The Elongation Catastrophe in Physical Self-Replicators

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Abstract

An insufficiently appreciated paradox in the origin of life is that the replication of information-carrying molecules requires the molecules to be very specifically shaped; but such specific molecules are hard to produce without natural selection. We demonstrate and investigate this problem by building a physical model of self-replication out of specifically shaped plastic pieces with embedded magnets, which float around on an air-hockey type table. We use a mechanism known as template replication, which works by the joining of complimentary strands, roughly analogous to the biological replication of DNA, except without the involvement of enzymes. Building a physical rather than a computational model forces us to confront several issues that have analogues in the microscopic, chemical world. In particular, in order to achieve a low mutation rate we must reduce as much as possible the formation of incorrect sequences, which can happen both spontaneously and as a result of strands joining in a misaligned way. The latter results in ever-lengthening sequences in a process known as the "elongation catastrophe". We present an overview of our design process, illustrating the many interdependent adaptations that had to be made to the pucks' shapes in order to solve these problems while maintaining a high rate of template replication. The chicken and egg question is how, in the pre-biotic world, could template replication be achieved without the presence of enzymes that require template replication in the first place? By building a real physical model a new answer to this question is suggested. We propose that early pre-biotic monomers required structural specializations that reduced the rate of formation of incorrect sequences, without the need of an encoded enzyme.

Introduction

In the highly evolved biology of today a complex array of encoded enzymes is necessary for the replication of DNA and RNA polymers. These enzymes were not available at the origin of life, and so nucleotide template replication had to be non-enzymatic (Szathmáry, 2000; 2006). The best example of non-enzymatic template replication we have so far is still the work of Guenter von Kiedrowski (1986) who made the first non-enzymatic template replicator consisting of the hexanucleotide sequence GGCGCC that catalyses the templated ligation of CGG and CCG trimers.

In such experiments, replication must be carefully distinguished from spontaneous self-assembly which is typically easier to achieve than replication in stochastic systems. In the von Kiedrowski experiment there is a low rate of self-assembly (specifically elongation/dimerisation) by non-templated ligation of CCG and CGG. To prove replication one must compare the rate of formation of GGCGCC in the absence and the presence of an initial seed of GGCGCC. The difference is the extent of true templated self-replication. Whereas self-assembly of random novel oligomers is fine for random search in sequence space, self-replication is crucial for evolution by natural selection, i.e. the production of offspring whose fitness correlates with parental fitness (Price, 1970). If most of the DNA in a proto-organism was self-assembled de-novo into random sequences and not replicated from the parent, the genome would be real garbage, as opposed to inherited junk.

This raises a paradox that is of no lesser importance than Eigen's paradox regarding the error catastrophe (Eigen 1971). Our logically anterior paradox deals with the fact that specificity of self-replication over self-assembly is a critical pre-requisite for an evolvable physical template self-replicating system. Without specific ligation, random de novo synthesized sequences invade a population of replicating evolved sequences. These random sequences compete with evolved sequences for monomer resources thus diluting out evolved information (i.e. sequences that had arisen from a

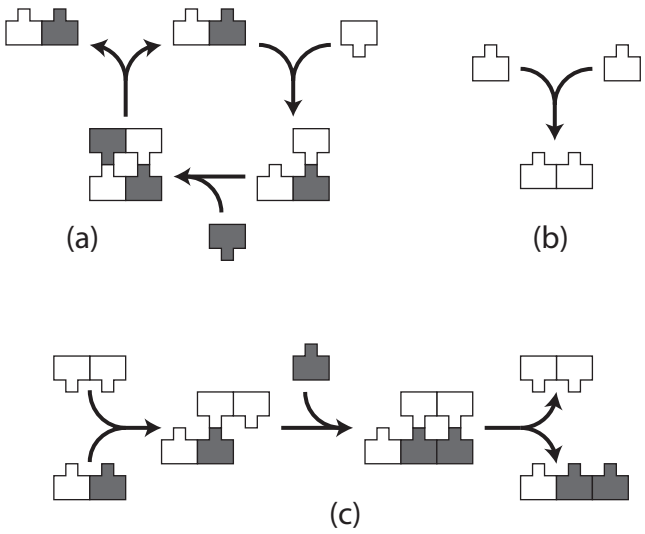

Figure 1: A generic illustration of template replication and two side reactions that must be avoided. (a) Homologous template directed ligation (self-replication) results in the correct duplicaton of a sequence. (b) A new (incorrect) sequence is formed by non-templated spontaneous ligation. (c) Elongation of the original sequence by partially homologous template ligation at staggered ends. See (Fernando, Von Kiedrowski et al. 2007) for a full analysis of the elongation catastrophe.

lineage of template replication events). In addition evolved sequences become trapped inside elongating strands (that cannot easily unzip or denature) such that they cannot easily experience another round of replication, see Figure 1. We call this the elongation catastrophe and it raises what we will call the elongation paradox (Fernando, von Kiedrowski et al. 2007). How can specific ligation be achieved without complex enzymes that require template replication with specific ligation in the first place?

