalyst.
- Gate 2
  - Signal has no effect on this case
  - Acts to *inhibit* the allosteric catalyst.

The said allosteric catalyst, when activated, would lead to the linear production of a molecule which would then be thresholded (using one of the methods described above) and exponentially increased. The thresholder and amplifier could also be part of the same unit.

The idea is that in the abscence of signal, the spontaneous gate dissociation for both the gates would cancel each other out and result in a 0 signal. If input is added, however, an unbalance would be caused in the amount of activator present, and, however, small that balance, the exponential amplifier would detect it.

Evidence from [2] on the behaviour of the allosteric catalyst in the presence of both activator and inhibitor indicates that this could be a viable mechanism, providing the thresholder is sharp enough. It seems, howevre, that an exces of gate II might be necessary.

Unfortunately, detailed kinetic data pertaining to the model in [2] is not available, and so simulations could not be run.

References

[1] DY Zhang, AJ Tuberfield, B Yurke and E Winfree, *Science* **318**, 1121 (2007)

[2] DY Zhang, E Winfree. Pre-publication.

[3] G Seelig, D Soloveichik, DY Zhang, E Winfree, *Science* **314**, 1585 (2006)