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of life**

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**The power without the glory:
multiple roles of hydrogen peroxide in mediating
the origin of life**

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Abstract

The HP crucible hypothesis proposed here holds that life began in a localised environment on Earth that was perfused with a flow of hydrogen peroxide from a sustained external source, which powered and mediated molecular evolution and the protocellular RNA world. In this article we consolidate and review recent evidence, both circumstantial and tested in simulation in our work and in the laboratory in others' work, for its multiple roles in the evolution of the first living systems:

i) it provides a periodic power source as the hydrogen peroxide/thiosulfate (THP) redox oscillator, ii) it may act as an agent of molecular change and evolution and mediator of homochirality, iii) the THP oscillator, subject to Brownian input perturbations, produces a weighted distribution of output thermal fluctuations that favours polymerisation and chemical diversification over chemical degradation and simplification. The hypothesis can shed light on the hero and villain roles of hydrogen peroxide in cell function, and on the singularity of life: of necessity, life evolved early an armory of catalases, the continuing and all-pervasive presence of which prevents hydrogen peroxide from accumulating anywhere in sufficient quantities to host a second origin. The HP crucible hypothesis is radical but based on well-known chemistry and physics, it is eminently testable in the laboratory, and many of our simulations provide recipes for such experiments.

Key words: Origin of life, Chemical evolution, RNA world, THP oscillator,
Non-equilibrium thermal fluctuations, Hydrogen peroxide

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1. Introduction

Over recent decades our understanding of biological evolution, and the theory itself, has made giant strides (Müller, 2017), but the same cannot be said for its beginnings, the origin of life. Even specific breakthrough advances, such as non-enzymatic RNA replication and growth and division of vesicles (Szostak, 2017), are but fragments of the greatest story never told. A sociological hindrance may be that origin of life research is inherently interdisciplinary and multi-faceted so there is little common language and no unified framework to guide theory and practical developments.

The problem may be rooted at a deeper level though. That relatively little progress has been made on understanding abiogenesis, despite best efforts over many decades by top laboratories, suggests that we may have been missing or overlooking some fundamental insight, that a radical conceptual leap may be needed. (Similar or related sentiments from recent literature are collected in Supplementary Material section S1.) In previous works (Ball and Brindley, 2014, 2015, 2016a,b, 2017) we have proposed that hydrogen peroxide was an important ingredient in the abiogenesis environment, and tested, in simulation, several ways in which it may have facilitated molecular evolution and the RNA world. In the first part of this hypothesis article we synthesize these results into the HP crucible hypothesis, and present an expository outline and evidential review. The HP crucible hypothesis holds that life began in a localised environment on Earth that was perfused with a flow of hydrogen peroxide from a sustained source, which powered and mediated molecular evolution and the proto-cellular RNA world in multiple ways. This is the first coherent exposition of the HP crucible hypothesis. Additionally, in this article we extend the reach of the HP crucible hypothesis downstream, to reason on how it may have shaped the tree of life.

The potential importance of hydrogen peroxide in pre- and proto-cellular evolution is illustrated by the recent work of Springsteen *et al.* (2018). The authors report experiments that generate a non-enzymatic, simpler analogue of the citric acid cycle (the most fundamental and universal metabolic cycle), in which glyoxalate is oxidised by hydrogen peroxide. They hypothesize that this cycle could have emerged prebiotically, giving references for possible sources and occurrence of hydrogen peroxide that we have also cited previously (Borda *et al.*, 2001; Liang *et al.* 2006). Their experiments allow us to draw proto-metabolism under the umbrella of the HP crucible hypothesis, together with polymerisation and replication. Further, Lineweaver and Chopra (2012) and Stueeken *et al.* (2013) recognise that the environment for abiogenesis was not necessarily inhabitable by the first organisms. The HP crucible hypothesis supports a stronger statement: that the physical environment where life began must have been radically different from any that has existed since and most likely was uninhabitable by cellular life.

What was this environment like? Liquid water may have persisted on Earth since ~4.3 Gya (Kasting and Catling, 2003; Sleep *et al.*, 2011), there is evidence for fossilized microorganisms that may be 4,280 Gy old (Dodd *et al.*, 2017), and at least parts, if not most, of the Earth's near-surface were likely teeming with varied cellular life by 3.7 Gya (Nutman *et al.*, 2016). Thus abiogenesis may have occurred within a time-window of only a few hundred thousand to some millions of years during which chemical evolution yielded pre-RNA (Kaur *et al.*, 2017) and the RNA world, lipids and peptides, and simple metabolic cycles (Springsteen *et al.*, 2018), which co-evolved to proto-cellular and fully cellular organisms. To us this suggests that life arose in a localised, relatively ephemeral, highly abiogenesis-favouring environment, and, having arisen, perforce rapidly evolved catalases which prevent any emergence of “life

2.0” (Davies, 2011), by effectively “pulling up the ladder” (Bains and Schulze-Makuch, 2015).

The HP crucible hypothesis constrains the general milieu or even the locality on Earth where life likely originated. Possible sources of hydrogen peroxide on the early Earth were discussed and reviewed in Ball and Brindley (2014). If produced by intense ionizing radiation from a natural fission reactor (Draganic, 2005; Adam, 2007) there may be an outside chance, in future, of locating the actual crucible of life because such leave a (diminishing) signature. The hydrothermal vent setting for the origin of life is supported by the H₂O₂-producing experiments of Borda *et al.* (2001) and Foustoukos *et al.* (2011), with significant H₂O₂ produced even in anoxic environments (Gil-Lozano *et al.*, 2017). The ionizing radiation that bathed the early Earth would have produced dilute H₂O₂ rain on land and sea and in the ocean surface layer (Draganic, 2005; Dartnell, 2011), but we lack hard evidence, at present, that H₂O₂ from these sources concentrated locally in continuous flow by, e.g., preferential evaporation of water, although this is amenable to experimental investigation.

In the following section we elaborate on the three key words in the title, with respect to the HP crucible hypothesis. Subsection 2.1 – the power: What was the driving force for the transitions from geochemistry to biogeochemistry to biochemistry? We review and interpret simulations in which the thiosulfate-hydrogen peroxide (THP) redox thermochemical and pH oscillator was used to drive RNA processes. Subsection 2.2 – the glory: Although hydrogen peroxide has the power – the enthalpy of the peroxide bond – it has been denied the glory, until recently being cast as life's villain. We restore it to its rightful place as a likely driver and mediator of life's origin. Subsection 2.3 – multiple roles: We place the stochasticity of THP oscillations at centre stage in the origin of life drama. We then discuss the properties of hydrogen peroxide with respect to their

possible effects on incipient life. In section 3 we draw the tree of life into the HP crucible hypothesis. We sum up briefly in section 4. Mostly, we exclude references that were cited in our previous works, instead citing newer relevant literature.

