INSTITUTE OF PHYSICS PUBLISHING PHYSICAL BIOLOGY
Phys. Biol. **1** (2004) P1–P8 PII: S1478-3967(04)70348-4

**PERSPECTIVE**

# Thermal force approach to molecular evolution

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Received 13 October 2003
Accepted for publication 9 December 2003
Published 12 February 2004
Online at stacks.iop.org/PhysBio/1/P1 (DOI: 10.1088/1478-3967/1/1/P01)

**Abstract**

Recent experiments are discussed where temperature gradients across mesoscopic pores are shown to provide essential mechanisms for autonomous molecular evolution. On the one hand, laminar thermal convection can drive DNA replication as the molecules are continuously cycled between hot and cold regions of a chamber. On the other hand, thermophoresis can accumulate charged biopolymers in similar convection settings. The experiments show that temperature differences analogous to those across porous rocks present a robust nonequilibrium boundary condition to feed the replication and accumulation of evolving molecules. It is speculated that similar nonequilibrium conditions near porous submarine hydrothermal mounds could have triggered the origin of life. In such a scenario, the encapsulation of cells with membranes would be a later development. It is expected that detailed studies of mesoscopic boundary conditions under nonequilibrium conditions will reveal new connecting pieces in the fascinating puzzle of the origins of life.

## 1. Boundary conditions

At a functional level, living organisms are often classified as systems which implement a combination of replication, mutation and selection. It is argued and expected that such a system will evolve into higher and higher levels of complexity. However, such a definition of life disregards the physical environment needed to drive molecular evolution. We will focus on such boundary conditions and seek those where molecular organization increases in a thermodynamic nonequilibrium setting. For example, we will show how a convection cell can accumulate DNA into single spots. Such a reduction of uncertainty (and we would call it a reduction of entropy were it not for the fact that entropy is, strictly speaking, not defined in such nonequilibrium conditions) is necessary to feed an evolutionary scheme of replication, mutation and selection.

To illustrate the point, consider a modern computer worm. Using the above criteria, it might be classified as a living creature, yet it can only thrive in a nonequilibrium environment of electrically driven and connected computer systems. Similarly, we can establish laboratory conditions which give birth to systems of replication, selection and mutation. Let us take as an example the work to select RNA molecules with favorable catalytic function out of a large pool of starting sequences [1–4]. It is motivated by the idea of an RNA world [5] origin of life where it is assumed that self-replicating RNA molecules can reach higher and higher levels of sophistication by molecular evolution. Proponents of scenarios of an origin of life based on an RNA world are clear in stating that the energy and entropy source of concentrated, activated nucleotides are needed and state that a possible source is not given. Yet based on thermodynamical arguments [6, 7], these molecules must originate from a nonequilibrium mechanism. Actively searching for these mechanisms will probably reveal new insights into possible and realistic settings for the origin of life. Experiments testing such boundary conditions can show novel connecting pieces in the puzzle on the origins of life.

The origin of life problem can either be pursued as a historical backward extrapolation which strips the molecular machinery as we know it today to its putative functional core without seeking boundary conditions which drive and sustain such a nonequilibrium system. Such a top-down approach

  

might miss important restrictions based on metabolism or thermodynamic boundary conditions. The approach taken here insists that a second experimental bottom-up approach based on biochemistry, physics and geology of systems far away from thermodynamical equilibrium can reveal unexpected complementary information. Unexpected interplays between the biopolymer DNA and the boundary conditions of a temperature gradient will be shown.

To be more specific, we show experimentally that core processes of evolution can be driven by thermal convection in pores of rock. Both accumulation and replication of DNA in a convective setting adds crucial mechanisms to several hypothesizes on an origin of life in pores near hot hydrothermal venting systems [34–42].

## 2. Two thermal forces: convection and thermophoresis

Two effects of the boundary condition of a locally elevated temperature will be studied by experiments. Both have an effect only if the temperature varies across a reaction chamber. The first effect, thermal convection, is driven by elevated temperature which induces thermal expansion and makes the same volume of liquid less dense. Gravity and differences in temperature lead in many geometries to an unstable situation and induce thermal convection: liquid typically moves up in hotter and down in colder regions. This gives rise to circular fluid movements.

Since liquids are viscous, the trajectories become laminar (i.e. nonturbulent and stable over time) if the fluid movements are slow and the convection dimensions small. For convection in water and temperatures ranging from 50 °C to 100 °C, laminarity typically occurs for convection cells smaller than a centimeter. Recently it was shown that a laminar convection could trigger the DNA replicating polymerase chain reaction (PCR) [8]. This demonstrates that replication reactions could be hosted elegantly in the temperature oscillation of laminar convection.

