In silico Support for Eschenmoser’s Glyoxylate Scenario

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Abstract

A core topic of research in prebiotic chemistry is the search for plausible synthetic routes that connect the building blocks of modern life such as sugars, nucleotides, amino acids, and lipids to “molecular food sources” that have likely been abundant on Early Earth. In a recent contribution, Albert Eschenmoser emphasised the importance of catalytic and autocatalytic cycles in establishing such abiotic synthesis pathways. The accumulation of intermediate products furthermore provides additional catalysts that allow pathways to change over time. We show here that generative models of chemical spaces based on graph grammars make it possible to study such phenomena is a systematic manner. In addition to reproducing the key steps of Eschenmoser’s hypothesis paper, we discovered previously unexplored potentially autocatalytic pathways from HCN to glyoxylate. A cascading of autocatalytic cycles could efficiently re-route matter, distributed over the combinatorial complex network of HCN hydrolysation chemistry, towards a potential primordial metabolism. The generative approach also has its intrinsic limitations: the unsupervised expansion of the chemical space remains infeasible due to the exponential growth of possible molecules and reactions between them. Here in particular the combinatorial complexity of the HCN polymerisation and hydrolysation networks forms the computational bottleneck. As a consequence, guidance of the computational exploration by chemical experience is indispensable.

1 Introduction

The Origin of Life is among the most fascinating and most interdisciplinary scientific problems. Despite a century of research, however, it still presents itself as an enigma. On the one hand, we still lack both a detailed understanding of the principles that govern the transition from non-living matter to living systems in general, and a clear historical scenario of the emergence of Life on Earth. See, e.g., Ruiz-Mirazo et al. for a recent review.
A key topic of prebiotic chemistry is to find synthetic routes to the bio-molecular building blocks of modern life that are chemically plausible given the environmental conditions on Early Earth. In particular, these synthetic routes need to start with material that has been reasonable abundant after the formation of the planet. This is a notoriously difficult research problem for several reasons: (1) The chemical search space is vast, in fact it is by far too large to enumerate exhaustively, and it cannot be confined to only those compounds that are well-characterised and described in current chemistry databases. (2) The constraints imposed by our current knowledge of the conditions on early earth are too vague to exclude large portions of the search space. (3) The problem of finding synthetic routes to target molecules itself, even for a fixed set of chemical reactions, is a very difficult combinatorial problem. (4) Experimental verification of potential routes is inherently slow. Consequently, conceptual guiding by skilled organic chemists is indispensable to direct research towards regions of prebiotic chemical space worthwhile of being explored in more detail. On the other hand, efficient formal, computational approaches are required to handle the combinatorial complexity in practice.

Recently, Albert Eschenmoser published a conceptual paper detailing the hypothetical relationships between HCN chemistry and the constituents of the reductive citric acid cycle. Eschenmoser suggested to look for catalytic and/or autocatalytic processes in the non-robust subspace of HCN chemistry since these have the potential to canalise this fragile chemistry towards the formation of $C_4$ and $C_6$ compounds, which in turn could function as precursors of α-keto acids and carbohydrates. In this scenario glyoxylate (CID 760) and its formal dimer, dihydroxyfumarate (CID 54678503), serve as the pivotal entry points into sugar chemistry. Both compounds can be formally viewed as “aquo-oligomeres” of carbon monoxide (CID 281) and open up a route to sugars that is independent of formaldehyde (CID 712) as basic building block. Eschenmoser furthermore pointed out that aldehydes have the potential to catalyse both the oligomerisation of HCN (CID 768) to the tetramer DAMN (CID 2723951), and the hydrolysisation of the cyanide groups ($\text{-CN}$) of the DAMN to the respective amide groups ($\text{-CONH}_2$). This type of chemistry makes oxaloglycolate (CID 524) (a tautomer of dihydroxyfumerate) and glyoxylate accessible from HCN. Finally, Eschenmoser proposes a hypothetical autocatalytic cycle feeding on glyoxylate in which oxaloglycolate (or its diamides) acts as “umpolung-catalyst” for its own production. Umpolung is an important process in organic chemistry where the reactivity of a functional group (e.g., of a carbonyl group C=O) is inverted via chemical modification. The majority of bonds in organic reactions are formed between atoms of different polarity. Heteroatoms usually polarise the carbon skeleton in consequence of their high electronegativity. Therefore the carbon atom of the carbonyl group is partially positively charged allowing carbanions (carbon atoms carrying a negative charge) to attack the carbonyl carbon atom to form a novel carbon-carbon bond. Umpolung now chemically modifies the carbonyl group in such a way that the polarity of the carbonyl group is inverted. This means that the carbonyl carbon atom, after umpolung, carries a negative charge and can, in contrast to its normal mode of reactivity, attack itself a positively charged carbon center to form a novel carbon-carbon bond. (for a review on “umpolung” in organic synthesis see e.g., Seebach [28])