2. H_2O_2 – “villain with a smiling cheek”

2.1 The power

The evolution of templated replication and biochemistry required power from a sustained source, as well as a continuous supply of nutrient, to counter the otherwise inevitable drift to equilibrium and collapse of biomolecules to tars and tholins (Deamer and Weber, 2010; Russell *et al.*, 2013; Pascal *et al.*, 2013; Sutherland, 2017). Molecular evolution and the RNA world most likely drew on thermal power directly (Muller and Schulze-Makuch, 2006), if the milieu was porous rock around a heat source such as a sub-marine hydrothermal vent (Sleep *et al.*, 2011; Lane and Martin, 2012) or a natural nuclear reactor (Ebisuzaki and Maruyama, 2017). That such a power source must have been periodic in temperature is now well-recognized (Spitzer, 2013; Spitzer *et al.*, 2015), because in the absence of fully developed cellular machinery thermal cycling is required for processes such as adsorption/desorption, polymerisation/hydrolysis, and RNA replication. Several mechanisms have been proposed: diurnal and seasonal cycles (Mamajanov *et al.*, 2014), tidal flows periodically activating natural nuclear reactors (Adam, 2016), periodic entrainment by hot geothermal streams (Szostak, 2016) and thermoconvection in rock pores (Kreysing *et al.*, 2015).

In Ball and Brindley (2014, 2015) we tested the novel idea that the thermal cycling needed to drive the RNA world may have been provided by thermochemical oscillations. In particular we identified the well-known thiosulfate-hydrogen peroxide (THP) redox oscillator, encouraged by the

substantial evidence of existence on the prebiotic world of the necessary reactants. (See reactions 1–2 in Supplementary Material section S2.) We used experimental rate data to show that the THP oscillator could power replication (by complementary strand pairing) of short RNA species, and further that the associated pH cycling may have played an important adjunct role. The reliance of all life on pH gradients to drive metabolism has prompted many researchers to investigate how this arose. Lane (2012) asks: “Some form of chemiosmotic coupling probably evolved very early in the history of life, arguably before LUCA (Lane *et al.*, 2010); the question is how, and why?” Lane’s hypothesis is that natural proton gradients across alkaline hydrothermal vents were harnessed by the earliest living cells. The HP crucible hypothesis provides a viable alternative (or perhaps complementary) possibility, since, in a bounded, spatially extended system, thermo-pH oscillations manifest as travelling waves and patterns. The gradients of a travelling thermo-pH wave powered by the THP oscillator can provide a proton motive force that may have driven metabolic processes in the RNA world.

This adds another dimension to the HP crucible hypothesis – it releases the RNA world and proto-cells from the narrow, confined environment of a hydrothermal vent, and at the same time provides the first intimation that

- i. the universal dependence of all life on the power of transmembrane pH gradients may have originated in the pre-cellular world, and
- ii. the vitally important functions of biologically produced hydrogen peroxide in cells may be rooted in the dynamic association of pre- and proto-cellular life with an abiotic hydrogen peroxide-rich medium.

Additionally, we found that pH cycling provided by the THP oscillator (reactions 2 in Supplementary Material section S2) improved ribozyme activity significantly over constant pH (Ball and Brindley, 2016b). Such pH cycling also

provides a possible resolution of the “replication versus ribozyme activity” paradox (Ivica *et al.*, 2013), which is that

- folded structures are necessary for ribozyme activity, but replication requires unfolded sequences.

In the dynamical THP medium a high temperature, low pH phase of the cycle may favour de-structuring of a ribozyme for replication, then replicated duplexes may adopt active ribozyme structures during a cooler, higher pH phase.

It is important to stress that, in none of these functions is thermochemical cycling necessarily exclusive; it could operate in a complementary, even resonant, manner with the non-chemical cycling mechanisms which have been proposed by others and referred to earlier.

2.2 The glory

We have shown that hydrogen peroxide could have provided the “power”, and much more, for the origin of life. However, the “glory” it deserves has not been accorded. Indeed, for more than a century, until the early 2000s, hydrogen peroxide was more sinned against than sinning, reviled and vilified as anti-life, its vital role in life from origin to present day misunderstood and even rejected. Biologists saw it as toxic to cell metabolism and gene transcription fidelity.

Chemists see it as destructive to molecular bonds: “If we drop some RNA into a flask of hydrogen peroxide it would be consumed within minutes!” No doubt *that* is true, but the origin of life environment was not, could not have been, flask-like. The dynamic, far-from-equilibrium flow environment we are contemplating here, with competing substrates for oxidation, is utterly different from the naysayers’ static flask that is left alone to equilibrate. In the THP oscillator-RNA system simulated in Ball and Brindley (2015), we quantified that a hydrogen peroxide molecule is ~65,000 times more likely to encounter a

thiosulfate ion than a G residue, the oxidizable base of the ribonucleotides. Given the intermittent spikes produced by the THP oscillator, RNA contact is likely to be even more fleeting. (We note also that the proto-metabolic cycles of Springsteen *et al.* (2018) were catalysed, rather than destroyed, by hydrogen peroxide.)

Since the 2000s there has been a steady rehabilitation of hydrogen peroxide (and other reactive oxygen species, ROS) with respect to its role in cellular life, and a much more nuanced view of its essential place in biology has emerged (Roy *et al.*, 2017). Its essential roles in metabolic processes and redox signaling in aerobic cells are reviewed by Sies (2017), who points out that, significantly, it is a *transcription-independent* signal molecule, and by Kuksal *et al.* (2017), and it is known to be produced by facultative anaerobes (Sekar and DiChristina, 2017; Lisher *et al.*, 2017).

Therefore, rather than dismissing hydrogen peroxide as being anti-life, important and valid questions that we urge to be investigated experimentally are:

- Taking as given the persistence of a localised environment, rich in hydrogen peroxide and the building blocks of life, in dynamic, non-equilibrium flow, what other components, conditions, and structures must be present, absent, or ongoing to kickstart molecular evolution?
- What types of nucleotide sequences are resistant to oxidation? Could exposure to hydrogen peroxide select for such sequences? How might the system dynamics evolve so as to minimize such exposure? What stabilizing agents may have been present?

As recommended by Spitzer (2013) and Agerschou *et al.* (2017), more experimentation using open driven systems should be encouraged, representing better the sustained, far-from-equilibrium conditions necessary for the origin and continuing existence and evolution of living systems.

