

## REVIEW ESSAY

## Prospects &amp; Overviews

# “Minimal metabolism”: A key concept to investigate the origins and nature of biological systems

Nino Lauber<sup>1,2</sup>  | Christoph Flamm<sup>3</sup> | Kepa Ruiz-Mirazo<sup>1,2</sup> 

<sup>1</sup> Biofisika Institute (CSIC, UPV/EHU), University of the Basque Country, Leioa, Spain

<sup>2</sup> Department of Philosophy, University of the Basque Country, Leioa, Spain

<sup>3</sup> Institute for Theoretical Chemistry, University of Vienna, Vienna, Austria

**Correspondence**

Kepa Ruiz-Mirazo, Biofisika Institute (CSIC, UPV/EHU), University of the Basque Country, Leioa, Spain.

Email: kepa.ruiz-mirazo@ehu.eus

**Abstract**

The systems view on life and its emergence from complex chemistry has remarkably increased the scientific attention on metabolism in the last two decades. However, during this time there has not been much theoretical discussion on what constitutes a metabolism and what role it actually played in biogenesis. A critical and updated review on the topic is here offered, including some references to classical models from last century, but focusing more on current and future research. Metabolism is considered as intrinsically related to the living but not necessarily equivalent to it. More precisely, the idea of “minimal metabolism”, in contrast to previous, top-down conceptions, is formulated as a heuristic construct, halfway between chemistry and biology. Thus, rather than providing a complete or final characterization of metabolism, our aim is to encourage further investigations on it, particularly in the context of life’s origin, for which some concrete methodological suggestions are provided. Also see the video abstract here: <https://youtu.be/DP7VMKk2qpA>

**KEYWORDS**

autonomous control, constructive self-maintenance, functional bootstrapping, metabolism, origins of life, prebiotic systems chemistry, rule-based computational chemistry

Metabolism is the kernel of life. — Arren Bar-Even

**INTRODUCTION**

Experimental evidence coming from several research groups in the last few years<sup>[1–7]</sup> is showing that central metabolic pathways, like glycolysis, the pentose phosphate cycle, the citric acid cycle, or close analogues, could run under prebiotic conditions, long before proteins and enzymatic control developed in biological organisms. Building a solid bridge from geochemistry and organic chemistry towards biochemistry still requires a lot of work, but we could be witnessing the first steps of a fundamental change in our scientific approximation to the problem of origins of life, brought about by a non-reductionist, systems conception that is expanding, since the turn of the century, both in chemistry and biology.<sup>[8–10]</sup> Leslie Orgel, one of the pioneers of the field of origins, had deep concerns about the feasibility of reaction cycles in prebiotic

conditions,<sup>[11,12]</sup> but he did acknowledge that “if complex cycles analogous to metabolic cycles could have operated on the primitive Earth, before the appearance of enzymes or other informational polymers, many of the obstacles to the construction of a plausible scenario for the origin of life would disappear”. By proving his primary intuition wrong, like the aforementioned results seem to be doing (although not quite there yet, as we will expand below), the scientific community would be opening the way to prove him right in the latter assertion, which is much more interesting and promising for the future of the field.

However, in order to design further experiments and research avenues in the most productive way, we should take some time to reflect, carefully, about what metabolism is. For many scientists, metabolism consists in the set of chemical transformations that sustain, at the most basic level, a living organism, by providing it with molecular building blocks and energy to synthesize/repair all of its components. In brief, metabolism would be the core chemistry of life,

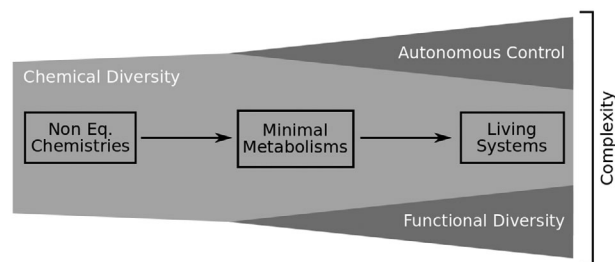
This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2021 The Authors. *BioEssays* published by Wiley Periodicals LLC

as it is realized in each cell,<sup>[13–15]</sup> or in a wider ecological context, across the whole biosphere.<sup>[16,17]</sup> This conception appears to be fairly congruent and secure, until one realizes that it is entirely dependent on how one answers the question of what life is, or what a living being is—which are tricky issues, still far from reaching consensus in the academic sphere.<sup>[18–20]</sup> An alternative approach would be to consider metabolism in its own terms: namely, as a chemistry that is strongly linked to biological phenomena, but not fully subordinate to them. From that standpoint, one could think about metabolic processes that are not necessarily part of a full-fledged biological organism. And this is of particular interest for those who consider that the idea of metabolism should play a relevant role in leading the investigation on the origins of life,<sup>[21–24]</sup> including ourselves.<sup>[25,26]</sup> Metabolism, under the hypothesis that it could operate before proteins and genetic mechanisms took the stage, would thus serve as a heuristic construct to guide prebiotic research.

If such a premise is accepted and metabolism is postulated to stand at the interface between chemistry and biology, then one is forced to address: (i) the criteria to distinguish minimal forms of metabolism from simpler sets of chemical reactions; and (ii) a plausible scenario from which more complex expressions of metabolism could progressively develop, all the way to genetically-instructed metabolisms, like the ones we observe today in nature. The first point is related to what several chemists interested in the origins of life are pointing at when they speak about the need to discover a major “system innovation”<sup>[27]</sup> or “emergent functions in complex reaction mixtures”<sup>[10]</sup>; that is, the initial key steps for the process of biogenesis to take off, once a collection of primary molecular building blocks concur in a prebiotic setting. The second point refers to later stages of that process, during which the incipient metabolisms would gain robustness and fidelity in their execution and reproduction, until they become full-fledged biological metabolisms: in other words, how to correlate the conjecture with real life, or with life as we know it, demonstrating that the principles of metabolic organization have deep physico-chemical roots and should constitute a central theoretical pillar for systems biology—as already suggested by a number of authors.<sup>[28–30]</sup>

These are precisely the two main questions that we will consider in the next pages, while putting forward a new conception of *minimal metabolism* (alternative to its more common “top-down” biological interpretation<sup>[31]</sup>), which will hopefully contribute to generate novel hypotheses and lines of work in prebiotic chemistry, as well as food for thought on the foundations of systems science. First, in the next section (“Collecting the key ingredients for metabolism”), the most relevant aspects to characterize metabolism will be brought to the fore, in connection to previous approaches to the problem. Since most of these “classical” approaches have been reviewed more extensively elsewhere,<sup>[32–34]</sup> we will just retrieve those insights that, according to our perspective, remain valid and useful for the current state of affairs. This will allow us to articulate, in section “Minimal metabolism”: tentative proposal and viability conditions”, the theoretical criteria to differentiate a minimal metabolism from a network of coupled chemical reactions, based on the capacity of the former to produce its own material constraining structures and establish, autonomously, a functional bootstrapping between the two: processes of synthesis and constraints. Our



**FIGURE 1** Minimal metabolisms would stand at the interface between non-equilibrium complex chemistries and biological systems. As major prebiotic transitions unfold, the complexity of the corresponding phenomena should increase, together with the capacity to develop autonomous control mechanisms. Whereas chemical diversity decreases (light grey area), in the sense that only a subset of all possible molecular compounds/types of reactions is exploited by living systems, functional diversity increases (darker grey area), in the sense that these systems manage to generate and couple together a wider (eventually, an open-ended) variety of inter-dependent components and transformation processes

aim will not be to come out with a neat, well-formalized or definite version of what “minimal metabolism” consists in. Rather, the goal at this stage is simply to offer a guideline, a prescription on how we should think about it in order to conduct fruitful scientific research that brings about a more accurate and comprehensive characterization of it in the future. Nevertheless, tackling the problem from this systems conceptual framework has several important implications, as will be shown in section “Methodological suggestions: a case for ‘rule-based’ computational approaches”, where we propose the methodology required for such an exploration, and in the conclusion, where we briefly indicate how minimal metabolisms, as they are here conceived, would naturally thrive and evolve towards more complex architectures, like the ones we observe in real living systems.

## COLLECTING THE KEY INGREDIENTS FOR METABOLISM

In line with our introductory remarks above, metabolism, though intrinsically linked to living systems, does not cover all their complex, multifarious phenomenology—which extends far beyond single-organism physiology, spanning evolutionary time scales. Therefore, metabolism will be treated here as *fundamental for life but not synonymous to life* (in contrast to other authors—e.g., classically:<sup>[35,36]</sup>; or, more recently:<sup>[29,34]</sup>). After insisting on this important point, illustrated in Figure 1, let us bring together those elements that characterize any metabolism, including minimal (hypothetical) forms of it that could be postulated as prebiotic.