Albert Eschenmoser arrived at hypotheses put forward in “The Chemistry of Life’s Origin” based on his extensive knowledge of organic chemistry. In this contribution we ask whether these scenarios could also have been found by formal computational methods and whether there are plausible alternatives, e.g., other autocatalytic processes hidden in the combinatorial chemical space of HCN chemistry. To this end we employ a computational framework we have developed specifically to explore very large chemical reaction networks. It combines a generative approach that implements chemical reactions as graph transformations with network flow analysis on the resulting hypergraph representing the reaction network [3, 4]. This framework allows, for example, to detect autocatalytic cycles [1] and to establish the existence of synthesis routes connecting a pair of compounds [5]. We will use it here to redraw and elaborate on Eschenmoser’s pictures by computational means.

2 Formal Approach

The vastness of chemical spaces renders the common methods of computational chemistry, from empirical potentials to full-fledged quantum chemistry, infeasible for large-scale explorations. A purely combinatorial approach that views molecules as graphs and chemical reactions as graph transformations, however, brings
2.1 Graph Grammars and the Double Pushout Approach

Despite the ubiquity of graphs in the chemistry literature, graph transformations [19] have been introduced only quite recently as an explicit models of chemical reactions [8]. They provide a more general and more versatile framework for specifying chemical reactions than earlier, matrix-centred approaches such as the Dugundji-Ugi theory [13, 18].

The basic idea behind graph transformations is that each transformation rule \( r \) specifies that a “left” graph \( L \) can be replaced by a “right” graph \( R \). The rule \( r \) can be applied to a graph \( G \) provided \( L \) is contained as a subgraph in \( G \). The application of \( r \) to \( G \) then consists in replacing the subgraph \( L \) within \( G \) by \( R \), resulting in a new graph \( H \). A simple example of such a rule is the addition of \( \text{Br}_2 \) to a \( \text{CH}_2=\text{CH}_2 \) double bond resulting in a dibromide \( \text{Br}^\text{−}\text{CH}_2\text{−CH}_2\text{−Br} \). Graph transformation systems generalise the string or term rewriting systems familiar in mathematics, computer science, and logic [7].

Several different mathematical frameworks have been explored for graph rewriting [14]. They vary in the exact definitions of how \( L \) has to be contained in \( G \), i.e., when the precondition for applying a rule is satisfied, and how exactly the replacement \( R \) is inserted into \( G \) to form the rewritten graph \( H \). We adopted one of the more restrictive frameworks known as the Double-Pushout approach (DPO) because it has several features that are appealing for applications to chemistry. In DPO a rule, usually called a production, consists of three graphs, the “educt” \( L \), the “product” \( R \), and a “context” \( K \) that remains unchanged during the reaction. The DPO formalism stipulates that \( K \) is contained as a subgraph in both \( L \) and \( R \). Since atoms are neither lost nor gained in chemical reactions, \( K \) in particular contains all atoms that take part in the reaction. Together, \( L, K, \) and \( R \) completely specify the formal transition state and the atom map of the transformation. A further advantage of DPO is that exchanging the roles of \( L \) and \( R \) automatically leads to the production for the reverse reaction. We refer to Andersen et al. [3] for more information on these technicalities.