Another, less well known effect of differences in temperature is thermophoresis. Particles can be moved simply by a gradient of temperature. In most cases the particles move from hot to cold against the gradient. Although this effect has been known in other systems for almost 150 years [9, 10] and well understood for dust particles, a description in liquids is still vague and phenomenological. Measurements of the thermophoretic effect on biologically relevant molecules like DNA [11] or proteins [12] were done only recently.

Interestingly, convection and thermophoresis, both triggered by the same temperature gradient, can lead to conditions where DNA accumulates in stagnation points of a convection [11]. We will discuss how both thermal forces—convection and thermophoresis—yield a natural boundary condition for molecular evolution.

## 3. Laminar convection driving exponential DNA replication

Evolution provided living cells with molecules which show an elaborate strength of surface interaction towards each other. Most of the reactions in a cell are based on a fine-tuned network of reversible binding reactions, ranging from proteins binding to their reaction partners to the Watson–Crick pairing DNA and RNA. Biological molecules neither stick too tightly to each other nor bind too loosely. Binding in early stages probably had the problem of being either too sticky, allowing for only one binding event with no chance to unbind again, or of being bound too loosely and thus being lost to the ocean. It will be shown that the temperature cycling of laminar convection in a closed chamber allows for reactions with less strictly balanced binding.

Besides catalytic reactions assisted by surfaces of solids [13–16] or proteins, a central binding/unbinding reaction is the templated copying of genetic material. An example is the replication of DNA, where, starting from a single strand, pieces of a second strand are bound and ligated. Only by separating both strands can the reaction be repeated. The temperature oscillation of a laminar convection is a natural and common setting which can perform this reaction cycle over and over in less than a minute.

An experimental demonstration is given below: the temperature oscillation of a mesoscopic convection can drive the exponential DNA replication of a polymerase chain reaction (PCR) [8]. It also serves as an experimental demonstration for RNA world scenarios where the unbinding of replicated RNA found no convincing solution [5, 17].

In a chamber with radius 2.5 mm and a thickness of 1 mm, a horizontal temperature gradient between 60 °C and 95 °C is applied by 75 mW infrared heating in the center (figure 1(*a*)). Replacing the infrared heating with a filament showed similar convection patterns in simulations, but is more difficult to implement experimentally. The geometry was optimized to yield a laminar convection flow with a Reynolds number of about 1. A major fraction of the volume—about 50%—undergoes the temperature cycling between 60 °C and 95 °C (figure 1(*b*)). These temperatures are needed for primer annealing in the colder region on the one hand and melting the double stranded DNA into two single strands in the hotter region on the other hand.

The reaction should, for each circulation, elongate single-stranded DNA in the outer 'cold' region to double-stranded DNA by using a polymerase protein, primers and activated nucleotides [18, 19]. Convection will automatically shuttle the elongated molecules back into the hot region where double-stranded DNA will become again two single-stranded DNA. As the convection shuttles them back into colder regions, a chain reaction of DNA duplication is started. It is expected to duplicate the amount of DNA for each round of the circulation.

The parameters of the experiment have been adjusted such that the convection allows enough time (≈12 s) for the elongation in the cold [8]. This condition was checked by monitoring the convection motion with fluorescent beads (figure 1(*c*)). Note that the circular geometry is only a simple way to use a heating spot—other geometries such as a linear horizontal temperature gradient should work equally well.

The used chamber circulates a volume of 20 $\mu$l with a typical time of 15 s. The convective PCR replicated a 96 base pair strand of DNA 1000-fold within 12 minutes. The