2.3 Multiple roles

In addition to its influence as the oxidant of the THP oscillator, hydrogen peroxide may play other roles in the drama of life's origin and early evolution. An indirect role – but one of crucial importance – comes about because the THP oscillations, in any realistic setting, are not perfectly uniform in amplitude and period, but are subject to random perturbations. Hydrogen peroxide also may have had more direct effects, chemical and structural in nature. In this section we focus on these aspects of the HP crucible hypothesis.

In the beginning was disorder

Although the necessity of an open, far-from-equilibrium flow environment to create and maintain life is well understood (Branscomb *et al.*, 2017), the effects of random fluctuations of a non-equilibrium flow on prebiotic chemistry have received much less attention. Plausible environments for life's origin were temporally messy because input flows through an inhomogeneous matrix were subject to random fluctuations, and chemically diverse because the resulting output fluctuations produced a wide range of compounds (Guttenberg *et al.*, 2017). “In this world, in fact, fluctuations are everything,” state Branscomb *et al.* (2017), and we agree. But their model contains only Brownian motions, or normally distributed, random thermal fluctuations, which are not special to far-from-equilibrium systems – they occur regardless of equilibrium status. Something *more* than their Brownian ratchets is needed to drive chemical processes into living ones.

The simulated, non-equilibrium flow experiments reported in Ball and Brindley (2017) mimic conditions in a hydrothermal porous rock setting by (i) using a “toy train” model for reacting flows through a system of interconnected, inhomogeneous rock pores (see figure S1 in Supplementary Material section S3),

and (ii) setting the *inputs* as normally distributed, random quantities, i.e.; subject to Brownian motions. Within this paradigm we simulated surface-catalysed dimerization of pre-biotically relevant monomers, driven by the THP oscillator. (The dynamical system model for the toy train is reproduced in Supplementary Material section S3.) We summarize the results in figure 1.

Of relevance here is the following result obtained rigorously by Bertola and Cafaro (2015):

- Gaussian perturbations through the boundary of a near-equilibrium, non-reactive, closed system do not induce Gaussian fluctuations in the outputs.

Their output temperature and entropy fluctuations followed a gamma probability density function (figure 1(b)). This is not surprising. A gamma distribution has positive skewness S and is left-weighted (the median $M < \text{the mean}$). In the form of the Maxwell-Boltzmann distribution it applies to the realm of dilute ideal gases at or near equilibrium.

What does the output fluctuation spectrum of our far-from-equilibrium, reactive open flow system look like? The *output* fluctuations of temperature oscillation maxima, as experienced by the monomers and dimers, yielded the distribution in figure 1(c), the smoothed envelope of the histogram shown in (d). The relevant summary statistics for the distribution are: median $M = 0.090$, skewness $S = 0.27$ and kurtosis $\kappa = 0.19$ (as calculated by Excel).

The contrast between the distributions in figure 1(b) and (c) is remarkable. Evidently the reactive mass flows, strong nonlinearity, and dynamic, far-from-equilibrium drive – all necessary (but not sufficient) conditions for life – alter the distribution (b) profoundly. What do these results mean?

i) Fluctuating inputs are *necessary* for a growing, diversifying prebiotic system. Such a system must sample a wide range of temperature extrema in order to produce a variety of products from reactions having different activation energies.

ii) The positive median and negative skewness of the output fluctuations weight the system towards *higher* temperature perturbations. Thus the precious, high activation energy synthetic reactions that the prebiotic, non-enzymic world must access in order to grow, polymerize and diversify are favoured.

But the temperature perturbations must not be *too* high, and must not occur *too* often! There would be optimum values of the summary statistics such that the system spends enough time at high enough temperatures to carry out essential high activation energy reactions, but not so much that the reactant is totally consumed or destroyed, or surfaces degraded. Characterizing this distribution in explicit form could effectively give us a “fundamental equation of life”, which may provide useful guidance in designing molecular evolution experiments: they should be messy, as suggested by Szostak (2011), but not too messy. It also suggests a new strategy in the search for habitable worlds and emergent extra-terrestrial life: sample the temperature fluctuations.

Applying this diagnostic to potential extra-terrestrial habitats could easily and cheaply eliminate unlikely ones – those that are not sufficiently out of equilibrium to support life and those where the temperature fluctuations are too extreme.

iii) We can see why a system close to equilibrium cannot give rise to or sustain life. Under output fluctuations that follow the gamma distribution of figure 1(b), a high activation energy reaction would undergo its low activation energy reverse with greater probability, and development of a more complex prebiotic world would be impossible.

Branscomb *et al.* (2017) state “There is no way to selectively cause the thermal fluctuations you want...”, but as evinced by the weighted fluctuation distribution rendered in figure 1 (c) and (d), life may have found a way of doing just that! Furthermore, this distribution adds support to the notion that life may be able to produce the fluctuations it requires simply by operating far (but not too far) from equilibrium.

Two (oxygen atoms) are better than one

Let us take a closer look at hydrogen peroxide, as medium and molecule, in terms of how its structure and properties may have facilitated abiogenesis.

The observed homochirality of the nucleic and amino acids of life on Earth has attracted much research (Plasson *et al.*, 2007; Blackmond, 2011). Proposed mechanisms for the emergence of homochirality are reviewed from a physics perspective by Walker (2017) and Goldenfeld *et al.* (2017). The hydrogen peroxide molecule too is chiral, due to quantum mechanically hindered rotation about the O–O bond (Hunt *et al.*, 1965; Zhang *et al.*, 2009). In Ball and Brindley (2016a) we used a simplified mathematical model to demonstrate a proposed two-tiered mechanism by which this axial chirality may have effected the D-homochirality of the nucleic acids.

Here we present a probabilistic argument for hydrogen peroxide mediated emergence of homochirality, illustrated qualitatively in the schematic drawing of figure 2. At t_0 there is a marginal excess of D-chains of marginally greater average length than L-chains. Although the hydrogen peroxide bulk medium is racemic, due to facile interconversion of the enantiomers, labelled M and P by convention, it may undergo liquid-liquid chiral phase separation (Latinwo *et al.*, 2016), and local enantiomeric excesses may occur due to enantiospecific H-bonding interactions with itself and other chiral species. Snapshots at subsequent

times show the effects of enantiospecific interactions of ribonucleotide chains with hydrogen peroxide: the L-chain distribution diminishes in size and average chain length, until it disappears, while the reinforcement effect biases the D-chain distribution to greater size and average length.