The minimal unit of template replication is a dimer (i.e. a polymer of length two) that can replicate three possible sequences, AB, BA, or AA(BB), as in Figure 1. The minimal unit of template replication has the capacity to replicate the specific configuration that it is in. It is this fact that allows template replication to potentially convey an unlimited amount of information (Szathmáry and Maynard Smith 1997) because of the compositionality of the genome (Fodor and Pylyshyn 1988) and to be evolvable due to the capacity for micro-mutation, i.e. small changes in the composition can generate correlated fitness variants (Price 1970). But there is a real danger with such a system that if ligation is not tightly controlled then novel random sequences can arise and evolved sequences can elongate (but not replicate) without limit, as in Figure 1(c).

Mutations must be able to occur in an evolvable system, but they must occur at a low rate in order to avoid Eigen's (1971) error threshold. A minimal evolvable system must therefore exhibit the replication of dimers with low rate of assembly of incorrect or elongated sequences. For this project we set ourselves the goal of producing a system where the average rate of replication of a seed dimer is greater than the rate of formation of all other sequences put together.

Interestingly, this elongation catastrophe was the fate of a 2D macroscopic system designed by Jarle Breivik for template replication that was faithful to some aspects of chemistry such as stochasticity and binding properties (Breivik 2001). He used 2D plastic shapes with embedded magnets and an oscillating temperature water bath. Unfortunately, despite the obvious ingenuity of the design, the original templates formed in an unseeded manner by spontaneous aggregation of "hydrogen bonded" pairs to form a double strand and no kinetic comparison between self-assembly and self-replication was made. From Figure 3 in Breivik's paper it appears that free ligation was responsible for the production of all the oligomers in that model by de novo synthesis of monomers in weakly bonded pairs. Strangely, the h-bonded pairs catalyze double p-bond formation, see Figure 3 in (Breivik 2001). It seems, no template replication was demonstrated, and if it did exist, it seems to occur much more slowly than the spontaneous formation of novel sequences. This is a problem for evolution by natural selection, not a feature. Breivik's system suffers severely from the elongation catastrophe and therefore could not be extended to undergo natural selection of sequences.

In fact, until now, to our knowledge it is still only the geneticist Lional Penrose and his son Roger Penrose (Penrose and Penrose 1957) who have shown a relatively specific type of ligation reaction in a physical system without resorting to electronic switches and other features that make specificity of ligation trivial and thus reduce their utility in abduction to chemistry or the potential for later miniaturization (Groß, Küchler et al. 2009). Penrose's devices use only gravity, collision, friction, and (passive and active) hooking. In the simplest model, two kinds of solid object A and B are agitated horizontally on a straight track. If seeded with either a AB or a BA dimer (AA and BB dimers cannot form in the Penroses' system) other monomers join together by being appropriately tilted, to form the identical dimer type, without novel AB or BA forms appearing spontaneously by un-catalysed ligation. E.F. Moore wrote of Penrose's design "If the reader attempts the problem of how to design the shapes of the units A and B so as to have the specified properties, the difficulties he will encounter in his attempt will cause him to more readily appreciate the ingenuity of Penrose's very simple solution to this problem." (Moore 1962).

However, 1D systems are severely limited in terms of extendibility to longer sequences to achieve unlimited heredity (Szathmáry and Maynard Smith 1997) because i. they may be constrained by the initial sequence of monomers along the chain (which is a problem if the identity of monomers cannot flip between A and B, which in some of Penrose's designs they can), and ii. information about the identity of units on the inside of a sequence must pass through all other bordering units before they can influence external monomers . Again, Lionel Penrose already carefully considered information transmission through units agitated in 1D, for example he invented in a length-dependent end-blocking device that prevents anything larger than 4-mers from forming, so avoiding the elongation catastrophe in one dimension. A more complete 1D self-replicator (still 1D because it is only agitated in the horizontal axis) was later invented by Penrose to allow the replication of dimers with more possible states/configurations defined by the arrangement of hooks stacked in the 2D axis orthogonal to the axis of agitation rather than perpendicular to that axis (Penrose 1959). So, in short, Penrose took the elongation catastrophe rather seriously.

Here for the first time we present a mechanical 2D stochastic self-replicator that has limited rates of non-catalysed spontaneous self-assembly (ligation) of monomers, and limited partial homologous templated ligation. Reducing the rates of these two side-reactions serves to some extent to curtail the elongation catastrophe. However, we note that our solution is hand-designed and partial. The elongation paradox is still not solved for the origin of life, i.e. we do not know how such infra-biological monomers could have arisen with these very specific capabilities; speculation on this based on this work is given in the conclusions.

We built plastic monomers containing magnets and passive hooks and sails, that floated on an air-hockey table, and were blown by fans on the perimeter of the table, see Figure 2. Spontaneous elongation (untemplated ligation) was reduced by careful design of the physical equivalent of the phosphodiester bond. In addition, partial homologous ligation was reduced by careful design of the template complex.