A basic feature, sometimes forgotten because it is rather uncontroversial, is the fact that metabolism is a non-reducible, systems construct. Indeed, all the traditional models on “minimal living organization”<sup>[37–41]</sup> conceive of it as a coupled set of diverse molecules and transformation processes that cannot be scaled down,

<sup>1</sup> The “hypercycle” model,<sup>[42]</sup> also a classical in theoretical biology, will not be discussed in this article, since it addresses the evolution of molecular replicators—not metabolism, in a proper sense. It does argue for the need that those molecular replicators get organized (“hyper-

by definition, to a particular component or reaction.<sup>2</sup> Although the way to represent the indivisibility of such a set varies from model to model (making use, respectively, of “complex mappings in category theory”, of the abstract idea of “topological domain”, of “coupled multi-component reaction cycles”, “bipartite networks”, or “functional/hierarchical relations among  $\lambda$ -calculus expressions”), all those pioneer works were elaborated in opposition to the strongly analytic and reductionist approaches of molecular biology, predominant at the time. This feature (the non-reducibility to molecular mechanisms) may seem obvious now, in the era of systems biology, but it has a fundamental consequence for our purposes here: metabolism must be defined in relational terms (i.e., through an account that includes—and typically highlights—the relationships among the different components of a system, not just the components themselves). What remains to be elucidated—and will be the focus of the next pages—is the type/s of constituents and processes involved, the conditions of viability, as well as the interactions and the architecture of relationships that characterize metabolic organization.

### Chemical diversity in open and far-from-equilibrium thermodynamic conditions

The major trouble that classical theoretical approaches to the problem of metabolism run into has to do with their high level of abstraction and strong inclination towards formalization. As we will explain in more detail below (specifically, in section “Methodological suggestions: a case for ‘rule-based’ computational approaches”), theoretical research in this context should advance hand in hand with experimental work, each providing constant feedback to the other. The reason behind is that one is forced to deal with complex chemical phenomena that involve a remarkable diversity of components and non-linear, dynamic interactions taking place in far-from-equilibrium heterogeneous conditions (with matter and energy coming in and out of the system), so it is extremely difficult to use first principles for prediction, or interpretation. Given the current lack of solid theoretical foundations to explore emergent phenomena in such a complex context, alternative methodologies are required, hybridizing *in vitro* and *in silico* approaches, and following the pathways that chemistry itself will show us. For the same reason, coarse-grain modelling is not reliable under such conditions. Yet, this is precisely what those classical models did, taking inspiration from chemical and biological knowledge (biochemistry and metabolic pathway analysis, in particular).

As a result, since then, the conceptual discussion on the basics of metabolism has been biased by highly abstract concepts, like “closure” (in various versions: e.g., “organizational closure”<sup>[35]</sup>; “closure to efficient causation”<sup>[36]</sup>; “closure in the space of catalytic tasks”<sup>[45]</sup>), and too radical simplifications or strong assumptions (like the idea that chemical couplings must be perfectly “stoichiometric”<sup>[39,46]</sup>, that

cyclically”) through cross-catalytic relationships, but reasoning for it on strictly evolutionary grounds.

<sup>2</sup> This is why one of the historical roots of the field of “systems biology” can be found, precisely, in some last century theoreticians of metabolism<sup>[9,28,43]</sup> (more specifically, in the field of “metabolic control analysis”<sup>[44]</sup>).

large populations of oligomers can spontaneously form, all with similar probabilities for catalysis,<sup>[40,47]</sup> or that chemical reactions operate as purely formal grammars<sup>[41]</sup>). The messy, heterogeneous and changeful chemistries from which metabolisms are likely to have developed is very far from those neat idealizations.<sup>[48]</sup> Even the chemistry taking place within extant, highly evolved cellular organisms probably remains quite distant—with plenty of noise, dissipative processes, nonlinearities, chemical damage, irregularities, redundancies, and promiscuities that minimal models tend to overlook. Hence, it is time to change the strategy on this problem. Experimental survey should take the lead, because there is an obvious urge to implement *in vitro* families of systems of intermediate complexity that are minimally stable, under well-defined conditions, before drawing any kind of generalization. Nevertheless, insights coming from theoretical biology (including the models we just criticized) will be required, as well, to illuminate that empirical search: the crux of the matter consists in selecting their most significant characteristics (without committing to other, dispensable ones). Stated in brief, this is what we are just getting at.

### Constructive self-maintenance: functional bootstrapping between synthesis and control

The synthetic and constructive attributes of metabolism, like its systems nature, are not under debate. In fact, according to the most common view in science, as remarked above, the main role of metabolism is the production of the material building blocks and the means of energy from which all living organisms continuously build and repair themselves. However, this fundamental, “enabling power” of metabolism cannot be maintained in time unless it is realized in a way that it feeds back on itself. In other words, metabolisms constitute real phenomena only if, in addition to a network of coupled chemical transformations providing matter/energy resources, a complementary functional relationship with—at least, part of—the actual products of those transformations is established. Such a functional relationship is accomplished thanks to the constraining effects that an adequate combination of products happen to exert on the underlying dynamics, responsible for their synthesis. In extant biology, each living cell constitutes a perfect example of this: within each of these autonomous entities, a large number of macro-molecules (e.g., proteins, RNAs, DNA) and supramolecular structures (e.g., lipid membranes), of the cell’s own making, operate on top of a complex network of reaction pathways.

To be fair, all the classical models introduced above contain, somehow, this core idea, although in diverse, distilled versions: in the autopoietic theory,<sup>[35,38]</sup> special emphasis is given to the compartment, the physical/topological boundary of the system, which is both a result and a condition of possibility for the internal network; similarly, in (M, R)-systems<sup>[36,37]</sup>, enzymes are, at a time, the products and the efficient kinetic controllers of all elementary reactions; in Kauffman’s reflexively autocatalytic sets,<sup>[40,49]</sup> enzymes are substituted by a more precarious and disordered ensemble of oligopeptides, but catalytic action is explicitly distinguished from synthesis/cleavage reactions—although both get deeply interconnected, as the network grows; in

the model of the chemoton,<sup>[39,46]</sup> the informational subsystem feeds from the metabolic one and, simultaneously, imposes a size constraint on the latter (through the length of the polymer produced, plus the strict stoichiometric couplings established among subsystems); finally, in Fontana's algorithmic chemistries,<sup>[41,50]</sup>  $\lambda$ -expressions develop into increasingly complex clusters, whose maintenance depends on hierarchical relationship with other, simpler expressions belonging to the set.

Nevertheless, by focusing on a fundamental but only partial aspect of the intricate, constructive self-maintenance that we observe in biological systems, each of these models, taken separately, could be missing the most important point: the "self" implied in "self-maintenance" may be implemented robustly just as a *combination* of several of those aspects, plus some more that we are currently missing. In their efforts to condense an immensely complex phenomenon, like the basic organization of any living being, into a minimalist formal scheme, these pioneers were offering different theoretical constructs (different, abstract "selves"), none of which is, in practice, sufficiently rich to stand on its own feet. The chemical implementation of some of those classical ideas led to several results of interest (e.g., Luisi's group work on autopoiesis<sup>[51,52]</sup> or Ghadiri's on peptide networks with autocatalytic properties<sup>[53,54]</sup>) but their implications and scope remained rather limited. More recent empirical approximations, from the field of systems chemistry, have also managed to keep "in the same pot" reaction networks and products that have catalytic—or some other constraining—effects on the actual network (see, e.g.,<sup>[55–58]</sup>). However, despite the relevance of these novel approaches (that should certainly be continued), they seem to be falling short, probably still too simplified.

From our view, the hypothesis that there could be a natural limit, a lower bound in the number and diversity of endogenously generated molecular constraints necessary to have a metabolism up and running should be more seriously considered. In fact, as argued in,<sup>[59]</sup> the concurrence of an irreducible core of different (kinetic, spatial, energetic) *control mechanisms*<sup>3</sup> might be key to trigger the emergence of the first autonomous functional systems. Without the combined effects of a basic set of catalysts, compartments, energy currencies (like some other research groups are more specifically searching for: e.g.,<sup>[69]</sup>) and without an underlying reaction network that is subject to their control, no effective functional bootstrapping may occur. Since this is a central tenet of our article, we will expand on it in the next section.