The mean chain length at t_0 of around 30 is arbitrarily chosen. On the basis of this discussion, we predict that the “true” evolving distributions, which could be obtained by simulation experiments, would evolve left-skewed and right-weighted for the D-chains and right-skewed left-weighted for the L-chains, as depicted in figure 2.

Let us assume enantiospecific D–P and L–M interactions. Then the molecular fraction D/P increases while the fraction L/M decreases over time, because facile interconversion means that M and P must remain constant and equal. When the enantiomeric excess $\phi \equiv (D - L)/(D + L) = 1$ the destructive risk posed by hydrogen peroxide to a homochiral RNA world may be reduced by up to 50%, in principle, over that to a putative racemic RNA world. In reality this reduction would be lower, but it only needs to be > 0 . So as increases the RNA world becomes fitter with respect to its environment, and emergence of homochirality *in itself* confers a significant advantage on replicating RNA species!

This mechanism is compatible with and complementary to some other mechanisms that have been proposed for prebiotic homochiral amplification; e.g., breakage and fusion of homochiral chains (Blanco *et al.*, 2011).

We also collected, from primary sources, and tabulated in Ball and Brindley (2016a) many of the relevant physical, thermodynamic, and electrical properties of hydrogen peroxide and its aqueous solutions, and found that, in particular, the higher surface tension and viscosity favour vesicle formation and division, that hydrogen peroxide solutions are more polar, H-bonded, and highly structured than

pure water, and that homochiral clusters may persist. None of these effects is dramatic, but we do not want high drama in the RNA world. (A dramatically larger surfactant effect would really gum up the prebiotic works!) In a continuous flow system small effects are non-destructive but cumulative over time – this is the essence of the mediation aspect of the HP crucible hypothesis.

3. Catalases and the singular tree of life

3.1 Burning bridges

If we accept that life may have required an HP crucible to originate, we must also ask: What aspects of subsequent evolution, that currently are a mystery, can be explained by the HP crucible hypothesis? What does it say about the tree of life? Let us pay attention to this, then, and see where it takes us.

That all life descended from a common ancestor has been widely accepted as a fundamental dogma of biology since Darwin and Pasteur. The homochirality of life supports a single origin, and by extension at a single locality, as does the near-commonality of the genetic code and basic metabolism; and independent tests using model selection theory provide strong corroboration (Theobald, 2010). Phylogenetic interrogations of genomic and protein databases have built up a solid picture of this last universal common ancestor (LUCA) at the bifurcation to Archarya and Bacteria around 4 Gya, and its functionality and capabilities (Woese *et al.*, 1990; Hedges *et al.*, 2014; Weiss *et al.*, 2016; Becerra and Delaye, 2016).

The “tree of life” is widely used both as metaphor and cartoon for the homology of life. Its branches vanish as they grow, so we can see and study directly only its existing tips and such vanished tips for which we have found fossils; the antecedent structure mostly is deduced. This deduced tree of life is

sketched in figure 3(a) in minimal form, where branches representing only the three domains, Bacteria, Archaea and Eukarya, arise from a single origin.

This fundamental dogma has been questioned though. There would seem to be no good reason, *prima facie*, why cellular life should have arisen just once on Earth. The existence of microbial “shadow biospheres” that arose from independent origins was proposed by Cleland and Copley (2005) and discussed further by Davies *et al.* (2009) and Davies (2011). Surely a shadow biosphere could coexist with the known biosphere – after all, the three domains of life, Bacteria, Archaea and Eukarya, rub along in more-or-less peaceful, often interdependent, coexistence to this day. Could there even exist a remnant RNA world ecosystem in some niche on Earth?

A few imagined shadow trees of life are sketched in figure 3 (b), (c), and (e) (also in minimal form, for obvious reasons). In (b) shadow life (perhaps of opposite chirality) originated contemporaneously with known life and persists (unobserved by us) to this day. No evidence for such a shadow biosphere has ever turned up, although we do not rule it out entirely. In (c) an independent, contemporaneous origin of life led to a shadow biosphere that coexisted for some time with the early stages of the known biosphere before becoming extinct, but not before some lateral gene transfer (LGT) had taken place. This assumes the shadow genetic code was nearly identical to the known genetic code, so we could never corroborate this scenario. In (d) a weak shadow tree was born when evolution was well-advanced and died before present, and (e) shows a recently originated shadow tree that persists (notionally) to this day.

The HP crucible hypothesis can explain why scenarios such as (d) and (e) are impossible:

- Quite simply, the all-pervasive presence of catalases (and peroxidases) produced by cellular life, since before LUCA, in all habitable niches would forestall any possible second origin. Since the HP crucible hypothesis holds that a medium rich in hydrogen peroxide was necessary (although not sufficient) for molecular evolution to proto-cellular structures, the unceasing action of catalases in oxidizing hydrogen peroxide effectively would prevent the development and persistence of such an environment again.

The evolution of catalases was usually assumed to have coincided with oxygenation of the atmosphere 2.5 Gya, attributed to rapid spread of oxygen-evolving photosynthetic organisms. But this view is shifting backwards in time (Slesak *et al.*, 2016; Olson *et al.*, 2017). Phylogenetic analysis was used by Slesak *et al.* (2016) to show that enzymatic antioxidant systems were functioning in in early anaerobic protocells at the time of LUCA. The HP crucible hypothesis lends powerful support to the early evolution of catalases. Calvin (1959) proposed that catalases developed from simple free Fe^{2+} , through iron-heme complexes to association with peptides to the active site of the fully translated catalase protein, and there has been no contradictory finding since.

Evolution can be thought of as the burning of a succession of small bridges: the results of a transformational evolutionary step usually destroy the pre-conditions for its own occurrence. This is described as “pulling up the ladder” in Bains and Schulze-Makuch (2015), and in nontechnical terms in the book by Schulze-Makuch and Bains (2017). But the first cellular life destroyed utterly the bridge across the biggest divide of all, that which spanned the cellular and the RNA worlds! Any chance of re-building it was permanently rubbed out by the necessary early evolution and subsequent persistence of catalases. Living cells *themselves* forbid the possibility that a competing biosphere, Life 2.0 (Davies, 2011), could arise and out-compete, coexist with, or devour Life 1.0!

3.2 Bottlenecks and bifurcations

If the tree paradigm applies to evolution of the biosphere from LUCA onwards, by continuity it must also apply to pre-LUCA evolution. What do the branches and twigs on a pre-LUCA tree of life actually represent? A single branch is the surviving coarse-grained thread, identified *a posteriori*, of a myriad successive bifurcations to smaller twigs, none of which survived competition with the lineage that became the living thread. A pre-LUCA tree of life is postulated in figure 4. It is rudimentary, and undoubtedly important details are lacking or incorrectly placed in the hierarchy, but it is a first pass and, anyway, its function is heuristic. Consider the branch, distinguished as a thick blue thread, that gives rise to LUCA.