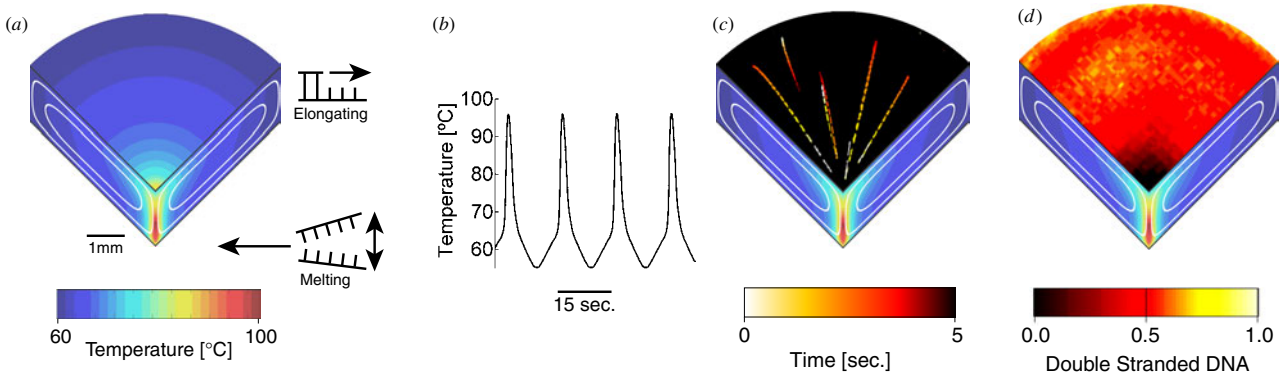


**Figure 1.** Laminar convection can drive DNA replication. (*a*) A temperature difference between heated center and cold periphery drives a laminar convection in a millimeter-sized chamber. The temperatures are chosen such that double-stranded DNA melts into two strands in the center at 95 °C. Each strand can be duplicated again into double-stranded DNA in the cold region with the help of a polymerase enzyme. The technologically very important polymerase chain reaction (PCR) reaction was chosen to show convective DNA replication. (*b*) A computer simulation predicts that most particles cycle within 15 seconds between the hot center at 95 °C and the colder periphery at about 60 °C. (*c*) The simulation is confirmed by imaging the movement of fluorescent beads within the liquid. They are radially attracted to the heated center, move outwards to the periphery and then come back again after an average 15 seconds. (*d*) Under these convection conditions, the finally replicated DNA can be seen with a fluorescent, intercalating dye already after 10 minutes. Since the dye only shows bright fluorescence for double-stranded DNA, the dark spot in the center confirms that the elevated temperature melts DNA there into single strands.

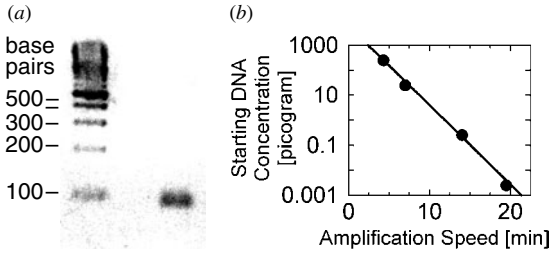


**Figure 2.** Exponential amplification by laminar convection. (*a*) A polymerase chain reaction (PCR), driven by laminar thermal convection, amplifies a single product. (*b*) The amplification is exponential and allows quantification of the initial DNA concentration using the principles of real-time PCR.

fluorescent image as viewed from above shows the amplified double-stranded DNA in figure 1(*d*). The black spot in the center confirms that DNA is molten into single strands to which the fluorescent dyes do not bind and therefore show low fluorescence.

The replicated DNA is confirmed to be a single product defined by the primer sequences and shows no amplification artefact (figure 2(*a*)). Laminar convection can drive the DNA replicating reaction of PCR at a speed about four times faster than a PCR reaction performed in a standard PCR cycler machine [8] where the whole reaction vessel has to be heated and cooled. In convection only the liquid is cycled which allows very fast temperature cycling.

Another interesting feature of laminar convection is that it really triggers an exponential chain reaction [8]. The time to replicate into a fixed amount of DNA needs logarithmically more time when the initial concentration of DNA is lowered as shown in figure 2(*b*). This is an important way to measure initial DNA concentration of a specific—for example viral—sequence, called quantitative or real-time PCR [20, 21]. Quantitative laminar convection PCR is therefore possible. Whether the same is feasible in a parallel developed Rayleigh–Bénard PCR approach remains to be seen [22].

Concerning molecular evolution, it should be clear that the experimental prerequisite of polymerase, primers and activated nucleotides are experimental substitutes for a yet unknown elongation reaction which might be based on a self-replicating RNA molecule. In the porous setting which we will discuss later, it can be imagined that the small feeding molecules diffuse through the porous chamber walls from neighboring convection cells or directly from hydrothermal flow channels. The replicated molecules could be trapped in the pore by thermophoresis or simply by their reduced diffusion constant. However, the laminar temperature cycling could also drive the replications of totally different systems to store genetic information. From the convection perspective, it is, however, revealing that the genetic molecules of RNA or DNA easily allow for temperature-driven replication. Other means of genetic memory where this would not be possible could easily be imagined.