Indeed, our system is a macroscopic close relative of von Kiedrowski's hexanucleotide replicators, because we have faced similar design challenges as in real chemistry, such as cyclisation and product inhibition. Guenter von Kiedrowski had to block the ends of his hexamers to prevent partial homologous ligation from catastrophically extending strands and depleting matter from the replicator cycle (Von

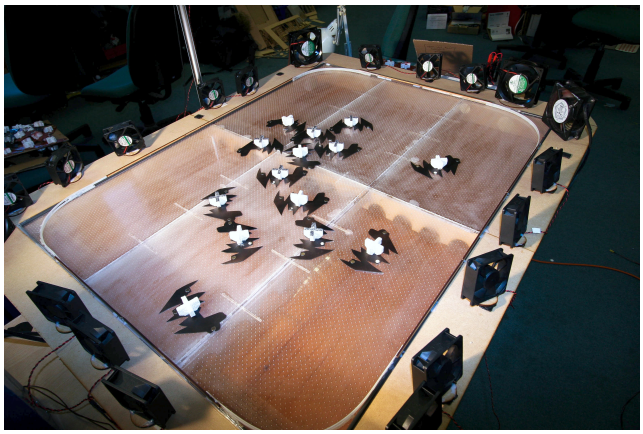

Figure 2: The design of the air-hockey style table containing the monomers. Sails on each monomer are blown by a perimiter of small fans. Another fan below the table passes air through small holes to suspend monomers over the table like small hovercraft.

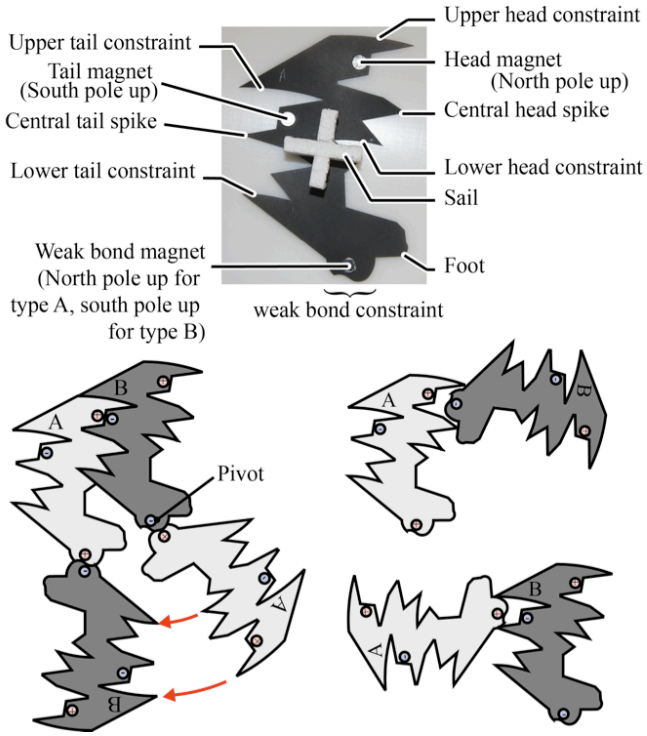

Figure 3: The design of the monomers. The top photograph shows the names used in the text for important parts of the design. In photographs the two monomers are distinguished by the colour of their polystyrene sails (white for A, black for B), whereas in diagrams, type B is shown in a darker shade of grey. The lower-left diagram shows the mechanism by which templated ligation takes place (but see also Figure 4). The lower right diagram shows how the design prevents the weak bond magnets from bonding to the strong bond magnets.

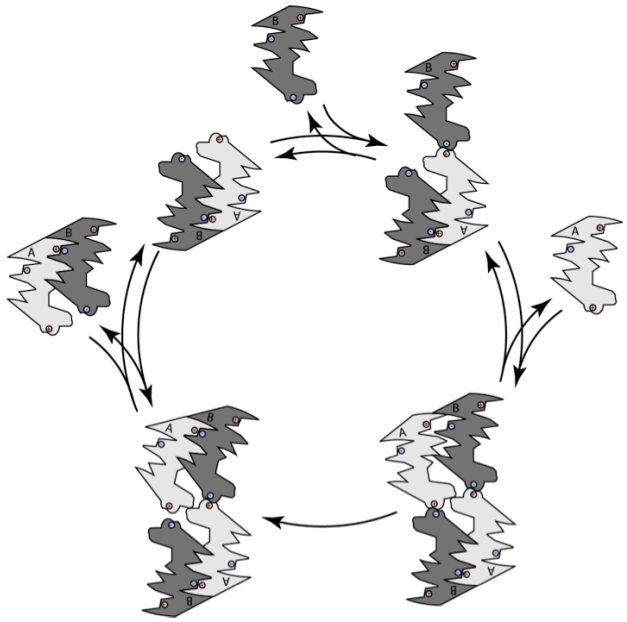

Figure 4: The autocatalytic cycle for replication of an AB dimer. (a) A type 'B' monomer joins to the dimer. (b) A type 'A' monomer joins to the other h-bond and swivels into place via the mechanism shown in Figure 1. Catalysis can also take place if the monomers join in the opposite order; in this case both monomers must swivel on their weak bonds, which often occurs when the configuration collides with another object. (c) A p-bond is formed by template directed ligation and, simultaneously, one of the h-bonds is broken. A collision with another molecule or the table edge is required in order for this step to occur. (d) Another collision breaks the remaining weak bond, and the two strands separate, completing the cycle.