A full picture of this branch would look quite a confusing mess, an open network of uncountable extinct twigs with, post genetic code, lateral gene transfers, but the simplified picture is helpful in guiding our thoughts about the tree of life concept in terms of the HP crucible hypothesis.

Imagine the tree growing upwards through a fluctuating hydrogen peroxide-rich flow, emanating at its source, which powers, directs and mediates the biomolecular species and functionalities that evolve, the homochirality, surface polymerisation, replication, catalases, etc. annotated in figure 4. As the tree grows and bifurcates the flow also becomes more dilute, due to source attrition, and action of catalases, enzymes must take over from hydrogen peroxide's direct drive and actions, and protective encapsulation is selected for, where salts are included and hydrogen peroxide excluded from the proto-cell. But living proto-cells had become dependent on the depleting non-biotic hydrogen peroxide, not least the pH gradients provided by the THP oscillator to power them! So abilities to exploit trans-membrane pH gradients were selected for.

We note that there may have been a period during which the intracellular solvent of proto-cells was an $\text{H}_2\text{O}_2/\text{H}_2\text{O}$ solution. Houtkooper and Schulze-

Makuch (2007) hypothesized that living organisms evolved on Mars that used a water/hydrogen peroxide mixture as an intracellular liquid, based on the presence of hydrogen peroxide in the Martian soil, which they proposed is of biogenic origin. Bonfio *et al.* (2018) explored experimentally a proposed path whereby iron-sulfur peptides in proto-cells pass electrons to non-biologically produced hydrogen peroxide (0.5 mM), which is then reduced to provide a pH gradient across primitive membranes. Hydrogen peroxide is singled out in particular by Taverne *et al.* (2018) as having played a major role in evolution of life on Earth, being both a driver and a consequence of evolution. These works strengthen the HP crucible hypothesis, adding experimental support and new evidence for a significant participation of hydrogen peroxide in very early cellular life.”

This discussion is purposely (and necessarily) speculative. It is intended to stimulate further ideas concerning possible feedbacks between an evolving biomolecular system and its energetic medium, and how these interactions themselves may have shaped emergent life. It is by setting out these speculations, too, that we can see, in principle, how hydrogen peroxide may have played an active role in shaping the tree of life by providing a pre-genetic selection pressure – as do biologically produced ROS to this day on genomes and epigenetically (Sies, 2017).

4. Conclusions

The HP crucible hypothesis can explain or provide a mechanism for several necessary (but not sufficient) conditions and processes for the origin of life on Earth, or so far unexplained universal characteristics of life:

- i. In a primordial medium rich in hydrogen peroxide, produced continuously by a localised source, the THP oscillator can provide the far-from-equilibrium drive and thermal periodicity necessary to power chemical evolution and the

- RNA world. The experiments of Springsteen et al. (2018) provide a possible example of this driving role.
- ii. Since the THP redox system also behaves as a pH oscillator, it can provide pH (or chemical potential) gradients in a spatially extended bounded matrix, which can augment the thermal drive. The universal dependence of all life on transmembrane pH gradients may have its origins in the THP oscillator.
 - iii. The Gaussian inputs that drive the THP oscillator in a natural setting produce a non-Gaussian distribution of output thermal fluctuations that is highly enabling for chemical evolution. This distribution is a “fundamental equation of life”, which can guide experiments and searches for habitable worlds.
 - iv. As a chiral molecule hydrogen peroxide has the potential ability to beget chiral segregation of biomolecules (by the Curie principle), and effect homochirality of the nucleic acids. Its structural and molecular properties in solution may facilitate chemical evolution and the RNA world. It effectively grooms the evolving pre- or proto-cellular world for survival in the absence of its external supply.
 - v. Evolution of living systems in an HP crucible may have stimulated the early (pre-LUCA) evolution of catalases and peroxidases, and may help explain how life's conflicted relationship with ROS arose.
 - vi. Life's origin in a hydrogen peroxide rich medium can explain the singularity of the tree of life. The rapid proliferation of cellular life in all habitable niches on Earth means that all-pervasive, efficient catalases have prevented any subsequent build-up of hydrogen peroxide.

There is much more work to be done on the HP crucible hypothesis. Inevitably we will be shown to be amiss, or lacking, in many details. There are also large gaps – for now. (For example, where, exactly, on the Earth did the

origin of life take place? We simply don't know.) But we stress that many aspects are amenable to experimental investigations, and the simulations we have reported in Ball and Brindley (2014, 2015, 2016a,b, 2017) in fact have provided recipes for experiments that are quite feasible in the hands of competent students and postdocs. Such experiments cannot of course *prove* anything about the origin of life, but they can demonstrate likely scenarios and guide our thinking on missing parts of the story.

The HP crucible hypothesis restores the full glory of hydrogen peroxide in life's long story: it was there in the beginning, and life – ancient and modern – cannot exist without it.

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Author Disclosure Statement

No competing financial interests exist.

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Figure captions

FIG. 1. (a) Gaussian (normal) and (b) gamma probability densities, (c) bezier-smoothed envelope of the temperature fluctuation histogram (d) from Ball and Brindley (2017).

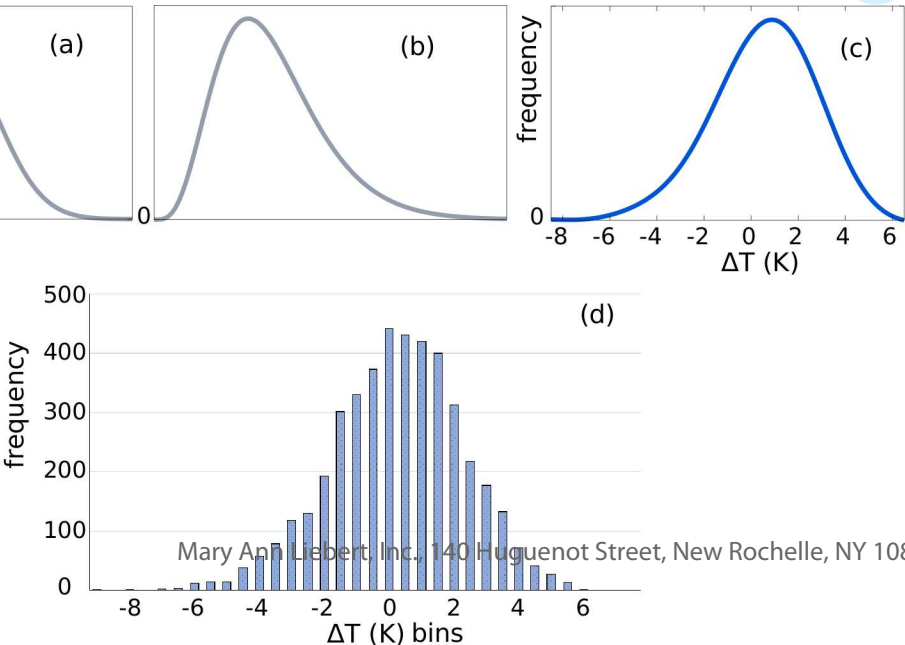
FIG. 2. Schematic drawing of hypothetical distributions of chain lengths of D polynucleotides and L polynucleotides at progressive, but arbitrary, times from t_0 to t_4 .