## "MINIMAL METABOLISM": TENTATIVE PROPOSAL AND VIABILITY CONDITIONS

No metabolism has been realized, so far, outside living organisms, deprived of the exquisite control of enzymes and additional cellu-

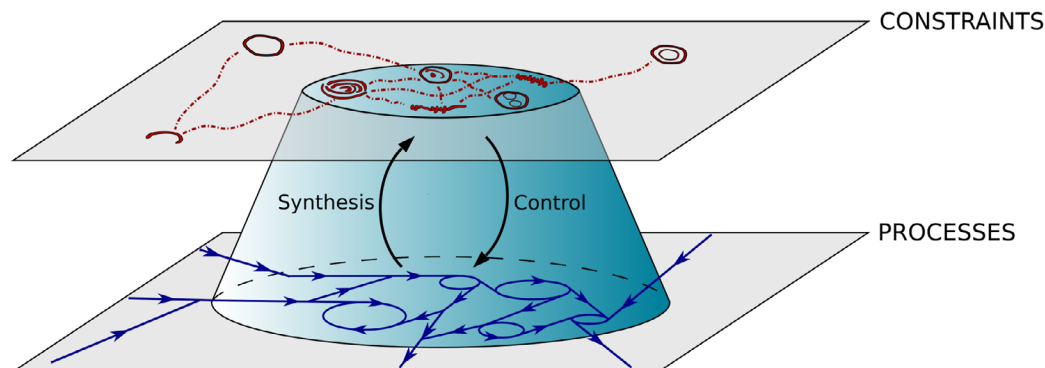
lar machinery. The evidence that the contrary could be plausible (at least, for some core pathways, or close chemical relatives, as we mentioned in the introduction:<sup>[1–7]</sup>), is promising but still inconclusive. In addition, there is an open, ongoing debate on whether prebiotic reactions to produce the first biomolecules, the basic building blocks for life (e.g., amino acids, lipids, nucleosides), should resemble current metabolic pathways or be completely different<sup>[27,70]</sup>. This resonates with some classical controversies among defenders of the heterotrophic versus autotrophic nature of the first metabolisms.<sup>[71,72]</sup> The question may not have an "all-or-none" answer (see:<sup>[13,73–75]</sup>, for intermediate positions). In principle, nobody can refute the possibility that the beginnings were, indeed, very different. However, it is harder to prove that case, because one must demonstrate, on top of the geological likelihood of such a divergent, primitive chemistry, what would be, then, the sequence of evolutionary steps required to converge towards extant biochemical pathways. The common justification that "natural selection would do the job, one way or another" is not tenable as a scientific argument, and less so the further away the corresponding chemistries stand from each other.<sup>4</sup> Instead, if someone eventually finds abiotic conditions under which a collection of cyclic and linear reactions resembling—if not equal to—extant metabolic pathways spontaneously appear and maintain themselves (without highly specialized/evolved catalysts, like enzymes), who will discard that option as a more parsimonious prebiotic scenario?

Regardless of the final answer that we may give to this preliminary question, the challenge of combining a set of transformation pathways to put together a complete metabolism should not be underestimated. Establishing an operational criterion for such a task, determining how to proceed and when it would be actually finished, is not obvious. In practice, if one tries to mimic current biochemistry too strictly, the enterprise might turn out to be as hard as putting together a minimal living cell (e.g., Craig Venter's last *Mycoplasma* construct<sup>[76,77]</sup>) from scratch. That approach certainly looks like a trap for "bottom-up" approaches. Among other reasons, because the number of extant metabolic pathways that are only viable—or mutually compatible—thanks to the presence of a specific set of enzymes produced by the cell is a big unknown, and so it will remain for a long time. Therefore, in order to tackle the implementation of simpler metabolisms, we consider that the strategy should be relatively flexible, searching for a moderate degree of resemblance with existing pathways, to ensure evolutionary continuity, but taking into consideration other, surely more relevant aspects.

What are these aspects? We will try to respond to this pivotal question here through a tentative but comprehensive enough proposal about the minimal requirements for metabolism. As we advanced in the previous section, the key premise will be the functional bootstrapping between synthesis and control. This implies distinguishing two levels of analysis in the system: processes vs constraints. Without this basic

<sup>3</sup> Our use of the term "control" in this paper is not the standard one, coming originally from cybernetics,<sup>[60]</sup> and typically identified with feedback mechanisms operating in biological cells<sup>[61]</sup>—but also in relatively simpler phenomena, like "dissipative structures"<sup>[62]</sup> or more recent non-equilibrium dynamic patterns.<sup>[63,64]</sup> We follow here a different tradition in theoretical biology, which relates the idea of "control" to functional constraints and hierarchical relationships established in intrinsically complex systems.<sup>[65]</sup> Thus, control for us involves an asymmetric causal connection that relies on the dynamic decoupling between processes and constraints,<sup>[65,66]</sup> which will be elaborated next; see also.<sup>[45,59,67,68]</sup>

<sup>4</sup> Natural selection cannot operate in the void: it is impossible to conceive catalyst optimization, for instance, without the prior existence of an underlying set of chemical reaction processes. These processes may change (co-evolve with the catalysts) over time, of course, but they must be there from the beginning.



**FIGURE 2** Graphical representation of the functional bootstrapping that we propose as the key requirement for a “minimal metabolism”, which involves both basic transformation processes and a diverse collection of constraining molecular structures that result from them and, at the same time, exert control on them. The particular shape and volume of the truncated cone is not relevant, since it is just a metaphorical illustration of the idea, but the non-reducibility hypothesis means that it remains truncated (top/bottom bases cannot shrink to a point)

hierarchical differentiation (schematically portrayed in Figure 2), it is impossible to capture and adequately interpret the heart of the problem, the meshwork of material relationships, of diverse nature, that are necessarily involved.

### A non-reductionist, hierarchical view of minimal metabolic organization

The distinction between processes and constraints (or boundary conditions), as such, is not novel, of course. Many authors make use of it, more or less explicitly, from those who do “constraint-based” modelling of metabolism (like Flux Balance Analysis<sup>[78,79]</sup> or other recent approaches<sup>[80]</sup>) to those who put forward the idea that metabolisms first appeared on inorganic surfaces, on geological settings rich in minerals and natural gradients of various kinds (e.g., <sup>[81–83]</sup>, or<sup>[16]</sup>). However, in such scenarios, constraints are equivalent to a set of exogenous (externally imposed) boundary conditions: i.e. they remain too decoupled from the actual chemistry that they modulate, virtually unaffected by it. Although certain sides of the problem may be addressed through these approximations, we consider that they are intrinsically limited for understanding how complex material organizations developed<sup>[84,85]</sup>.<sup>5</sup>

Thus, in our account, the two levels of Figure 2 would be populated by a diversity of molecular compounds in mutual dynamic transformation, although their relative stability, modes of interaction and causal effects would differ. In a first approximation, one could think about the “lower level” as the one in which basic metabolites diffuse,

bump into each other, react and transform into other chemical species. In turn, the “higher level” would be one in which some more elaborate products of those reactions (e.g., oligomers, supramolecular structures), whose characteristic lifetimes are longer than typical reaction times, come together to exert a number of constraining actions on the former.<sup>6</sup> We can say that it is a *functional* bootstrapping because the recurrent loop between synthesis and control (the complex interconnection between those two circular areas of each plane) is precisely what causally explains the self-maintenance of the system. In other words, our understanding of the concept of function is physiological (or “dispositional”/“organizational”<sup>[90–93]</sup>, applied, more precisely, to the context of proto-cellular development<sup>[94,59]</sup>).

The main novelty of this proposal is the irreducible molecular and interactive diversity at each level and, thereby, for the whole system. More explicitly stated, we put forward that any effective functional bootstrapping between synthesis and control, our key condition for metabolism, requires an inherent variety of components and interactions at each level of description. Depending on the physico-chemical couplings and mutual reinforcement relationships established among those various components and transformation processes, a number of different cyclic, self-constructing organizations could be put together, with different degrees of dynamic robustness. The challenge of determining specific sets of reactants, transformation processes, constraints and overall conditions that realize this functional bootstrapping should be a primary goal for upcoming investigations. To address that search, we just want to highlight that the graphical metaphor of the “truncated cone” (Figure 2) means that the exploration should involve chemical mixtures that bring about irreducible

<sup>5</sup> Some of these authors conceive metabolism basically as non-equilibrium geochemistry, and assume that this could unfold and build itself up into high levels of complexity, all the way to the synthesis of DNA-RNA-protein machinery<sup>[83]</sup> or even ecosystem architectures,<sup>[16]</sup> without the development of mechanisms for autonomous “micro-environment” control. Although that possibility is certainly worth exploring, we remain strongly skeptical about it. Endogenous compartmentalization processes, especially when they are tightly coupled with chemistry, seem to be fundamental enablers of metabolic organization<sup>[26,32,84,85]</sup> not eventual outcomes of it. The vectorial character of all forms of metabolism,<sup>[86,87]</sup> the relevance of organic interfaces with water in order to trigger off key prebiotic processes<sup>[88]</sup> and the need to control fundamental internal variables (like pH, osmotic strength or electrolyte composition) to ensure macromolecular function<sup>[89]</sup> support this latter view.