## 4. Thermophoretic trapping

Living cells work hard to achieve and sustain a high concentration of molecules. This is necessary to allow fast reactions and simultaneous interactions between many molecules. The need for high concentration was one of the reasons to consider and pursue molecular evolution at surfaces [13–16]. Furthermore, accumulation into some $\mu$m makes fast molecular transport cheap and fast by diffusion. Both conditions are thermodynamically highly unfavorable, yet both are basic prerequisites for living cells.

In the following, experiments will be discussed which demonstrate that biomolecules can be accumulated near a surface from solution with the help of temperature gradients. The accumulation is based on the effect of thermophoresis which moves molecules parallel to temperature gradients.

The thermophoretic effect is phenomenologically similar to charged molecules moved by a gradient of the electrical potential [23]. Thermophoresis—also called thermal diffusion or the Soret effect—was only recently measured for biological molecules [11, 12]. It turns out that for the case of DNA, it is a strong effect which, following recent theories [24], is a result of DNA's high charge density.

The description of thermophoresis in solution is described phenomenologically with a thermal diffusion coefficient DT at the level of Fick's law:

$$j = -D\nabla c - D_{\mathrm{T}} c(1-c)\nabla T. \tag{1}$$

The concentration $c$ is given as molar fraction of the considered molecule versus the solvent molecules, the current density as $j$ and the diffusion constant as $D$. We see that a temperature gradient $\nabla T$ induces a flow of particles very similar to diffusion driving particles along a concentration gradient $\nabla c$.

Piazza [24] recently refined a more general approach by Ruckenstein [23]. He argues that the thermophoresis of charged particles is dominated by the interfacial tension of its Debye layer. The derivation starts with the fact that the Debye length $\lambda_{\mathrm{DH}}$ increases with the square root of the temperature: $\lambda_{\mathrm{DH}} \propto \sqrt{T}$. Then an electrostatic interfacial tension $\gamma$ is associated with the interface between particle and the surrounding liquid. It scales as $\gamma \propto -\lambda_{\mathrm{DH}}^{-1}$. A particle will move in the direction in which the interfacial tension decreases. Therefore ionic particles will move from hot to cold since the Debye length $\lambda_{\mathrm{DH}}$ is shorter and the interfacial tension more negative in the colder region. Basically, ionic thermophoresis is interpreted as the Marangoni effect between particle and solvent. A quantitative derivation [24] leads to the relation

$$\frac{D_{\mathrm{T}}}{D} = \frac{3\pi\lambda_{\mathrm{B}} Z_{\mathrm{eff}}^2}{4Ta^3}\lambda_{\mathrm{DH}}^2 \tag{2}$$

with $\lambda_{\mathrm{B}}$ the Bjerrum length, $a$ the particle radius, $Z_{\mathrm{eff}}$ the dimensionless effective charge of the particle and $\lambda_{\mathrm{DH}}$ the Debye–Hückel length. The quadratic scaling of thermophoresis with the Debye length was demonstrated by Piazza [24] using Micelles.

It is typical for theories of thermophoresis that the thermal diffusion coefficient $D_{\mathrm{T}}$ is proportional to $1/T$. If this holds generally, an integration of equation (1) in a resting fluid results in a remarkable exponential dependence of the steady-state concentration $c$ on temperature $T$ and diffusion constant $D$:

$$\frac{c}{c_0} = \exp\left[-\frac{D_{\mathrm{T}}}{D}(T - T_0)\right]. \tag{3}$$

The boundary conditions are given by the concentration $c_0$ and temperature $T_0$. Thus it can be expected that thermophoretic techniques are very sensitive to the diffusion constant $D$. Also the concentration difference achieved can reach exponentially increased levels as the temperature difference $T - T_0$ rises.