FIG. 3. Known (a), possible (b) and (c), and impossible (d) and (e) trees of life.

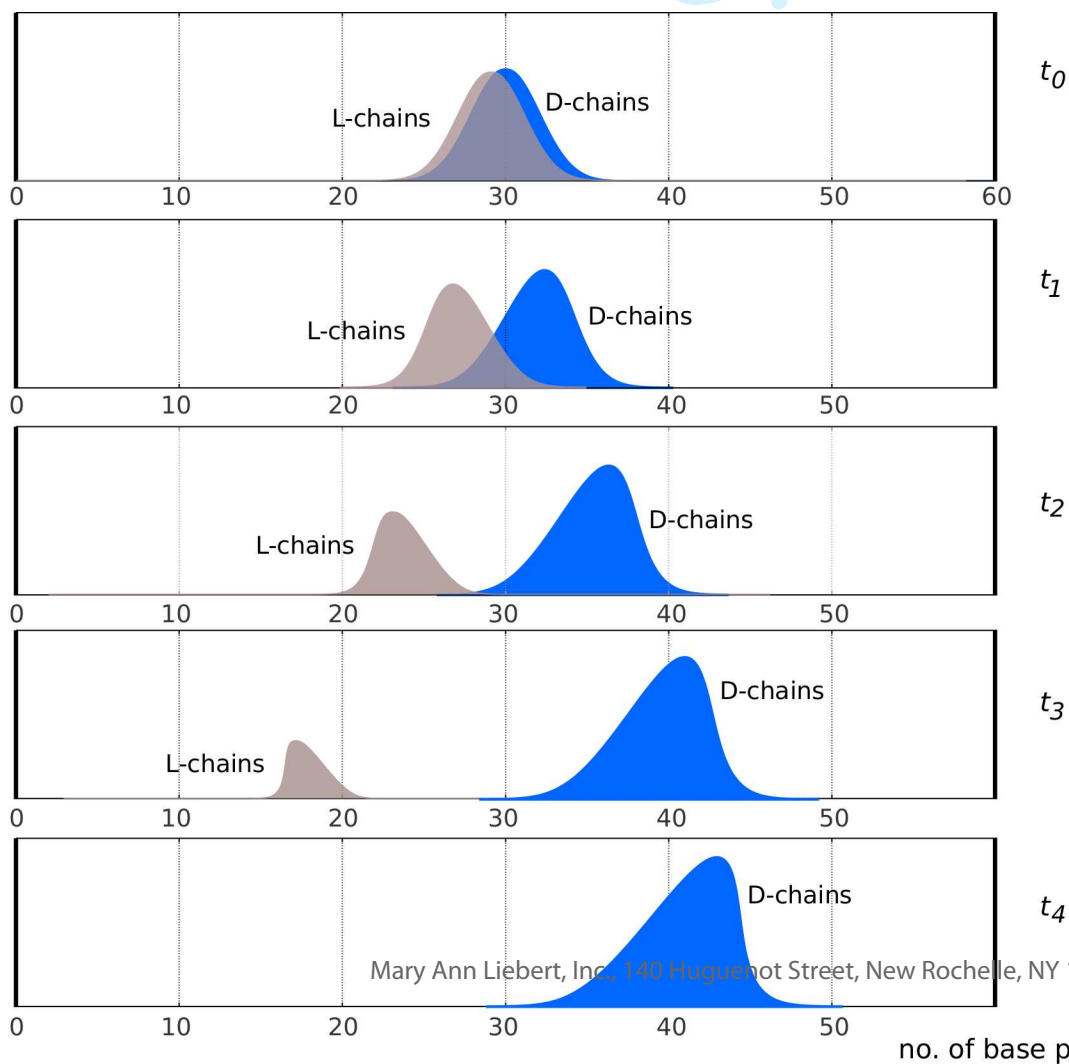
FIG. 4. A postulated partial tree of emergent life leading to LUCA. The branch that produces LUCA is a thick blue thread; grey or pale blue twigs do not survive. Possible LGT is indicated by the dotted red line.

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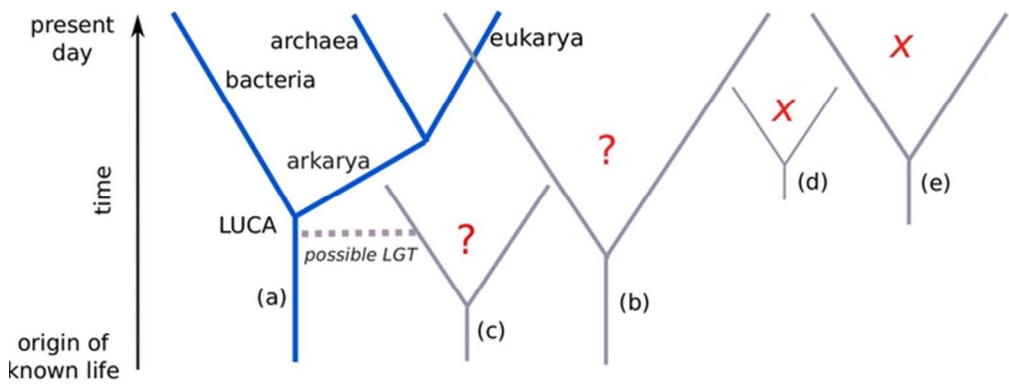


FIG. 3. Known (a), possible (b) and (c), and impossible (d) and (e) trees of life.