<sup>6</sup> The usual examples of endogenous constraints are enzymes (which operate locally, and with high specificity, on a reaction process), or lipid membranes (with more global and relatively less specific effects on the collection of molecules they encapsulate and the ongoing chemical transformations). The differences in stability span, at least, a few orders of magnitude: fractions of second for reactive species (in the slowest cases); beyond seconds, for constraints. This asymmetry is also reflected in their characteristic copy numbers, which tend to be remarkably lower for the constraining molecules than for the constrained ones. Thus, an effective link between components/processes belonging to both levels requires amplification and buffering mechanisms, in addition to the direct, physico-chemical effects of the (internally produced) constraints.



**BOX 1.****Chemical example**

Consider a reactive system producing all kinds of amino acids in a periodically changing environment (e.g., in aqueous, wet-dry cyclic conditions). Making peptide bonds under such conditions would not be easy, even though the formation of polymers were, in principle, thermodynamically favored (namely, the polymeric states on the complex energy landscape of the reactive system would not be accessible from the monomeric states due to kinetic hindrance). However, if  $\alpha$ -hydroxy acids were added to the mixture, ester bonds would then be readily formed under aqueous conditions, and the subsequent substitution of the alcoholic group in the ester bond by the amino group of an amino acid to form a thermodynamically more stable peptide bond would also be easier and faster (similar ideas can be found in proposals like the “thioester world”<sup>[13]</sup>). At first sight, the reactive system would have turned messier, since all kinds of random polymers with different mixing degrees of ester and peptide bonds might emerge. Polymers, however, can generate intricate structures (through folding), which are capable of implementing novel physico-chemical functionalities, becoming a target of selection. Indeed, if recurrent cycles of oligomerization and disintegration were established in the system (analogous to anabolic-catabolic cycles), a tiny imbalance in the thermodynamic stability of the ester vs peptide bond could effectively trigger the progressive enrichment of peptide links present in the polymer backbone, over successive rounds of synthesis and decay. As a result of that “self-cleaning” process, so to speak, polymers with novel functional traits (e.g., enhanced or more specific catalytic effects, including the avoidance of dissipation through side-reactions) would emerge and become exploitable by the reaction mixture. In turn, these (higher-level) functionalities would unlock previously inaccessible areas on the energy landscape of the reactive system, making it also less dependent from externally provided resources. Thus, we see how a combination of factors, which involve both description levels (reaction network and constraints), are required to give a complete explanation of the phenomenon. From this richer perspective, molecular recycling, for instance, stands out not only as key to avoid wasteful dynamics, but also to allow for highly sensitive control mechanisms or to bring about more autonomous (internalized) behavior. These possibilities would be further enhanced by the presence of autocatalytic loops and asymmetries in some key reaction mechanisms. Namely, cases in which the reversibility of some reaction steps is provided through different transformation pathways, forward and backward, which can afford new control options, as recently highlighted in the context of “dissipative self-assembly” phenomena<sup>[95]</sup>; see also.<sup>[96]</sup>

combinations of molecules and supramolecular structures with qualitatively different constraining effects on the reaction processes. For instance, in accordance with<sup>[59]</sup> (or earlier<sup>[85]</sup>), one should investigate chemical networks that produce catalysts (for kinetic control), self-assembling amphiphiles (for spatial control), common intermediaries to couple endergonic-exergonic processes (for energetic control), and template structures (for variability control). But many different combinations and material implementations might be possible, within that general scheme; and alternative schemes ought to be tried as well, of course.

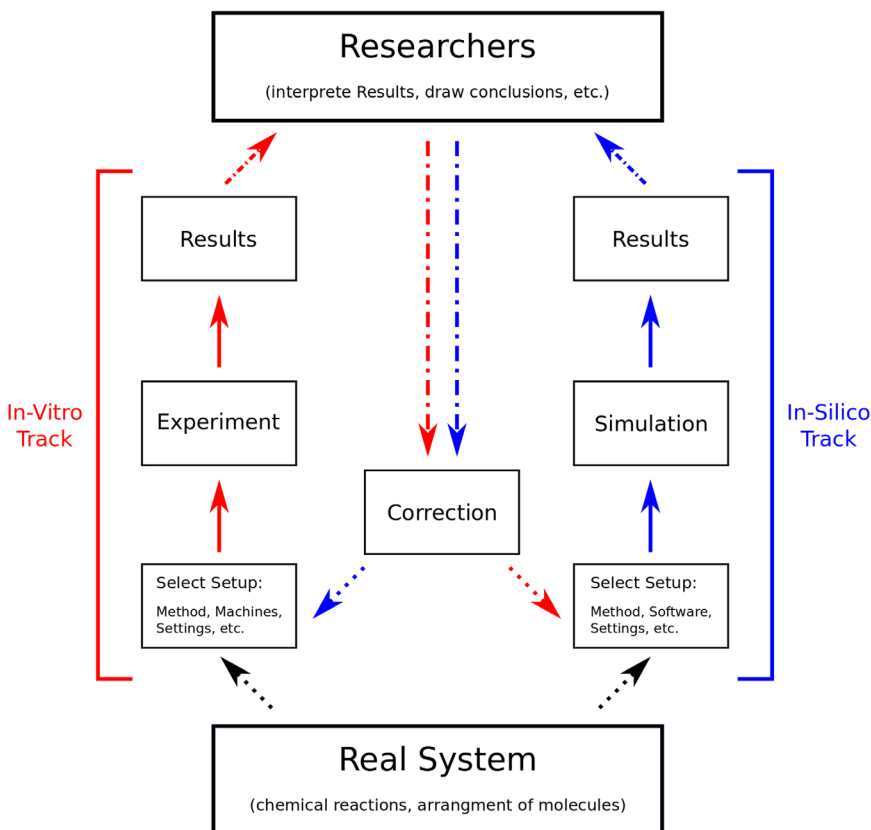
### Metabolic viability involves autonomous control development

Adopting this two-level (i.e. minimally hierarchical) perspective has important implications in terms of how we conceive—and, thus, how we propose to investigate—the viability conditions for proto-metabolic systems, as well as their evolutionary development (a concrete chemical example is given in Box 1, for illustrative purposes). With regard to the underlying network of physico-chemical transformation processes, among other features that one may consider (see<sup>[48]</sup> for an interesting review), the fundamental requirement that stands out, from our approach, is controllability. Not an external (e.g., human) or ubiquitous (e.g., thermodynamic) type of controllability but, rather, the implementation of reaction networks that develop, locally, their own control

mechanisms and transform themselves through those mechanisms. This brings forward an important shift of focus that should be more explicitly addressed in the field. After all, why do metabolic processes take place in non-equilibrium conditions? Why do they tend, so often, to get organized in cycles? Why do they establish couplings through a small set of common molecular intermediaries? A unifying answer to all these questions is that the network becomes much easier to control under those circumstances. Hence controllability, endogenous or *autonomous* controllability, appears as an essential and pervasive feature, to be more carefully examined through experimental work.

This dual perspective also prompts thinking about the development of metabolism in terms of a co-evolution or a co-determining process between reaction networks and concurrent molecular constraints operating on them, which can be helpful in order to identify the potential bottlenecks involved. For instance, it makes us realize that chemical transformations, if they are to become metabolic, should imply, right from the beginning, a landscape with significant kinetic (and probably also energetic) barriers. Although it might seem counterintuitive, one should investigate reactions that do not run, or hardly run, but nevertheless hold the potential to be run more efficiently, provided that they start producing the suitable catalysts, or that they couple with other, enabling processes. Direct thermodynamic control of a reaction is thus to be avoided—or very cautiously used—by experimentalists. This line of reasoning stems from having a complete enough picture of the situation, in which the endogenous synthesis of (kinetic, spatial, energetic) control mechanisms and their

**FIGURE 3** Tentative scheme of the interaction between in-silico and in-vitro approaches. A system (complex natural phenomenon) motivates investigation. By combining the appropriate experimental setup (machines, measurement devices and settings, etc.) and the appropriate computational platform (simulation method, software to use, selection of parameters and constraints, etc.) a richer understanding and more effective characterization of the real system will be achieved. Both experiments and simulations produce complementary results that help the research team advance in their interpretation of the real system/phenomenon and in the design of subsequent research steps (introducing corrections to either the experimental or the computational setup—or both). Explanation of arrow relations: dotted arrows indicate influences, normal arrows indicate productions, dash-dotted arrows indicate interpretations. This graph was originally inspired by<sup>[20]</sup> (in particular, Figure 1 and the contents of section 4, in there)



effects on the underlying chemistry ought to be the main target of the experiments. But, in turn, this points toward the need to reformulate quite radically the way of carrying out empirical research on the origins of metabolism, as we will discuss next.