The phenomenological equation (1) of thermophoresis can also be interpreted at the level of particle velocity. Since the current density $j$ is the product of concentration $c$ and velocity $v$ in $j = cv$, equation (1) means that a particle is propelled by a temperature gradient to a velocity given by $v = -D_{\mathrm{T}}\nabla T$. With the thermal diffusion coefficient for DNA [11] of $D_{\mathrm{T}} = 0.5 \times 10^{-8}$ cm$^2$ (s K)$^{-1}$, a temperature gradient of 1 K $\mu$m$^{-1}$ leads to a sizeable velocity of about $v = 0.5$ $\mu$m s$^{-1}$. In terms of electrophoresis, this velocity would compare to a moderate electrical field of 0.2 V cm$^{-1}$. To measure thermophoresis of biological molecules, traditional experimental setups need big volumes and large numbers of molecules. A novel approach using two types of fluorescent dyes was taken to assess thermophoresis in small volumes at almost single-molecule levels [11]. In a 10 $\mu$m thin chamber, the elevated temperature of a local infrared heating is measured with a temperature-sensitive dye (figure 3(*a*)). It is compared to measurements of DNA concentration with a second dye as shown in figure 3(*b*). The chamber geometry and the low heating reduce convection to a minimum.

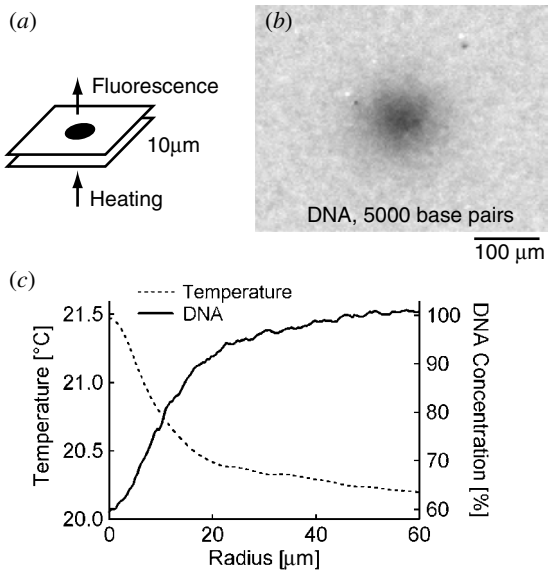


**Figure 3.** DNA is repelled by heat in resting water [11]. (*a*) In a thin chamber, a spot is heated by infrared illumination. Both temperature and DNA concentration are monitored by fluorescence. (*b*) The concentration image shows that DNA is depleted from the heated spot. (*c*) Quantitative profiles of temperature and concentration show that a 1.5 °C increase in temperature induces a drop of DNA concentration down to 60%. The profiles allow the deduction of the thermal diffusion coefficient for DNA.

The experiments show that the concentration of the DNA is reduced by 60% at the heated center although the temperature difference only amounts to about 1.5 °C (figure 3(*c*)). From the concentration image and the temperature image it is possible to infer the local concentration $c(T)$ depending on its local temperature $T$ in steady state. This result can be used with the integrated theory of equation (3) to extract the thermal diffusion coefficient of DNA [11] with values around $D_{\mathrm{T}} \approx 0.5 \times 10^{-8}$ cm$^2$ (s K)$^{-1}$.

Furthermore, this value does not depend very much on the length of DNA and is notably weaker than for hydrophobic molecules in nonaqueous solutions [25]. For example, polystyrene in toluene [26] gives a 20-fold higher thermal diffusion coefficient with $D_{\mathrm{T}} \approx 1 \times 10^{-7}$ cm$^2$ (s K)$^{-1}$. From the exponential dependence of thermophoresis in equation (3) one can expect a 1000-fold concentration ratio for DNA, 5000 base pair long, under a temperature difference of 50 °C (figure 4).

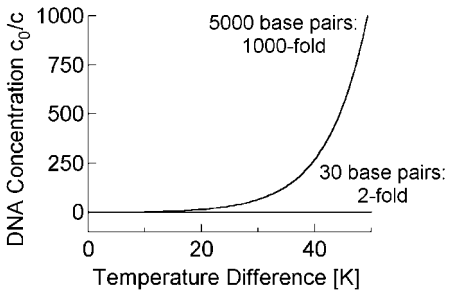


**Figure 4.** Exponential thermophoresis. Theoretically, it is expected that thermophoretic repulsion grows exponential when the applied temperature difference is increased. Based on this, one expects a 1000-fold concentration difference for 5000 base pairs DNA and 50 K temperature difference. Since shorter DNA shows markedly smaller effects, thermophoresis might be used to sort DNA by length.

Until now, care has been taken to minimize convective flow in the experiments. However, interesting effects can be seen by combining convection with thermophoresis. This combination has a considerable technological history, starting with the gas separation tube of Clusius [27], followed by thermogravitational columns for molecules in liquids in various ways [28–31]. These columns are about a centimeter in radius and more than a meter long, leading to equilibration times of many hours, if not days, even for small molecules or gases. However this effect applied along elongated vertical cracks of rock can provide large-scale downward concentration gradients as the fluid moves up in the hotter region where the molecules are thermophoretically depleted. Thermophoresis was also used to separate beads and polymers in nonaqueous solutions in thermal field-flow fractionation systems [32, 33].