The application of a production to a graph \( G \) is called a derivation. In a compressed representation

\[ \text{http://www.organic-chemistry.org/namedreactions/} \]
we can depict it as in Fig. 1. The production is shown by the two highlighted subgraphs (red bonds and green atoms) in the graphs denoted as $G$ and $H$. The highlighted patterns in $G$ correspond to an instance of precondition $L$, so that the production can be applied. In $H$, thus, $L$ is replaced by $R$. The full DPO representation of the rule application, along with the 18 chemical reaction mechanisms considered in this contribution, can be found in the appendix (the rule depicted in Fig. 1 is denoted as $r_{18}$ in the appendix).

An important technical consideration is that the graphs considered in our framework are usually not connected. Chemical production rules indeed often describe the formation of new bonds between two or even more educt molecules or may lead to splitting of a single molecule into multiple fragments. The graphs $G$ and $H$, hence, are not just molecules but rather multi-sets of molecules taken from a certain chemical space.

The application of a production of a given graph $G$ is a computationally non-trivial task since it requires the solution of a subgraph isomorphism problem namely that of finding $L$ as a subgraph of $G$. This is a well-known NP-hard problem [11]. Nevertheless, it can theoretically be solved efficiently for chemical graphs since they are almost always planar and the pattern $L$ is small, see Eppstein [15]. Our implementation uses the VF2 algorithm [12] for this purpose.

2.2 Generation and Exploration of Chemical Spaces

The set of chemical reactions, modelled as production rules, can be applied iteratively to a set of starting compounds. In principle, this procedure generates the universe of all molecules and all reactions between them that can be theoretically constructed from a given “chemical repertoire”. In each step, the chemical space grows. The production rules are intentionally modelled in a generic way, such that the same rule can be applied to many different combinations of compounds and even in many different ways on the same set of compounds. The generative approach thus very quickly leads to huge chemical spaces and the brute-force approach can only be applied for a very limited number of steps. A practical issue in constructing a chemical space is that we need to determine whether the result of a derivation is a new molecule, and hence needs to be added to the space, or whether it is a compound that we have already seen. This requires a large number of solutions of the graph isomorphism problem. For chemical systems, this issue is usually dealt with by using canonical SMILES strings [32]. Since errors in the canonicalisation algorithm have surfaced [20], we again use the VF2 algorithm [12] to verify results.

In order to control the computational effort, we employ methods from programming language design in computer science and use these to define a high-level strategy language. This will allow us to apply exploration strategies and expand the chemical spaces in a much more controlled way. For example, in contrast to a brute-force expansion, we can decide to only apply a smaller subset of rules to particular subsets of compounds specified in terms of certain graph-theoretical properties. Furthermore, the framework allows us to prune uninteresting parts of the chemical space by filtering the results of derivation again based on graph-theoretical properties. For a more formal introduction to the strategy framework we refer to Andersen et al. [4].

Eschenmoser [17] presented several schemes to investigate the relationship between HCN and the precursors of amino acids. To model some of his schemes, we followed strict strategies, i.e., we modelled specific reactions paths based on an explicit sequence of rule applications. The reason for this restriction is that a brute-force strategy in which all rules from the HCN chemistry are applied in an iterative way expands the chemical space so quickly that the computational resource are exhausted long before the products of interest are reached. Still, even a strict strategy with a pre-determined order in which production rules are applied, can lead to a plethora of different compounds because each individual rule potentially can be applied in many different ways. This follows from the fact that $L$ often admits several different subgraph isomorphisms into $G$.