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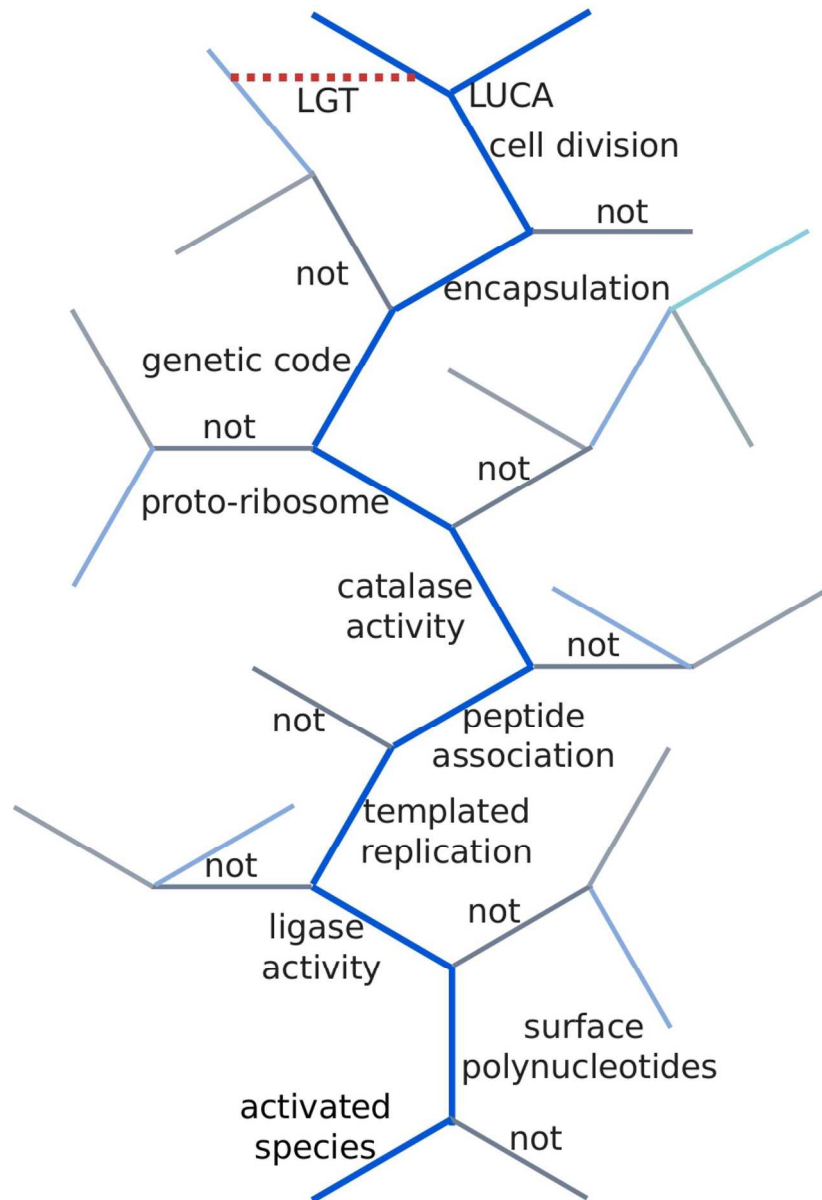


FIG. 4. A postulated partial tree of emergent life leading to LUCA. The branch that produces LUCA is a thick blue thread; grey or pale blue twigs do not survive. Possible LGT is indicated by the dotted red line.

117x171mm (300 x 300 DPI)

Supplementary material for
The power without the glory: Multiple roles of
hydrogen peroxide in mediating the origin of life

Rowena Ball*
John Brindley†

S1 Life, where is thy sting!

In the main article we suggest that some new fundamental insight or radical conceptual leap is needed before progress can be made on understanding the origin of life. Many other researchers have recently expressed similar sentiments:

- Walker (2017): “Despite the significance of the problem [of the origin of life], we currently have few scientific windows into its resolution... This suggests a radical rethink of the problem of origins may be necessary.”
- Cronin and Walker (2016): “A radical rethink is necessary, one that explores not just plausible chemical scenarios but also new physical processes and driving forces.”
- Spitzer et al. (2015): “Origin of life research has been slow to advance not only because of its complex evolutionary nature but also because of the lack of agreement on fundamental concepts.”
- Jheeta (2017): “Are we any nearer to obtaining a complete answer to the pertinent question of the origin of life? The information disseminated at this latest meeting basically yields up a miniscule yes, but a humungous no.”
- For Trevors et al. (2012) the origin of life is one of the big questions that require *transformative* research and bold hypotheses that challenge orthodoxy, especially if the existing knowledge is fragmented and incomplete.

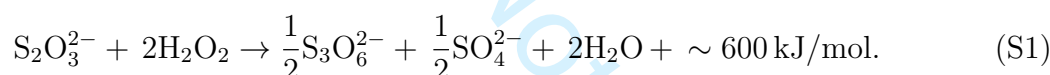
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†School of Mathematics, University of Leeds, Leeds LS2 9JT UK. Email J.Brindley@leeds.ac.uk.

- [Pross \(2012\)](#): “Despite the profound advances in molecular biology over the last half-century, we still do not understand what life is, how it relates to the inanimate world, and how it emerged... over the past half-century considerable effort has been directed into attempts to resolve these fundamental issues, but the gates to the Promised Land seem as distant as ever. Like a mirage in the desert, just as the palm trees signaling the oasis seemingly materialize, shimmering on the horizon, they fade away yet again...”
- [Davies \(2011\)](#): “We have little or no idea beyond a few general scenarios and just-so stories how life actually emerged from non-life... Unlike Darwin’s theory of evolution, we have no accepted theory of life’s origin, only a collection of scenarios and conjectures.”

S2 The THP oscillator

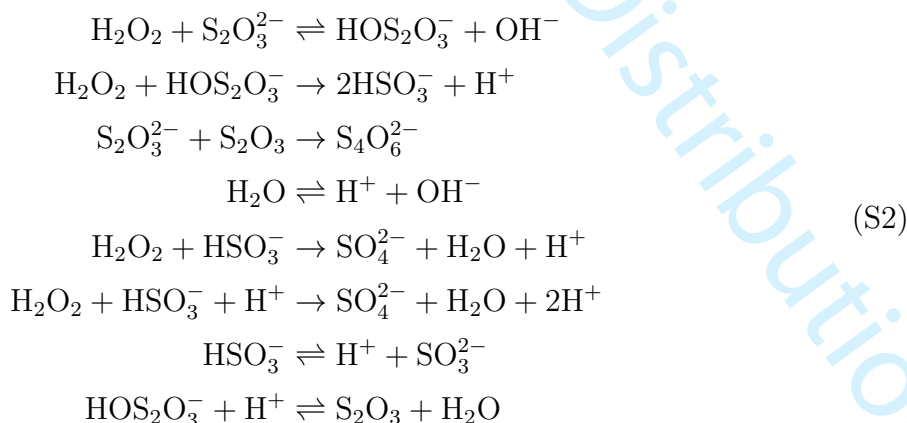
Thermochemical oscillations can occur in a nonequilibrium, bounded system when there is sufficient competition between the nonlinear rate of heat release due to reaction and the linear rate of heat removal through the boundary. Fundamentally they are relaxation oscillations, and physically they are due to the relatively high specific heat of most liquids [Ball \(2011\)](#). In a continuous flow system thermochemical oscillations can be self-sustained.