Kiedrowski 1986). However, in our system we have not explicitly blocked the ends, but have designed all the monomers so that end-blocking is 'emergent'.

The primary advantage of a physical system over a computer simulation is it forces us to confront the problems of template replication by changing the design of the monomers, rather than by changing the simulation to reduce the problems. Similarly, while real chemical monomers can have mechanica‹lly-implemented internal states, our self-imposed restriction of no electronic components prevents us from being able to implement any arbitrary mechanism, regardless of how easily it could be implemented mechanically in chemical systems.

Next we describe the design of the pucks (monomers) and then we conduct a classical seeding experiment to distinguish self-replication from self-assembly. This is the first demonstration of a 2D template replication system that is capable of low rates of spontaneous elongation yet high rates of self-replication (without the use of monomers containing electronically implemented finite state machines).

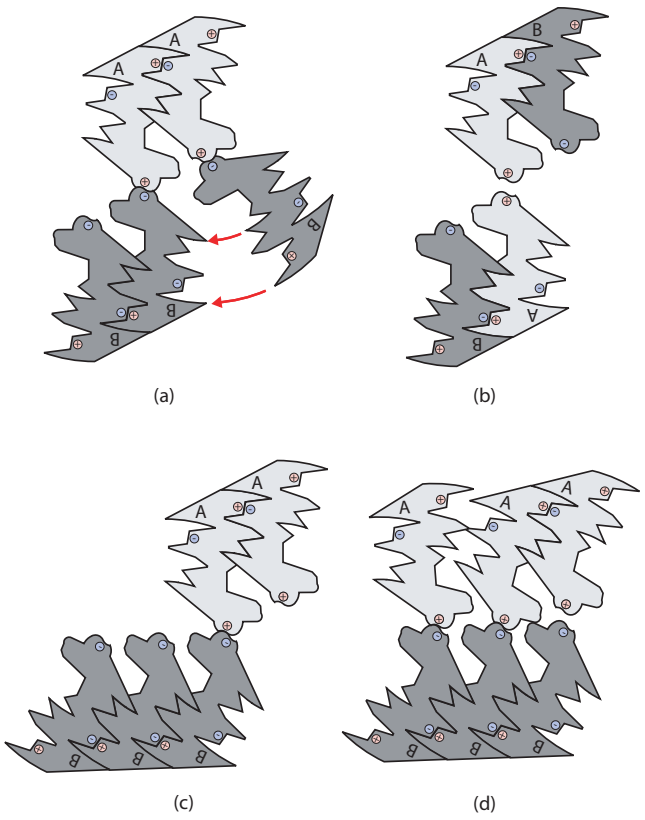

Figure 5: (a) The formation of a BBB trimer due to partial homologous ligation. The production of AAA and BBB trimers in this way is relatively common in our system (see Figure 9). (b) Staggered bonding is not possible between two AB dimers (or two BA dimers) because it would require the formation of an h-bond between magnets of the same polarity. (c) It is in theory possible for a further partial homologous ligation to extend a BBB trimer into a BBBB 4-mer. However, we did not observe this in any of our trials. We suspect this is because the two polymers have a high moment of inertia about the weak bond's pivot point, destabilising the bond and making it likely to break. (d) Polymers of length greater than two cannot replicate in the same way as dimers, because the "foot" mechanism does not allow the strong bond constraints to align with the weak bond pivot.

Methods

A frictionless table, similar to those used for air-hockey, was purpose built and consisted of a flat plastic surface perforated with an array of 1.5mm diameter holes, spaced at intervals of 10mm. An enclosure underneath this surface was pressurized with a powerful fan to produce a steady jet of air from each hole, allowing suitably shaped objects to float above the table surface. Surrounding the table was a set of approximately 20 small fans that could be arranged to cause a stochastic motion of the pucks, albeit with a significant rotational element, see Video A in Supplementary Material. There was no "temperature" oscillation as in Breivik's experiment, i.e. the fans always rotated at the same speed. The walls of the table allowed approximately elastic collisions. The puck design is shown in Figure 3.

Pucks are 1.5mm thick and made of plastic. The bases of the pucks are flat allowing a hovercraft type low friction floating of the puck above the table. The pucks were fabricated using a Versalaser cutter. Rapid fabrication of new designs was possible for prototyping. Pucks contain molybdenum disc magnets that can be oriented with the north or south pole facing upwards, allowing specification of attractive or repulsive interaction pairs.