According to equation (2), thermophoresis of charged particles works against the temperature gradient [11, 12, 23, 24] and leads to an attraction of molecules to cold regions. We can turn this depletion from hot spots into an accumulation by opposing the thermophoretic velocity with the fluid flow of the solvent. The most natural fluid flow under temperature gradient conditions is thermal convection. We will see that the repulsion from the hot spot can be countered by the flow and yields a strong accumulation when convection is allowed. Convection is triggered by applying stronger heating and increasing the chamber thickness from 10 $\mu$m to 50–500 $\mu$m.

The convection geometries are very similar to the one used in the laminar convection PCR experiment shown before. Under these conditions, DNA is highly accumulated into a microscopic spot near the convergent stagnation point of the convection at the lower chamber wall surface [11] (figure 5(*a*)). There, DNA accumulates in a small ring or point depending on the chamber and heating geometry (figures 5(*b*) and (*c*)). In this stagnation point, the fluid flow in all directions is slow. Still, the nearby convection flow will transport molecules near this point since the flow lines converge. A main role is attributed to the vertical temperature gradient which pushes the DNA to the colder wall against the slowly rising convection near the chamber wall. Increasing the horizontal temperature gradient by more focused heating gives rise to the ring geometry seen in figure 5(*b*).

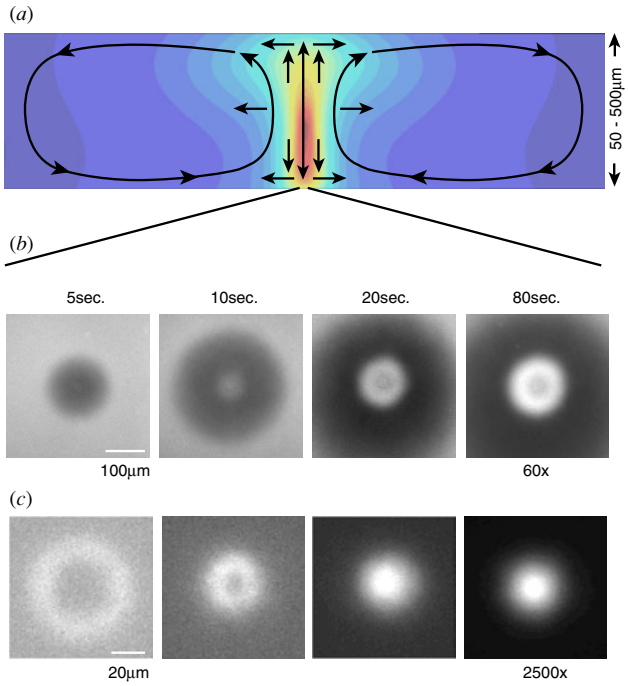


**Figure 5.** Thermophoretic trapping of DNA. (*a*) In the stagnation point of a convection flow, thermophoretic trapping can strongly accumulate DNA. The molecule velocities of thermal convection (black) and thermophoresis of DNA (red) are shown superimposed. They lead to accumulation of DNA in the lower stagnation point of the convection. (*b*) Accumulation is 60-fold for a 50 $\mu$m chamber and (*c*) about 2500-fold for a 500 $\mu$m thick chamber. Thus both replication by thermal cycling and thermophoretic accumulation can be achieved in a similar setting.

From fluorescence measurements and estimations of the flow it is deduced that the accumulation reaches more than 1000-fold enhanced DNA concentration as compared to the bulk concentration in the liquid. This probably is an indication that the exponential law of equation (3) indeed holds (see figure 4) and that the accumulation is a direct effect of the temperature gradients in the low-flow regions of the stagnation point of the flow.

Various tests have been performed to show that the accumulation is indeed based on thermophoresis of single DNA molecules. They include measuring the diffusion constant of DNA after switching off the heat, using a DNA-specific fluorescent dye in the experiments and changing to large salt concentrations which quench the accumulation the same way it quenches thermophoresis of DNA. Note again that a similar effect is expected if we apply the temperature gradient by a heated filament.

## 5. Thermally driven molecular evolution?

For molecular evolution to thrive, boundary conditions have to provide an autonomous nonequilibrium environment for replication, selection and mutation.