At high reactant concentrations, long residence times, or with poor heat removal, the THP thermochemical oscillator undergoes reaction [S1](#), in which deep, highly exothermic oxidation to trithionate occurs:



Isothermal, purely chemical oscillations can occur if the reacting system is effectively third or higher order in the chemistry. In a nonequilibrium flow system they, too, may be self-sustained.

At low reactant concentrations the reactions ??, in which light oxidation to tetrathionate occurs in the presence of acid/base, give rise to the isothermal THP pH oscillator:



The combined THP thermochemical-pH oscillator consists of reaction [S1](#) and the acid-base equilibria of reactions ??. Time series from dynamical simulations of the THP oscillator are rendered in [Ball and Brindley \(2014, 2015\)](#).

S3 Model for fluctuating flow through inhomogeneous rock pore

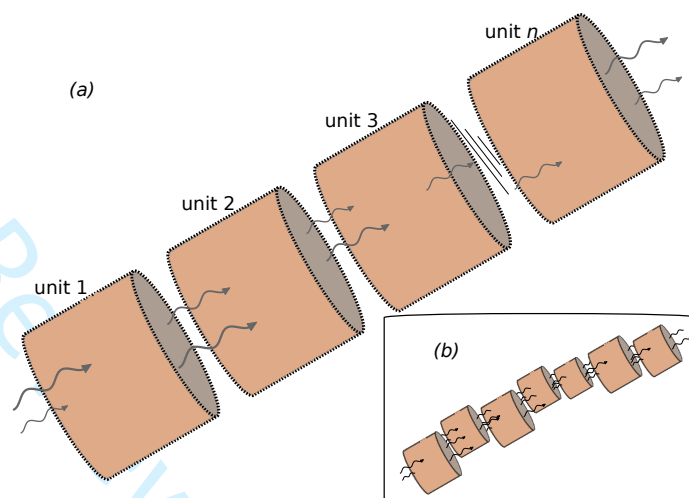


Figure S1: Sketch of the CSTRs-in-series setup in which n units are coupled serially. THP reactants for the flow in at one end; dotted outlines indicates porous walls through which THP reactants also enter the system in cross-flow. The cross-flow mean residence times may differ between units, which allows the model to account for the more general situation shown in (b), where the pore volumes are nonuniform.

Figure S1 and the following text are reproduced from [Ball and Brindley \(2017\)](#), for convenience of the reader.

Mass and enthalpy balances yield coupled differential equations for evolution of the temperature and concentrations of species at the outlet of each unit in figure S1a. The dynamical equation for the temperature in unit i ,

$$V\bar{C}\frac{dT_i}{dt} = V \sum_p (-\Delta H_j) r_{j,i}(T) + \bar{C}(\tilde{F}T_{i-1} - \tilde{F}T_i) + \bar{C}(\tilde{\phi}_i\tilde{T}_a - \tilde{\phi}_iT_i) + L(\tilde{T}_a - T_i), \quad (S3)$$

includes the following contributions on the right hand side: The first term is the total rate of heating due to reaction enthalpies, where $r_{j,i}(T)$ is the rate of reaction j in unit i and the summation is over the p reactions that contribute to the enthalpy. The second and third terms account for the heat transported by the inflow and cross-flows respectively. The fourth term is the rate of heat loss to an environment that is assumed to remain at a fixed ambient temperature T_a .

Mass balances in each unit i yield dynamical equations for the concentrations,

$$V\frac{dc_{x,i}}{dt} = V \sum_m \sigma r_{j,i}(T) + \tilde{F}c_{x,i-1} - \tilde{F}c_{x,i} + \tilde{\phi}_i\tilde{c}_{x,a} - \tilde{\phi}_ic_{x,i}, \quad (S4)$$

where $c_{x,i}$ is concentration of reactant or intermediate x in unit i , the summation is over the m reaction rates that involve x , and $\sigma = 1$ for intermediates that are produced and $\sigma = -1$ for reactants and intermediates that are consumed. The rate coefficients $k_{j,i}$ of the reaction rates $r_{j,i}$ have Arrhenius temperature dependence,

$$k_{j,i}(T) = z_j \exp\left(\frac{-E_j}{RT_i}\right). \quad (S5)$$

Nomenclature is defined in table S1. The input parameters are subject to stochastic fluctuations, which we modelled by treating the inflow rate, cross-flow rates, input concentrations and ambient temperature as normally distributed random quantities $\tilde{q} = \bar{q} + \delta q$, where $\bar{q}$ is the mean, δq is the random fluctuation and the variance $s = w\bar{q}$. The walls are non-uniform in composition and thickness, and present spatially variable resistance to cross-flows. To model this heterogeneity each mean cross-flow coefficient $\bar{\phi}_i$ is independently variable.

Table S1: Nomenclature of equations S3–S5, and numerical values of fixed quantities and parameters used in simulations reported in [Ball and Brindley \(2017\)](#).

Symbol (units)	definition	Quantity	Value
c or $[\dots]$ (M)	concentration		
$\bar{C}$ ($\text{J K}^{-1}\text{l}^{-1}$)	average volumetric specific heat	$\bar{C}$	3400
E (kJ mol^{-1})	activation energy		
F ($\mu\text{l s}^{-1}$)	inflow rate	F	0.48
ϕ , ($\mu\text{l s}^{-1}$)	cross-flow rate		
ΔH (kJ mol^{-1})	reaction enthalpy		
k ($\text{M}^{-1}\text{s}^{-1}$ or s^{-1})	reaction rate constant		
L (mW K^{-1})	wall thermal conductance	L	2.0
R ($\text{J mol}^{-1}\text{K}^{-1}$)	gas constant		8.31447
T (K)	temperature	T_a	283
V (μl)	unit reaction volume	V	10.0
z ($\text{M}^{-1}\text{s}^{-1}$ or s^{-1})	pre-exponential factor		
Subscripts			
a	of the ambient or wall temperature		
i	of reactor unit i , where $i = 1 \dots 12$		
j	of reaction j , where $j = \text{R1} \dots \text{R6}$		
x	a reactant or intermediate species		

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