The final design has the following features. The strong 'phosphodiester bonds' must not form spontaneously. This is achieved by embedding the magnets deep within the puck and producing a lock and key type join which can only form if the pucks collide at a very specific orientation. This orientation tends to occur only when the two monomers are 'hydrogen bonded' to a dimer template, and not when two pucks collide against each other as untemplated monomers. Once the pucks make the p-bond the magnets are very close together so the bond is strong. Thus the p-bond is difficult to form due to steric constraints but once formed is strong due to close magnets and mechanical rigidity. The h-bonds consist of an interaction between magnets that are further apart when the bond is formed, i.e. the bond is weaker. Also, there is a curve on the surface of the bond to allow pucks to rotate when h-bonded. This rotation brings the two h-bonded monomers into the appropriate configuration for the p-bond to form.

To reduce product inhibition, the pucks are shaped in such a way that two p-bonded dimers cannot be joined at both h-bonds. Thus, as the p-bond forms it breaks one of the two hydrogen bonds. The remaining h-bond is sufficiently weak that the two dimers can separate and undergo another round of replication.

There are two types of monomer, labelled 'A' and 'B', which differ only in the orientation of the magnets that form their h-bonds. 'A' type monomers can only form weak (h-)bonds with 'B' type monomers, and vice versa. Strong (p-)bonds can be formed between any pair of monomers, giving rise to four types of strong-bonded dimer, 'AA', 'AB', 'BA' and 'BB'. Template replication produces a new dimer that is both the compliment and the reverse of the original. This results in three separate autocatalytic cycles: {AB}, with the reaction AB + A + B → 2AB; {BA}, with the reaction BA + A + B → 2BA; and {AA, BB} with the reactions AA + 2B → AA + BB and BB + 2A → AA + BB.

Misalignment with the generation of a staggered or dangling end as they are often called, can cause 'AA' dimers to be extended via catalysis to 'AAA' dimers, and similarly for the 'BB' type, by partial homologous ligation (see Figure 5). However, in all the experiments conducted we did not observe the production of 4-mers by partial homologous ligation. Importantly misalignment did not tend to occur for 'AB' and 'BA' dimers, which cannot catalyse partial homologous ligation dependent elongations unless another species of dimer is also present in the system. The explanation is given in Figure 5.

In summary there are the three principles that we used to limit the elongation catastrophe in this simple system.

1. Impossibility of formation of non-complementary h-bonded pairs.

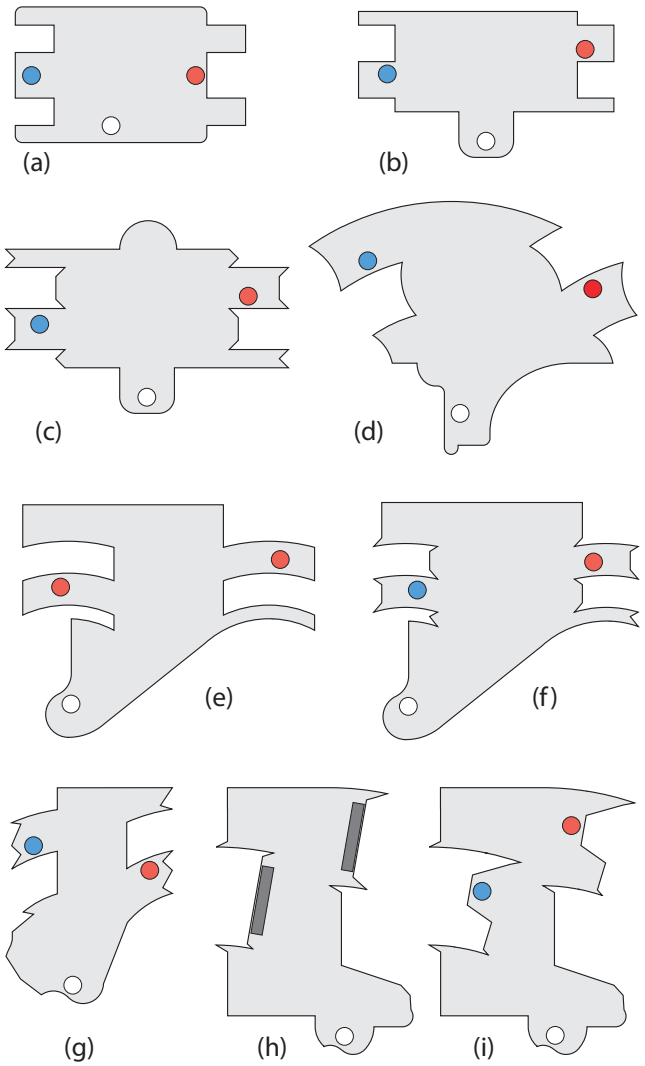

Figure 6: A selection of unsuccessful iterations of the design, illustrating the ways in which various issues were solved. The designs are shown in chronological order. See text for details. Magnets are shown in red or blue depending on whether the north or south pole is oriented upwards. The weak bond magnets, whose orientation depends on the polymer type, are shown in white.

2. A high moment of inertia at the pivot point of a staggered end.
3. Improper alignment of p-bond passive hooks during an attempted templated ligation for N-mers where N > 2.