### METHODOLOGICAL SUGGESTIONS: A CASE FOR “RULE-BASED” COMPUTATIONAL APPROACHES

As argued above, minimal metabolisms are complex dynamic systems that cannot be directly inferred from (currently known) first-principles, so we suggest that they ought to be investigated through a combination of in vitro and in silico exploratory strategies. This goes beyond the classical—yet important—claim that theoretical modelling should be contrasted with empirical data, which has also been made in the metabolism research camp (see, e.g.<sup>[97],[98]</sup> or<sup>[99]</sup>). More precisely, we consider that in all empirical work related to the origins of life the development of theoretical chemistry (including rigorous computational model implementation, in executable code, with predictive power) is mandatory, to double-check our intuitions and preliminary assumptions. Furthermore, simulation models should be used to explore the limitations of the theoretical concepts under conditions that are not accessible to the experiment. Therefore, in silico methods must not be simply subordinated to data management (like they tend to be, in recent times, across many other fields), but developed hand-in-hand with in vitro approaches, since most research in prebiotic chemistry and biogenesis still needs to be hypothesis-driven.<sup>[100]</sup>

Figure 3 gives an overview on how we think this should operate, in practice. Beyond standard work-cycles (i.e. subsequent rounds of design, performance, and interpretation of experiments, in vitro or in silico), a deeper and more systematic interbreeding between the two types of methodology is proposed. In other words, instead of immediately trying to fix or redesign an in vitro experimental setup according to the newly obtained data and insights, the latter should be used to elaborate a closely related in silico theoretical model. Computer simulations parameterized and constrained by the experimental data allow (in addition to other regular predictions and interpretations) collecting observations about the system that do not necessarily translate into direct variables/magnitudes accessible through the current experimental setup. Such results and the debate generated (naturally, as these are compared with the evidence collected about the real system) provide a much richer discussion platform to make progress. The process would repeat from there, performing as many cross-iterations as required.

Thus, we would have a cyclic, hybridizing process in which experimental work advances hand in hand with theoretical research, each providing constant feedback to one another. Although this may not be a radically novel idea in science, we consider that a special effort should be made in that direction in the prebiotic research camp, with each type of approach/methodology pushing the other to move out from its initial “comfort zone” and trying to address and suggest new relevant aspects of the phenomenon. Some experimentalists interested in the origins of life have recently switched gears from the characterization of single molecules and their reactions to the investigation of the behavior of

**BOX 2.****“Rule-based” computational approaches**

In the theoretical analysis of complex molecular mixtures, a given dynamic behavior is typically expressed by an aggregate variable, at the macroscopic level, which translates into a characteristic distribution of (reactive) collisions of molecules, at the microscopic level. This means, that model building at the macroscopic level is accompanied by an information loss, in the sense that a distribution of molecular interactions at the microscopic description level is abstracted into a single numeric parameter value at the macroscopic description level. A change of the macroscopic parameter value may influence the behavior of the macroscopic system description, but the connection (i.e. how this change translates into modifications of the “physical implementation” of the system at the microscopic level – namely, in terms of the distribution of reactive collisions of molecules) is lost. This detachment between the microscopic and macroscopic descriptions makes it especially hard to design, interpret or engineer complex reactive mixtures. Current “rule-based” modelling approaches express knowledge about local interactions between the building blocks of the system precisely as explicit rules (see<sup>[103,104]</sup> or the chemistry oriented graph-rewrite framework MØD<sup>[105]</sup>). In this context, it is reasonable to assume that the collection of local rules captures every necessary aspect of the system at the microscopic level. Specified in that way, the system can be simulated at that microscopic level without any preconceived notion about how the macroscopic behavior arises in it. Thus, the aggregation of microscopic behavioral patterns into macroscopic observables can be studied in depth, advancing the understanding on how the system may be engineered at the microscopic level (i.e., the physical implementation level), to achieve specific macroscopic behaviors.

Accordingly, a big advantage of rule-based approaches over classical ones is that they allow a more direct and precise investigation of the impact of local modifications on the emergence of the global system-level behavior. Furthermore, since the application of rules depends on specific structural patterns, as preconditions, this type of model is ideally suited to identify subsequent rule applications, necessary to obtain a particular event of interest as a result of a sequence of elementary steps (e.g., the synthesis of a complex molecule from simpler building blocks). This methodology is called “mechanistic causal analysis”,<sup>[106,107]</sup> and permits extraction of meaningful pathways or mechanisms from ensembles of trajectories in rule-based simulations. The most recent theoretical advances in this line of work have been implemented as the Kappa Calculus,<sup>[108]</sup> a rule-based approach that operates on site graphs and has already been showcased (e.g., for the analysis of the synchronization of the molecular oscillators implementing the circadian clock of cyanobacteria<sup>[109]</sup>). Within this general framework (including the combination of platforms like MØD and the Kappa Calculus) there is also potential to identify, as simulations are run, different types of molecular interactions occurring in the system, discriminating constraining relationships from standard reaction mechanisms, which would be key to investigate minimal metabolisms, in the way they were here characterized.

complex molecular mixtures under varying conditions.<sup>[101]</sup> However, we have not observed a similar shift in the computational approaches to study prebiotic chemical systems, despite some interesting exceptions (e.g.<sup>[102]</sup>). Classical modelling techniques, like describing the system through a set of differential equations, are still very popular. It is quite surprising that novel formalisms, like *process algebras* and *rule-based modelling approaches*, which were specifically developed to cope with complex, combinatorial and concurrent systems, have been so seldom adopted, up to date. In Box 2 we briefly explain what these consist in and why we defend that they should be further pursued, in particular to carry out research on minimal metabolisms.

As an example in place, let us comment an interesting study that involves the use of this type of algorithmic methods, in conjunction with *in vitro* techniques, recently published by the Grzybowski Lab.<sup>[110]</sup> In a first stage, this group carried out a thorough computer analysis of prebiotically plausible chemical reaction pathways, starting from a few simple compounds that hypothetically existed in an early-Earth environment. Reaction patterns were translated into formal rules that, together with those initial molecules, generated a basic grammar, which was then used to iteratively expand the network of all possible compounds at reach, in principle, from those starting conditions. Thus,

they computationally recreated the chemical reaction space that can be theoretically obtained from a certain set of prebiotic building blocks. Up to this point, a very nice example to illustrate how the rule-based strategies (as described in Box 2) are perfectly applicable in this context. An additional strength of their approach relied on the fact that the selection of initial molecules, as well as the reaction rules, were consistent with previous *in vitro* studies from the field of prebiotic chemistry. Furthermore, they used their findings as a basis to implement new *in vitro* experiments, as well, in order to confirm the predictions and gain further insights. Therefore, this case is a good illustration of how the proposed workflow (sketched in Figure 3), can be realized successfully. However, as an approach to reach the complexity of minimal metabolisms, we do not find it satisfactory, yet: structural analysis (knowledge about the topology of prebiotic reaction networks) is fundamental but not sufficient. Functional bootstrapping requires also a dynamic perspective, through which it should be possible to draw causal distinctions, identify different types of relationship within chemical reaction space (in particular, control mechanisms on top of chemical transformations), as we will briefly recapitulate next, to conclude. In any case, the pending task to develop new analytic techniques to characterize non-equilibrium chemistries



that generate their own material controllers and rules, within the same “reacting pot”, should be definitely promoted in this research field.

## CONCLUDING REMARKS

Metabolism involves coupled chemical transformation processes that synthesize constraining molecular structures, which exert diverse types of dynamic control on these processes, to achieve relatively robust, whole system self-maintenance in non-equilibrium conditions. This is the real heart of biological complexity, the *kernel of life* as it was beautifully expressed in our opening quote, so it should become the main target of investigation in the field of origins, where minimal expressions of that core idea ought to be pursued and materialized in the near future. Reductionist molecular approaches have contributed (and will keep contributing) to prebiotic research, of course, but we need to conceive alternative ways of simplifying, of modelling those first biologically relevant steps, starting already from complex chemical mixtures. The empirical and theoretical tools required for such a challenge are still under development, but that’s where the efforts of the field should be focused.