The experiments discussed show that thermal forces can drive replication in the temperature oscillation of laminar convection [8]. Laminar convection cycles molecules and

particles robustly along a fixed path. This could allow for strong binding to templates since they can easily detach again at the peaks of temperature of the convection, to be prepared for another cycle of replication.

The experiments also show that a simple small scale thermal convection can significantly enhance the concentration of DNA in small spots the size of some tens of micrometers [11]. This concentration effect is achieved simply by dissipating heat across a closed chamber: thermophoretic forces drive the molecules towards cooler chamber walls, helped by the convergent stagnation point of the convection flow. In this situation, only thermal energy enters and leaves the environment. This is sufficient to create for example a locally enhanced concentration of molecules.

Both nonequilibrium effects can be found in the same chamber geometry. The selection of molecules by thermophoretic trapping at the same time boosts reaction kinetics by enhanced concentration and allows many-body interactions with higher probability. It yields a selection environment to preserve longer biopolymers with a potentially exponential characteristic (equation (3), figure 4). The temperature cycling allows for many template-based or surface-catalytic reactions which would remain inaccessible under isothermal conditions.

Both effects can be expected in a wide variety of mesoscopic, porous geometries at a length scale between 10 $\mu$m and 1 cm, encapsulating volumes over 9 orders of magnitude from picoliters to milliliters. At this time one can only speculate on the dynamics and synergetic effects which can be expected from complex chamber systems of interconnected pores made of material with different thermal conductivities.

A proposal for a physical location for such a scenario would follow the lines of Corliss, Russell, Hall, Cairns-Smith, Matsuno, Martin and others [34–37] in pinpointing hydrothermal systems at the deep-sea floor [38, 39, 41, 42]. Hydrothermal venting systems create large temperature gradients across porous precipitates and rocks as hot water (50 °C to 370 °C) is pumped by a large-scale convection which is heated by magma. This high temperature circulation meets 2 °C cold ocean water in a large variety of dendritic, complex flow geometries as shown in figure 6(*a*). In such a macro- and microfluidic piping system [38–42], hot and cold water come very close and sustain strong temperature gradients across porous rocks in a variety of geometries. We give a rendering of such a situation in figure 6(*b*). The expected temperature gradients [43] match the boundary conditions used in the experiments.

This network of chambers also holds the potential to increase the efficiency of thermophoretic trapping, leading to accumulation also of shorter polymers. For example, a diffusive connection between two chambers which connects the accumulated spot of the top chamber with a chamber below should allow a multiplied accumulation as the molecules are thermophoretically trapped through a sequence of chambers. A similar mechanism was demonstrated for gases by the 'accumulation swing' of Clusius [44]. Note that the accumulation along such a porous system will establish a net downward flow of molecules, away from the diluting and therefore hostile ocean.

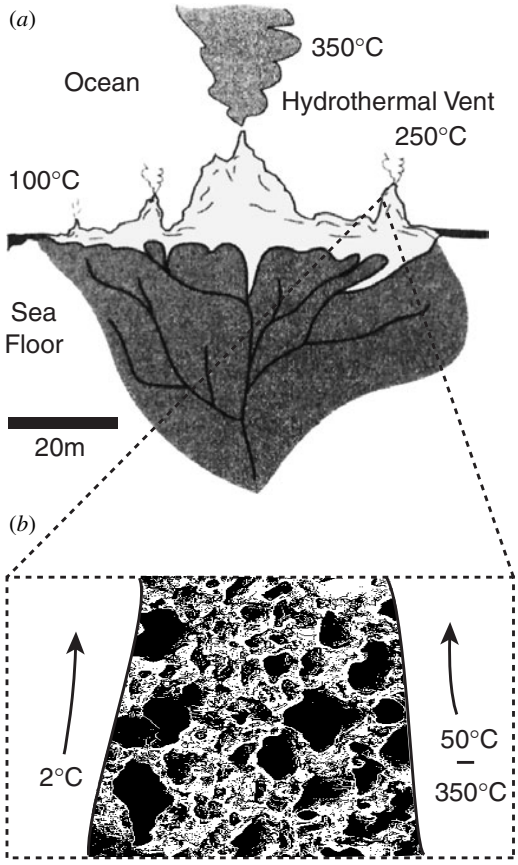


**Figure 6.** Hydrothermal scenario for molecular evolution of life. (*a*) Hydrothermal vents at the deep-ocean floor volcanically heated water at various temperatures and in various geometries. Reprinted by permission from Hannington *et al* [40]. (*b*) Artistic rendering of pores subjected to the steep temperature gradients created between large scale flows of 2 °C cold ocean water and hot water ejected by hydrothermal vents. Close proximity of both circulations have been observed [38, 39, 43]. In such a geometry, both convective replication and thermophoretic trapping could drive molecular evolution.