We also employ the very useful technique of an expansion step with a subsequent closure of the chemical space, i.e., we apply (potentially all) chemical reactions to a set of chemical compounds, and subsequently infer all possible reactions between all compounds. When applying this strategy to a given network that fulfills a certain property (e.g., being autocatalytic), then the space that results from an expansion step with a subsequent closure operation can be used as an input to find neighbouring solutions to the given specific chemical transformation motif. How to find such motifs is discussed in the next section.
2.3 Detection of Chemical Transformation Motives

A chemical transformation motif is a subnetwork of a chemical space with the following properties: (1) The motif has a well-defined boundary to the outside, i.e., the educts, the products, and the food molecules are predefined. (2) The motif is stoichiometrically balanced, i.e., each chemical reaction contributes an integer flux that reflects how often a specific reaction in the chemical space is used within a specific pattern. Formally, the chemical spaces are hypergraphs, and a chemical motif can be expressed as a hyperflow on this hypergraph. Conceptually, integer hyperflows are similar to the flows obtained from Flux Balance Analysis, see e.g., Orth et al. [22]. The insistence on integer flows, however, allows to infer mechanistically important properties. E.g., this approach allows to distinguish between autocatalytic subsystems that need to be seeded by their products and those that can “auto-start”.

In order to bias the search for chemical transformation motifs or the search for alternatives to predefined motifs, we use an objective function. The probably most natural objective function will provide a maximum parsimonious solution by minimizing the number of reactions that are used to realise a sought transformation motif. Other possible objective functions include the fluxes on the reactions, or they use a quantification for the reactions that reflect the likelihood of reactions happening. For finding chemical transformation motifs we formulate the question to be answered as an Integer Linear Programming (ILP) problem. The technical details of this approach are far beyond the scope of this paper and we refer to Andersen et al. [6].

3 Results

In his conceptual paper [17] Eschenmoser proposed to re-investigate the non-robust parts of HCN chemistry, and emphasises glyoxylate as key a compound for the emergence of a primordial metabolism. A recent experimental study explored the aldol-type chemistry of dihydroxyfumarate, a tautomer of oxaloglycolate, and small aldehydes including glyoxylate [26]. It demonstrates that under moderate-pH conditions (pH 7–8) in water, biologically relevant ketoses are formed selectively with high yield. A follow-up study [9] revealed that, in contrast, tartrate (CID 875) and oxalate (CID 971) are produced under high-pH conditions. Dehydration and decarboxylation of tartrate, both well-known reactions, readily yield oxaloacetate and pyruvate. Although high-pH conditions are considered problematic in the prebiotic context, these studies nicely illustrate that even under consistent basic conditions even the most abundant products depend very sensitively on details of the reaction conditions. In silico explorations of the chemical space in the vicinity of experimentally known or theoretically inferred reaction sequences may drastically speed up the search for novel and unconventional routes to biologically relevant molecules. To demonstrate this point, we modelled the necessary HCN reaction chemistry proposed in Eschenmoser’s paper [17] with the help of our graph grammar framework. In this manner we can explicitly construct the reaction network surrounding the suggested pathways, so that we can explore potential alternatives in the chemical space where these routes are embedded. The hypothetical reaction sequences generated by the graph grammar framework can be refined whenever experimental evidence is collected. The 18 reaction types used in this contribution are listed in the appendix. We have deliberately specified as little context as possible to guarantee a broad applicability.

3.1 Overview

Fig. 2 summarises our contribution. It merges several of Eschenmoser’s schemes [17] and integrates them with our findings. The figure shows only key compounds and is intended to facilitate the navigation through the much more detailed analysis of the individual components that we have modelled and analysed with our graph grammar framework.

All results given in this section are based on the automatic computational inference of chemical transformation motifs within a large chemical space. Solutions of the various underlying optimisation questions correspond to automatically inferred detailed descriptions of the corresponding mechanism for the question asked. These computational results contain a plethora of additional information that we neither use nor discuss in this paper. For example, a complete set of atom maps is obtained as a by-product. From these,
3.2 Pathways from the HCN-tetramer to Oxaloglycolate