Understanding subsequent transitions towards genetically-instructed metabolisms (i.e., real, much more robust and efficient, full-fledged metabolisms) will not be easy, either. How on earth could these complex systems (complex but natural systems, after all) bring about a translation apparatus, for instance (with ribosomes, genetic code, etc)... is simply mind-blowing. Nevertheless, what appears crystal clear to us is that a translation apparatus would make, literally, no sense without metabolism. The advantage of proposing an early systems transition in the problem of origins of life is that later major-transitions look at least feasible. Minimal metabolisms would transform into more complex versions by developing new layers on top of that elementary chassis we described by means of a truncated cone in Figure 2. The process will surely require many generations of self-reproducing protocells undergoing prebiotic selection processes, but as far as the architecture of metabolism is concerned, we should think about it as a co-evolution between an increasingly complex network of chemical transformations and an increasingly complex set of molecular constraints (constraints on top of constraints, meta-constraints). A second, interesting advantage of this type of proposal, proceeding stepwise in the genealogical context of origins-of-life research, is that it should help us decipher the principles of organization underlying the hyper-complex system behavior that we observe, all at once, in living cells.

## ACKNOWLEDGMENTS

This work was carried out as part of a Horizon 2020 Marie Curie ITN (“ProtoMet”—Grant Agreement no. 813873 with the European Commission), within which NL obtained a PhD fellowship. KR-M also acknowledges support from the Basque Government (Grant IT 1228-19) and the Spanish Ministry of Science and Innovation (PID2019-104576GB-I00). The authors would like to thank the critical feedback of two anonymous reviewers, in addition to the comments and sugges-

tions from Daniele de Martino, Alvaro Moreno and Juli Peretó, who read a previous version of the manuscript.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

## ORCID

Nino Lauber  <https://orcid.org/0000-0003-4449-6565>

Kepa Ruiz-Mirazo  <https://orcid.org/0000-0002-0157-4394>

## REFERENCES

- Keller, M. A., Turchyn, A. V., & Ralser, M. (2014). Non-enzymatic glycolysis and pentose phosphate pathway-like reactions in a plausible Archean ocean. *Molecular Systems Biology*, 10(4), 725.
- Keller, M. A., Zylstra, A., Castro, C., Turchyn, A. V., Griffin, J. L., & Ralser, M. (2016). Conditional iron and pH-dependent activity of a non-enzymatic glycolysis and pentose phosphate pathway. *Science Advances*, 2(1), e1501235.
- Coggins, A. J., & Powner, M. W. (2017). Prebiotic synthesis of phosphoenol pyruvate by  $\alpha$ -phosphorylation-controlled triose glycolysis. *Nature Chemistry*, 9(4), 310–317.
- Muchowska, K. B., Varma, S. J., Chevallot-Beroux, E., Lethuillier-Karl, L., Li, G., & Moran, J. (2017). Metals promote sequences of the reverse Krebs cycle. *Nature Ecology & Evolution*, 1(11), 1716–1721.
- Muchowska, K. B., Varma, S. J., & Moran, J. (2019). Synthesis and breakdown of universal metabolic precursors promoted by iron. *Nature*, 569(7754), 104–107.
- Springsteen, G., Yerabolu, J. R., Nelson, J., Rhea, C. J., & Krishnamurthy, R. (2018). Linked cycles of oxidative decarboxylation of glyoxylate as protometabolic analogs of the citric acid cycle. *Nature Communications*, 9(91), 1–8.
- Trent Stubbs, R., Yadav, M., Krishnamurthy, R., & Springsteen, G. (2020). A plausible metal-free ancestral analogue of the Krebs cycle composed entirely of  $\alpha$ -ketoacids. *Nature Chemistry*, 12, 1016–1022.
- Ruiz-Mirazo, K., Briones, C., & de la Escosura, A. (2014). Prebiotic system chemistry: new perspectives for the origins of life. *Chemical reviews*, 114(1), 285–366.
- de la Escosura, A., Briones, C., & Ruiz-Mirazo, K. (2015). The systems perspective at the crossroads between chemistry and biology. *Journal of Theoretical Biology*, 381, 11–22.
- Kroiss, D., Ashkenasy, G., Braunschweig, A. B., Tuttle, T., & Ulijn, R. V. (2019). Catalyst: can systems chemistry unravel the mysteries of the chemical origins of life? *Chem*, 5(8), 1917–1920.
- Orgel, L. E. (2000). Self-organizing biochemical cycles. *Proceedings of the National Academy of Sciences of the United States of America*, 97(23), 12503–12507.
- Orgel, L. E. (2008). The implausibility of metabolic cycles on the prebiotic Earth. *PLoS Biology*, 6(1), e18.
- de Duve, C. (1991). *Blueprint for a cell: The nature and origin of life.* Burlington NC: N. Patterson.
- Morowitz, H. J. (1992). *Beginnings of cellular life: Metabolism recapitulates biogenesis.* New Haven, CT: Yale University Press.
- Harold, F. M. (2001). *The way of the cell: Molecules, organisms, and the order of life.* Oxford: Oxford University Press.
- Braakman, R., & Smith, E. (2013). The compositional and evolutionary logic of metabolism. *Physical Biology*, 10(1), 011001.