It should be noted that high salt concentrations quench the thermophoretic effect and therefore the accumulation of charged biomolecules [11, 24]. The limits are about 500 mM for monovalent ions and 30 mM for divalent ions [11]. Whereas thermophoresis is strong under physiological salt conditions, the salt of today's ocean water reduces thermophoretic effects considerably. Estimations of the salt concentration in the Hadean ocean are under debate and might have been lower or higher than today's [45]. However, water from hydrothermal vents [38, 39] is for example depleted of $MgCl_2$ and other ions and therefore should enable thermophoretic trapping.

Thermophoretic trapping and laminar thermal convection not only affects molecules, but also small particles such as, for example, clay. Due to their smaller diffusion constant $D$, thermophoretic effects lead to much larger concentration differences (equation (3)) and trigger strong thermophoretic

trapping. Particles which would be otherwise suspended can form an accumulated matrix held in place by thermophoretic trapping. This matrix might enhance the trapping of biological molecules by virtue of reducing their diffusion constant. Whether this combination of effects can be achieved remains to be seen since experiments in nonaqueous systems near a glass transition [46] show that $D_T$ scales with $D$ and in such a case would not affect the thermophoretic trapping efficiency.

It has to be stressed that thermophoretic trapping is a flow equilibrium of particles being trapped and released again by diffusion to the convection flow. From the perspective of a single particle, the trapping therefore is only transient. When released again to the convection flow, the particle continues to be subject to temperature cycling. This allows the same molecules to bind/unbind to each other or to surfaces in the convection flow and to accumulate thermophoretically in the stagnation point with high concentration.

It is interesting to note that, even in one convection chamber, the laminarity of convection produces trajectories which are isolated from each other by diffusion. For many geometries and molecules the diffusion is much slower than the temperature cycling. A single chamber can thus host a number of thermal cycling conditions which interact at time scales slower than the convection itself. Therefore different species of molecules could replicate in parallel under different conditions and allow for coevolution and evolution of subpopulations.

Increasing evidence has been found that cellular life in rock pores is common. Work over the last 20 years has confirmed that a vast number of prokaryotes—if not their majority [47]—live and thrive in pores of rock deep in the earth's mantle. These slowly growing creatures manage to survive on scarce nutrition sources and some of them can stand temperatures in excess of 110 °C, found both at depths and at hydrothermal vents. Such life in rock pores deep underground gives a realistic backdrop for a pore-based scenario of the origins of life. One is tempted to change the meaning of the oft-cited words of Richard Feynman [48]: 'There's plenty of room at the bottom'.

The given thermal force scenario is compatible with ideas proposed by Russell, Hall and Martin [36, 37]. They argue that life might have originated in pores, driven by gradients of pH and electrical potential at hydrothermal mounds developed over submarine seepages. It should be noted that at these locations, temperature gradients are abundant and could also allow for the discussed scenario of convective replication and thermophoretic trapping.

Russell and Martin argue, based on biochemical evidence [36], that membrane metabolism is a comparably late development in evolution. One might therefore imagine that life first evolved in pores and enclosures at the ocean floor, driven by various gradients—including thermal gradients—only later to evolve into autonomous cells bordered by lipid membranes. Only then was life ready to explore the diluted, unfriendly space of the oceans.

We have to apologize that we had to skip over many chemically motivated arguments and questions. The experiments discussed also give rise to many new questions. Can, for example, a fast small-scale convection be used to polymerize by cooling as demonstrated in the free-flow experiments of Matsuno [35]? Can an RNA world version of convective PCR be created and used to show a basic evolutionary dynamics of code generation? How much can thermophoretic trapping enhance the speed and make molecular evolution more stable? We believe that only a fully interdisciplinary interplay between biochemical top-down approaches and geobiophysical bottom-up searches for convincing boundary conditions will reveal new approaches and solutions for a molecular understanding of the evolution of life.

## Acknowledgments

We thank Noel Goddard, Koichiro Matsuno and Michael Russell for reading the manuscript. DB was supported by the Emmy Noether Program of the Deutsche Forschungsgemeinschaft.

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