In combination these three factors significantly reduced the elongation catastrophe by limiting partial homologous ligation. The curved passive hooks previously described also helped by reducing the extent of non-catalysed ligation.

A Phylogeny of Designs

A number of issues had to be solved simultaneously in order to produce a successful design. It took approximately 30 iterations to produce the final design, some of which can be seen in Figure 6. We have listed the issues that needed to be solved below.

i. The strong (p-)bonds must be unlikely to form spontaneously, i.e. the problem of reducing spontaneous generation.
ii. There must not be any reactions that catalyse p-bond formation, other than the intended template mechanism. For example, if two pairs of monomers joined by h-bonds come together, they must not line up at the right angle to form p-bonds.
iii. Once formed, the strong bonds must be strong enough that they rarely break. (In the final design they were strong enough not to break at all.)
iv. The strong bonds must form easily when catalysed by the weak bonds.
v. The weak (h-)bonds must form easily.
vi. The weak bonds must also break easily. This facilitates strand separation, as well as freeing up monomers that have become weak-bonded to other monomers, which would otherwise not be able to participate in catalysis.
vii. Once a dimer has catalysed the creation of another, the two 'strands' must be able to separate, i.e. the problem of product inhibition.
viii. The magnet in the weak bond must not be able to attach strongly to the magnet in the head or tail of another puck. Such unwanted bonds inhibit catalysis by occupying the bond points, and can also give rise to configurations that can catalyse the wrong type of dimer.
ix. The puck must be able to float effectively on the table. Designs with long thin protruding parts, or uneven weight distributions, can drag on the table's surface.
x. The pucks must not tend to jump off the table's surface and become stacked on top of one another. This tends to happen if two magnets with the same polarity are forced close to one another, or if the design features spikes that are too sharp.

Of these, issues i and ii were by far the hardest to solve. In most of our designs, including the final one, the strong bond works by requiring the two pucks to collide at a very precise angle. In many of the designs, if the collision occurred at a slightly different angle, a strong bond would often form anyway. This is because the head and tail magnets would tend to make the pucks slide into place to form a strong bond, or else the two pucks would sit together in a configuration where they could easily be nudged into the right position to form a strong bond. This was solved in the final design by adding long spikes to the strong bond constraints, in such a way that the magnets tend to pull the pucks away from, rather than towards, the strong bond configuration if the pucks are not correctly lined up. However, the pucks do still occasionally collide at the right angle to form a strong bond.

Since we could not substantially reduce the rate at which this occurs, we instead focused on increasing the rate of catalysis. We addressed issue iv by designing the weak bond to act as a pivot that guides the strong bonds into place.

Issue v was solved by making the weak bond protrude as much as possible from the body of the puck. This increases the range of relative angles at which two pucks can be oriented while still being able to form a weak bond. Issue vi was addressed by making the weak bond into a pivot that can swing fairly freely. As the joint hinges the two magnets are pushed further apart, so that the bond can break if it swings far enough. This could be fine-tuned by making very small changes to the magnets' positions. The "foot" mechanism was introduced to solve issue vii.

Issue viii was solved in the final design by the "spikes" in the head and tail sections (see Figure 3). These also help with issue i. The remaining issues were solved primarily by trial and error.

Figure 6 shows a selection of previous iterations of the design, illustrating some of these problems and how they were solved. Design (a) was ineffective because weak bonds formed only rarely. This is because the pucks have to be fairly specifically oriented with respect to one another in order for the weak bond magnets to come in range of each other. Additionally, the weak bond magnet of an 'A' type monomer can bond strongly to the tail magnet of another monomer, blocking catalysis. These two problems are solved in design (b) by making the weak bond protrude from the body of the puck, and by re-designing the strong bond so that the magnets are recessed away from the puck's edge. However, it is relatively easy for strong bonds to form spontaneously in this design, and they can also be catalysed by the edge of the table. The spikes added to the strong bonds in design (c) help to prevent spontaneous strong-bond formation, but they also interfere with the catalysis mechanism. This design also features a 'hump' on the opposite side to the weak bond; this is to prevent the edge of the table from catalysing bonds. Design (d) is the first to feature a weak bond that is designed to pivot around a particular point, with a correspondingly curved set of strong bond constraints. However, strong bonds can still form spontaneously quite easily, and weak bond formation is relatively rare.

Design (e) has a strong bond that is held together using repulsion rather than attraction (hence the head and tail magnets are of the same polarity). Unfortunately this tends to result in the magnets jumping off the table to stack on top of one another, since this is energetically preferable to being near one another in a repulsive configuration. The weak bonds have also been re-designed to be easier to form. Design (f) is similar but uses attracting magnets again; its main problems are that strand separation is very slow, and spontaneous strong bond formation is still an issue. Design (g) is the first to feature a mechanism to break one of the weak bonds when a strong bond is catalysed (two dimers cannot fit together in such a way that they are joined at both weak bonds). However, the spontaneous formation of strong bonds is still an issue, as is the formation of unwanted bonds between the weak and strong bond magnets. Design (h) uses Velcro rather than magnets for the strong bonds in an attempt to solve these issues. This idea was discarded because Velcro produces a loose joint, which means the strong bonds do not align accurately enough for catalysis to take place. However, we realised in testing this design that making the lock-and-key structures on the strong bonds wider helps to prevent spontaneous strong bond formation.