17. Smith, E., & Morowitz, H. J. (2016). *The origin and nature of life on Earth: The emergence of the fourth geosphere*. Cambridge: Cambridge University Press.
18. Bich, L., & Green, S. (2018). Is defining life pointless? Operational definitions at the frontiers of biology. *Synthese*, 195(9), 3919–3946.
19. Mariscal, C., & Ford Doolittle, W. (2020). Life and life only: A radical alternative to life definitionism. *Synthese*, 197(7), 2975–2989.
20. Amilburu, A., Moreno, Á., & Ruiz-Mirazo, K. (2020). Definitions of life as epistemic tools that reflect and foster the advance of biological knowledge. *Synthese*, <https://doi.org/10.1007/s11229-020-02736-7>.
21. Dyson, F. J. (1985). *Origins of life*. Cambridge: Cambridge University Press.
22. Morowitz, H. J., Kostelnik, J. D., Yang, J., & Cody, G. D. (2000). The origin of intermediary metabolism. *Proceedings of the National Academy of Sciences of the United States of America*, 97(14), 7704–7708.
23. Luisi, P. L. (2006). *The emergence of life: From chemical origins to synthetic biology*. Cambridge: Cambridge University Press.
24. Shapiro, R. (2007). A Simpler Origin for. *Life. Sci. Am.*, 296: 46–53.
25. Ruiz-Mirazo, K., Peretó, J., & Moreno, A. (2004). A universal definition of life: autonomy and open-ended evolution. *Origins of Life and Evolution of the Biosphere*, 34(3), 323–346.
26. Piedrafitá, G., Monnard, P.-A., Mavelli, F., & Ruiz-Mirazo, K. (2017). Permeability-driven selection in a semi-empirical protocell model: The roots of prebiotic systems evolution. *Scientific Reports*, 7(3141), 1–10.
27. Sutherland, J. D. (2017). Opinion: Studies on the origin of life – The end of the beginning. *Nature Review. Chemistry*, 1(2), 0012.
28. Booger, F. C., Bruggeman, F. J., Hofmeyr, J.-H. S., & Westerhoff, H. V. (2007). *Systems biology. Philosophical foundations*. Amsterdam: Elsevier.
29. Cornish-Bowden, A., Cárdenas, M. L., Letelier, J.-C., & Soto-Andrade, J. (2007). Beyond reductionism: Metabolic circularity as a guiding vision for a real biology of systems. *Proteomics*, 7(6), 839–845.
30. Westerhoff, H. V., Winder, C., Messiha, H., Simeonidis, E., Adamczyk, M., Verma, M., Bruggeman, F. J., & Dunn, W. (2009). *Systems Biology: The elements and principles of Life. FEBS Letters*, 583(24), 3882–3890.
31. Harold, J. (1984). Morowitz the completeness of molecular biology. *Israel Journal of Medical Sciences*, 20: 750–753.
32. Moreno, A., & Ruiz-Mirazo, K. (1999). Metabolism and the problem of its universalization. *Bio Systems*, 49(1), 45–61.
33. Hofmeyr, J.-H. S. (2007). The biochemical factory that autonomously fabricates itself: A systems biological view of the living cell. In: F. C. Booger, F. J. Bruggeman, J.-H. S. Hofmeyr, & H. V. Westerhoff (editors), *Systems biology. Philosophical foundations*. pp. 217–242, Amsterdam: Elsevier.
34. Cornish-Bowden, A., & Cárdenas, M. L. (2020). Contrasting theories of life: Historical context, current theories. in search of an ideal theory. *Bio Systems*, 188, 104063.
35. Maturana, H., & Varela, F. J. (1980). *Autopoiesis and cognition: The realization of the living*. Dordrecht: D. Reidel Publishing Company, (First Edition, in Spanish: Ed.ial Universitaria, Santiago, 1972).
36. Rosen, R. (1991). *Life itself: A comprehensive inquiry into the nature, origin and fabrication of life*. New York: Columbia University Press.
37. Rosen, R. (1971). Some realizations of (M, R)-systems and their interpretation. *Bulletin of Mathematical Biology*, 33(3), 303–319.
38. Varela, F. J., Maturana, H. R., & Uribe, R. (1974). Autopoiesis: The organization of living systems, its characterization and a model. *Bio Systems*, 5(4), 187–196.
39. Gánti, T. (1975). Organization of chemical reactions into dividing and metabolizing units: The Chemotons. *Bio Systems*, 7(1), 15–21.
40. Kauffman, S. A. (1986). Autocatalytic sets of proteins. *Journal of Theoretical Biology*, 119(1), 1–24.
41. Fontana, W., & Buss, L. W. (1994). The arrival of the fittest: Toward a theory of biological organization. *Bulletin of Mathematical Biology*, 56(1), 1–64.
42. Eigen, M., & Schuster, P. (1979). *The hypercycle: A principle of natural self-organization*. Berlin, Heidelberg: Springer.
43. O'Malley, M. A., & Dupré, J. (2005). Fundamental issues in systems biology. *BioEssays*, 27(12), 1270–1276.
44. Fell, D. (1997). *Understanding the control of metabolism*. London: Portland Press.
45. Kauffman, S. A. (2000). *Investigations*. Oxford: Oxford University Press.
46. Gánti, T. (2003). *The principle of life*. Oxford: Oxford University Press, (First Edition, in Hungarian: Gondolat, Budapest, 1971).
47. Kauffman, S. A. (1993). *The origins of order: Self-organization and selection in evolution*. Oxford: Oxford University Press.
48. Peretó, J. (2012). Out of fuzzy chemistry: From prebiotic chemistry to metabolic networks. *Chemical Society Reviews*, 41(16), 5394–5403.
49. Doynne Farmer, J., Kauffman, S. A., & Packard, N. (1986). Autocatalytic replication of polymers. *Physica D*, 22(1-3), 50–67.
50. Fontana, W., & Buss, L. W. (1996). The barrier of objects: From dynamical systems to bounded organizations. In: J. Casti & A. Karlqvist (editors), *Boundaries and Barriers*. pp. 56–116, Reading MA: Addison-Wesley.
51. Bachmann, P. A., Walde, P., Luisi, P. L., & Lang, J. (1990). Self-replicating reverse micelles and chemical autopoiesis. *Journal of the American Chemical Society*, 112(22), 8200–8201.
52. Luisi, P. L., & Varela, F. J. (1989). Self-replicating micelles—A chemical version of a minimal autopoietic system. *Origins of Life and Evolution of the Biosphere*, 19(6), 633–643.
53. Lee, D. H., Granja, J. R., Martinez, J. A., Severin, K., & Ghadiri, M. R. (1996). A self-replicating peptide. *Nature*, 382(6591), 525–528.
54. Kauffman, S. A. (1996). Even peptides do it. *Nature*, 382(6591), 496–497.
55. Patel, B. H., Percivalle, C., Ritson, D. J., Duffy, C. D., & Sutherland, J. D. (2015). Common origins of RNA, protein and lipid precursors in a cyanosulfidic protometabolism. *Nature Chemistry*, 7(4), 301–307.
56. Gibard, C., Bhowmik, S., Karki, M., Kim, E.-K., & Krishnamurthy, R. (2018). Phosphorylation, oligomerization and self-assembly in water under potential prebiotic conditions. *Nature Chemistry*, 10(2), 212–217.
57. Monreal Santiago, G., Liu, K., Browne, W. R., & Otto, S. (2020). Emergence of light-driven protometabolism on recruitment of a photocatalytic cofactor by a self-replicator. *Nature Chemistry*, 12(7), 603–607.
58. Post, E. A. J., & Fletcher, S. P. (2020). Dissipative self-assembly, competition and inhibition in a self-reproducing protocell model. *Chemical Science*, 11(35), 9434–9442.
59. Ruiz-Mirazo, K., Briones, C., & de la Escosura, A. (2017). Chemical roots of biological evolution: The origins of life as a process of development of autonomous functional systems. *Open Biology*, 7(4), 170050.
60. Wiener, N. (1948). *Cybernetics: Or control and communication in the animal and the machine*. Cambridge MA: MIT Press.
61. Stelling, J., Sauer, U., Szallasi, Z., Doyle, F. J., & Doyle, J. (2004). *Robustness of Cellular Functions Cell*, 118: 675–685.
62. Nicolis, G., & Prigogine, I. (1977). *Self-organization in nonequilibrium systems: From dissipative structures to order through fluctuations*. New York: Wiley.
63. Epstein, I. R., Vanag, V. K., Balazs, A. C., Kuksenok, O., and Dayal, A., & Bhattacharya, P. (2012). Chemical oscillators in Structured Media. *Accounts of Chemical Research*, 45(12), 2160–2168.
64. Semenov, S. N., Kraft, L. J., Ainla, A., Zhao, M., Baghbanzadeh, M., Campbell, V. E., Kang, K., Fox, J. M., & Whitesides, G. M. (2016).