Design (i) is close to the final design and works fairly effectively. Its two remaining problems are that unwanted weak-strong bonds can form (although they are quite weak), and monomers can be attracted together by the strong bond magnets in such a way that a strong bond can form if they are nudged in the right way. These problems is solved in the final design by the addition of the central head and tail spikes (see figure 1), and by making the other spikes a lot larger.

We produced a total of 14 monomers, seven of type 'A' and seven of type 'B'. A total of 48 experiments were performed with the final design, each lasting 25 minutes. 36 of these were seeded trials, meaning that one dimer was added to a system containing the remaining 12 monomers. The system is allowed to run for a few minutes before adding the dimer, to ensure that the initial conditions do not affect the outcome. After the dimer was added we counted the number of each type of polymer every 2.5 minutes.

Of the 36 seeded trials, 12 were seeded with an 'AB' type dimer, 12 with type 'BA', 6 with type 'AA' and 6 with type 'BB'. Since 'AA' and 'BB' are two phases of the same

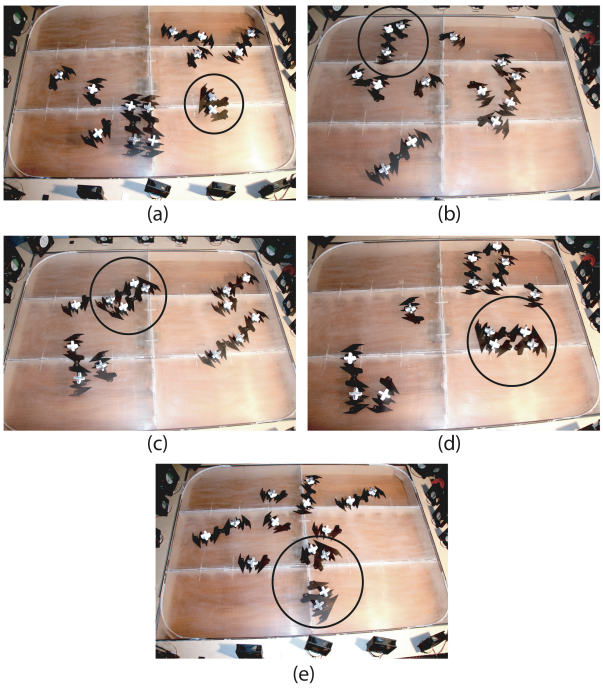

Figure 7: Photographs showing one round of the self-replication cycle. (a) An 'AB' dimer (circled) is placed into a system containing 6 'A' monomers (with white-topped sails) and 6 'B' monomers (black-topped sails). (b) A 'B' monomer joins to the 'A' part of the dimer via a weak bond. (c) An 'A' monomer joins via a weak bond to the 'B' part of the dimer, and its head constraints interlock slightly with the other monomer's tail constraints. (d) A collision with the table's edge or another molecule pushes the two monomers together, so that they form a strong bond. This breaks one of the two weak bonds. Note that both dimers are of type 'AB'. (e) Further collisions break the remaining weak bond, and the two strands separate. This completes the autocatalytic cycle.

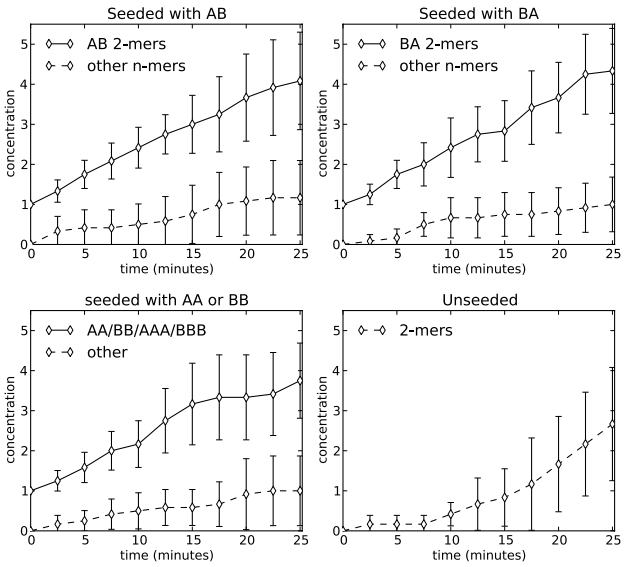

Figure 8: Time series plots showing the results of letting the system run for 25 minutes, seeded with one dimer of a particular type, or with no dimer. In this plot, all polymers apart from those of the seed type are lumped into a single category. In the case of the trials seeded with AA or BB, we count AA, BB, AAA and BBB as a single category, since these can all be produced by the catalysis process from the seed type. Each plot shows the average over 12 trials. The error bars show a 95% confidence interval.