- Autocatalytic, bistable, oscillatory networks of biologically relevant organic reactions. *Nature*, 537(7622), 656–660.
65. Pattee, H. H. (1973). The physical basis and origin of hierarchical control. In: H. H. Pattee, (ed.), *Hierarchy Theory. The Challenge of Complex Systems*. pp. 73–108, New York: G. Braziller.
  66. Pattee, H. H. (1977). Dynamic and linguistic modes of complex systems. *International Journal of General Systems*, 3(4), 259–266.
  67. Umerez, J., & Mossio, M. (2013). Constraint. In: W. Dubitzky, O. Wolkenhauer, K.-H. Cho, & H. Yokota (editors), *Encyclopedia of systems biology*. pp. 490–493, New York: Springer.
  68. Montévil, M., & Mossio, M. (2015). Biological organisation as closure of constraints. *Journal of Theoretical Biology*, 372: 179–191.
  69. Bonfio, C., Godino, E., Corsini, M., Fabrizi de Biani, F., Guella, G., & Mansy, S. S. (2018). Prebiotic iron-sulfur peptide catalysts generate a pH gradient across model membranes of late protocells. *Nature Catalysis*, 1(8), 616–623.
  70. Ralser, M. (2018). An appeal to magic? The discovery of a non-enzymatic metabolism and its role in the origins of life. *Biochemical Journal*, 475(16), 2577–2592.
  71. Lazcano, A., & Miller, S. L. (1999). On the origin of metabolic pathways. *Journal of Molecular Evolution*, 49(4), 424–431.
  72. Morowitz, H. J. (1999). A theory of biochemical organization, metabolic pathways, and evolution. *Complexity*, 4(6), 39–53.
  73. Krishnamurthy, R. (2018). Life's biological chemistry: a destiny or destination starting from prebiotic chemistry? *Chem Eur. J.*, 24(63), 16708–16715.
  74. Krishnamurthy, R. (2020). Systems chemistry in the chemical origins of life: the 18th camel paradigm. *J. Syst. Chem.*, 8: 40–62.
  75. Peretó, J. (2019). Prebiotic chemistry that led to life. In: V. M. Kolb (ed.), *Handbook of astrobiology*. pp. 219–233, Boca Raton: CRC Press.
  76. Hutchison, C. A., Chuang, R.-Y., Noskov, V. N., Assad-Garcia, N., Deerinck, T. J., Ellisman, M. H., Gill, J., Kannan, K., Karas, B. J., Ma, L., Pelletier, J. F., Qi, Z.-Q., Alexander Richter, R., Strychalski, E. A., Sun, L., Suzuki, Y., Tsvetanova, B., Wise, K. S., Smith, H. O.... Craig Venter, J. (2016). Design and synthesis of a minimal bacterial genome. *Science*, 351(6280), aad6253–aad6253.
  77. Breuer, M., Earnest, T. M., Merryman, C., Wise, K. S., Sun, L., Lynott, M. R., Hutchison, C. A., Smith, H. O., Lapek, J. D., Gonzalez, D. J., de Crécy-Lagard, V., Haas, D., Hanson, A. D., Labhsetwar, P., Glass, J. I., & Luthy-Schulten, Z. (2019). Essential metabolism for a minimal cell. *eLife*, 8: e36842.
  78. Orth, J. D., Thiele, I., & Palsson, B. Ø. (2010). What is flux balance analysis? *Nature Biotechnolog*, 28(3), 245–248.
  79. Goldford, J. E., Hartman, H., Marsland, R. et al. (2019). Environmental boundary conditions for the origin of life converge to an organosulfur metabolism. *Nat Ecol Evol* 3, 1715–1724.
  80. Akbari, A., Yurkovich, J. T., Zielinski, D. C., & Palsson, B. Ø. (2021). The quantitative metabolome is shaped by abiotic constraints. *Nature Communications*, 12, 3178.
  81. Wächtershäuser, G. (1988). Before enzymes and templates: Theory of surface metabolism. *Microbiological Reviews*, 52(4), 452–484.
  82. Wächtershäuser, G. (1990). Evolution of the first metabolic cycles. *Proceedings of the National Academy of Sciences of the United States of America*, 87(1), 200–204.
  83. Martin, W., & Russell, M. J. (2003). On the origins of cells: A hypothesis for the evolutionary transitions from abiotic geochemistry to chemoautotrophic prokaryotes, and from prokaryotes to nucleated cells. *Philos. Trans. R. Soc. Lond. B*, 35(1429), 59–85.
  84. Ruiz-Mirazo, K. (2020). Boundary versus enabling conditions for the origins of life: Comment on "Mineral self-organization on a lifeless planet" by García-Ruiz et al. *Phys. Life Rev*, 34–35, 96–98.
  85. Ruiz-Mirazo, K., & Moreno, A. (2004). Basic autonomy as a fundamental step in the synthesis of life. *Artificial Life*, 10(3), 235–259.
  86. Mitchell, P. (1961). Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. *Nature*, 191(4784), 144–148.
  87. Harold, F. M. (1986). *The vital force: A study of bioenergetics.*, New York: W.H. Freeman.
  88. Serrano-Luginbühl, S., Ruiz-Mirazo, K., Ostaszewski, R., Gallou, F., & Walde, P. (2018). Soft and dispersed interface-rich aqueous systems that promote and guide chemical reactions. *Nat. Rev. Chem.*, 2(10), 306–327.
  89. Hofmeyr, J.-H. S. (2019). Basic biological anticipation. In: R. Poli (ed) *Handbook of anticipation.*, Cham: Springer, pp. 219–233.
  90. Mayr, E. (1982). *The growth of biological thought.*, Cambridge MA: Harvard University Press.
  91. Cummins, R. (2002). Neo-teleology. In: A. Ariew, R. Cummins, & M. Perlman (editors), *Functions: New Essays in the Philosophy of Psychology and Biology*. pp. 157–172, Oxford: Oxford University Press.
  92. Mossio, M., Saborido, C., & Moreno, A. (2009). An organizational account of biological functions. *The British journal for the philosophy of science*, 60(4), 813–841.
  93. Roux, E. (2014). The concept of function in modern physiology. *Journal of Physiology*, 592(11), 2245–2249.
  94. Shirt-Ediss, B., Murillo-Sánchez, S., & Ruiz-Mirazo, K. (2017). Frsaming major prebiotic transitions as stages of protocell development: Three challenges for origins-of-life research. *Beilstein Journal of Organic Chemistry*, 13: 1388–1395.
  95. Boekhoven, J., Hendriksen, W. E., Koper, G. J. M., Eelkema, R., & van Esch, J. H. (2015). Transient assembly of active materials fueled by a chemical reaction. *Science*, 349(6252), 1075–1079.
  96. Das, K., Gabrielli, L., & Prins, L. J. (2021). Chemically-fueled self-assembly in biology and chemistry. *Angewandte Chemie*, 60, 2–26.
  97. Majewski, R. A., & Domach, M. M. (1990). Simple constrained-optimization view of acetate overflow in *E.Coli*. *Biotechnology and Bioengineering*, 35: 732–738.
  98. Covert, M. W., Schilling, C. H., Famili, I., Edwards, J. S., Goryanin, I. I., Selkov, E., & Palsson, B. O. (2001). Metabolic modeling of microbial strains in silico. *Trends in Biochemical Sciences*, 26(3), 179–186.
  99. De Martino, D., MC Andersson, A., Bergmiller, T., Guet, C., & Tkacik, G. (2018). Statistical mechanics for metabolic networks during steady-state growth. *Nat Comm*, 9, 2988.
  100. Ruiz-Mirazo, K. (2019). Reaction: a plea for hypothesis-driven research in prebiotic systems chemistry. *Chem*, 5(8), 1920–1922.
  101. Ashkenasy, G., Hermans, T. M., Otto, S., & Taylor, A. F. (2017). Systems chemistry. *Chemical Society reviews*, 46: 2543–2554.
  102. Yu Zubarev, D., Rappoport, D., & Aspuru-Guzik, A. (2015). Uncertainty of prebiotic scenarios: The case of the non-enzymatic reverse tricarboxylic acid cycle. *Scientific Reports*, 5(8009), 1–7.
  103. Danos, V., Feret, J., Fontana, W., & Krivine, J. (2007). Scalable simulation of cellular signaling networks. In *Lecture Notes in Computer Science (APLAS 2007)*. pp. 139–157, Heidelberg, Germany: Springer-Verlag Berlin Heidelberg.
  104. Harris, L. A., Hogg, J. S., Tapia, J.-J., Sekar, J. A. P., Gupta, S., Korsunsky, I., Arora, A., Barua, D., Sheehan, R. P., & Faeder, J. R. (2016). BioNetGen 2.2: Advances in rule-based modeling. *Bioinformatics*, 32(21), 3366–3368.
  105. Andersen, J. L., Flamm, C., Merkle, D., & Stadler, P. F. A software package for chemically inspired graph transformation. In: R. Echahed & M. Minas (editors), *Graph Transformation: 9th International Conference, ICGT 2016, in Memory of Hartmut Ehrig, Held as Part of STAF 2016, Vienna, Austria, July 5–6, 2016, Proceedings*, pp. 73–88, Cham, 2016. Springer International Publishing. (for more information on the software-package see: <https://cheminf.imada.sdu.dk/mod/>)
  106. Danos, V., Feret, J., Fontana, W., Harmer, R., Hayman, J., Krivine, J., Thompson-Walsh, C., & Winskel, G. (2012). Graphs, rewriting and pathway reconstruction for rule-based models. In Leibniz

- International Proceedings in Informatics (FSTTCS 2012), pp. 276–288, Dagstuhl, Germany: Dagstuhl Publishing.
107. Laurent, J., Yang, J., & Fontana, W. (2018). Counterfactual resimulation for causal analysis of rule-based models. In *IJCAI'18: Proceedings of the 27th International Joint Conference on Artificial Intelligence*, pages 1882–1890, Stockholm, Sweden: AAAI Press.
108. Boutillier, P., Maasha, M., Li, X., Medina-Abarca, H. F., Krivine, J., Feret, J., Cristescu, I., Forbes, A. G., & Fontana, W. (2018). The kappa platform for rule-based modeling. *Bioinformatics*, 34(13), i583–i592, (for more information on the software-package see: <https://kappalanguage.org/>)
109. Forbes, A. G., Burks, A., Lee, K., Li, X., Boutillier, P., Krivine, J., & Fontana, W. (2018). Dynamic influence networks for rule-based models. *IEEE Transactions on Visualization and Computer Graphics*, 24(1), 184–194.
110. Wolos, A., Roszak, R., Żadło-Dobrowolska, A., Beker, W., Mikulak-Klucznik, B., Spólnik, G., Dygas, M., Szymkuć, S., & Grzybowski, B. A. (2020). Synthetic connectivity, emergence, and self-regeneration in the network of prebiotic chemistry. *Science*, 369(6511), eaaw1955.

**How to cite this article:** Lauber, N., Flamm, C., & Ruiz-Mirazo K. (2021). 'Minimal Metabolism': A key concept to investigate the origins and nature of biological systems. *BioEssays*, 43, e2100103. <https://doi.org/10.1002/bies.202100103>