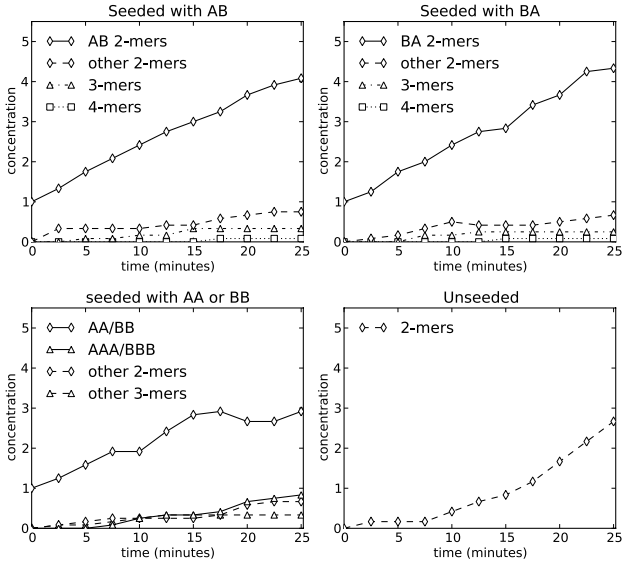

Figure 9: Time series data from the same trials as Figure 8, with the reaction products split up by length. Note in particular the drop in concentration of AA and BB dimers towards the end of the trial as they are converted into AAA and BBB via elongation at staggered ends. (Error bars are omitted because they would be overlapping)

replicator, the latter two are plotted below as a single set of 12 trials.

The control experiment involves initializing the system with seven 'A' type monomers and seven 'B' type ones, and is again run for 25 minutes. 12 such experiments were conducted.

Results

The results are summarised below and in Figures 8 and 9. We count as a side reaction the production of any oligomer other than the seed type. In the AA/BB case we count AAA and BBB as copies of the original rather than as side-products, because there is no mechanism to prevent the formation of these 3-mers, and because they can still catalyse the production of new BB or AA dimers.

In 19 out of the 36 seeded trials, no side reactions took place during the 25 minutes of the trial. In these successful trials, an average of 4.3 duplicates (or, in the AA/BB case, elongations) of the seed were created in addition to the seed itself. The maximum possible number of copies is 6 in the AB or BA case, or 5 in the AA/BB case, with a miss-matched pair of monomers left over. This best-case performance was achieved in four of the trials.

In the remaining 17 seeded trials a side reaction produced an oligomer of a different species from the seed. In some trials this did not substantially disrupt the replication of the seed, but in others, particularly if the side reaction happened early in the trial, the side product produced more replicates than the seed dimer, effectively out-competing it by using up the monomer supply. Under some circumstances it is also possible for the side product to join to a dimer of the seed type in a staggered fashion (as in Figure 5), catalysing its elongation into a different species. For these reasons the mean number of duplicates of the seed after 25 minutes was only 1.7 in the 17 trials where side reactions occurred, or 3.1 over all 36 seeded trials. In four out of the 12 unseeded trials there were no side reactions, meaning that only monomers were present after 25 minutes. Over all 12 unseeded trials, an average of 2.7 oligomers were produced, of various species.

Time series data are shown in Figures 8 and 9, averaged over each of the four sets of 12 trials. In figure 8 all the side reaction products are lumped into a single category. The error bars show that the domination of duplicates of the seed over all other species is statistically significant to within a 95% confidence interval at every time step.

Conclusions

The hexanucleotide replicator of von Kiedrowski was not evolvable because no mutant of the original sequence was capable of self-replication. Furthermore the ends of the molecules were blocked so that elongation was impossible. Breivik's model was not evolvable for the opposite reason; there was too much spontaneous generation and an elongation catastrophe. Here we have shown a way to achieve something in between, that at least has the potential for evolvability.

There is no doubt that the elongation catastrophe will be faced in all nanoscale self-replicating systems as well. Of-course, technology may allow such problems to be solved

somewhat trivially if monomers are allowed to contain switchable electromagnetic bonds (Groß, Küchler et al. 2009) and can implement a finite state machine (Griffith, Goldwater et al. 2005) thus avoiding issues of product inhibition and mismatching by simply allowing bonds to be arbitrarily made or formed based on perfect local information. However, this arbitrary programmability limits their utility in providing insight into possible molecular mechanisms of non-enzymatic template replication that depend on carefully evolved steric and force constraints, which is one of our main motivations here.

Of course, real molecular systems happen on vastly different spatial and temporal scales: our system has 14 monomers whereas a small chemical system might have 10^{20}. In chemical systems interactions might occur only in a tiny majority of collisions, which we had to avoid in our experiments as it would have made the time scale too long. Nevertheless we believe the insights we have gained are useful.

The implication for the origin of life is that it is possible to produce monomers that self-limit to some extent the lengths of strands that can be self-assembled according to the mechanisms shown in Figure 5. It may be the case that such primitive methods may have been among the first evolved to combat the elongation catastrophe. The production of this physical model has (at least for us) been helpful as E.F. Moore said it would be.

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