At first glance the conversion of the HCN-tetramer into oxaloglycolate seems to be a straightforward hydrolisation process. Eschenmoser’s paper [17] and Fig. 2 illustrates this process in strongly abstracted form with just 3 steps. A mechanistic model, however, shows that 11 steps are required. Fig. 3 summarises a superposition of all pathways of minimum length starting at DAMN and ending in oxaloglycolate. The figure emphasises the combinatorial nature of the HCN hydrolysation chemistry. Although all connections in the graph in Fig. 2 seem to be 1-to-1, this is in fact not the case. The simple molecules H₂O, NH₃, and HCN were suppressed in the drawing to reduce cluttering. The intermediate compounds selected as representatives in the overview figure (see Fig. 2) are highlighted in Fig. 3. A closer inspection of the structure of the network in Fig. 3 shows that it closely resembles a Cartesian graph product [25]. This feature is based on the fact that the temporal order of several of the intermediate steps can be permuted. A consequence of the product-like structure is a high level of confluence, i.e., the fact that a large number of partially overlapping alternative
pathways lead from the same educts to the same products. The hydrolysation chemistry of DAMN behaves like a dynamic combinatorial library that can adjust the internal fluxes upon change of the source or sink reactions.

### 3.3 The Autocatalytic Production of Glyoxylate

Eschenmoser [17] discussed the usage of glyoxylate as a starting material for the autocatalytic production of oxaloglycolate. Glyoxylate itself is produced via slow hydrolysation chemistry from the HCN-dimer iminoacetonitrile (CID 14496737). The production of oxaloglycolate and a potential downstream primordial metabolism therefore also depends on HCN as food source. If an alternative pathway for the production of glyoxylate becomes accessible, the dependent downstream metabolism remains unaffected and formally adapts to the new precursor of glyoxylate as food source. However, the production of glyoxylate from its precursor molecule is the rate-limiting step for the downstream processes. An autocatalytic cycle feeding on a slow hydrolysation process can not exhibit exponential growth characteristics and thus cannot canalise the reaction network. Eschenmoser therefore asked how glyoxylate could be produced more efficiently from its precursor compound(s).

We searched for potential autocatalytic cycles that produce glyoxylate from HCN. Even in the very strict modelling approach we applied, several autocatalytic solutions were found. Two arbitrarily chosen solutions with a minimum number of reactions have been analysed in more detail, see Fig. 4. The HCN hydrolysation chemistry contains many more solutions in particular if the constraint on the minimality of the autocatalytic cycle is relaxed. Both solutions produce two glyoxylate molecules from a single glyoxylate (acting as autocatalyst) and HCN as food source. The solutions are depicted as hyperflows, i.e., any reaction has an assigned integer value (shown as edge label). As usual H$_2$O and NH$_3$ (with a balance of +3 and −2, resp.), are suppressed from the drawing. The food molecule for both autocatalytic cycles are produced via slightly different pathways (Fig. 4a via iminoacetonitrile, Fig. 4b via formamide). Although the chemistry in the two cycles differ, the last step which produces two copies of glyoxylate are identical in both solutions. It involves amide hydrolysis which, depending on the reaction conditions, can be a slow process. During one turnover, both cycles consume two HCN molecules. We want to emphasise that these solutions only possess the proper topology for being autocatalytic cycles. We do not make any assumption about the kinetic rates or potential draining reactions which both can dampen the flux around the cycle to such an extend, that the autocatalytic behaviour is lost. However, our approach would allow to include such properties via additional constraints or an adequate modification of the objective function that is used in our optimisation approach.

### 3.4 The Autocatalytic Production of Oxaloglycolate

Eschenmoser [17] proposed an hypothetical autocatalytic cycle, that produces oxaloglycolate from glyoxylate. This transformation requires that the glyoxylate dimer or its amides acts as “umpolung-catalyst”. With the help of the strict expansion strategy we reproduced this model computationally. The corresponding autocatalytic cycle is depicted in the overview figure (simplified in Fig. 2 as two dashed orange (hyper-)edges). The right-hand side of Fig. 5 shows the detailed reaction pathway generated by the graph grammar approach. More precisely, it represents an optimal hyperflow in the chemical space in which both glyoxylate and oxaloglycolate are produced autocatalytically. This pathway illustrates the coupling of two autocatalytic cycles, one producing oxaloglycolate from glyoxylate and the other producing glyoxylate from HCN. The overall pathway consumes 4 HCN in order to produce one oxaloglycolate.

A cascading of autocatalytic reaction cycles has the advantage, that the downstream cycles are not rate limited by the upstream processes that produce their food molecules, and that symmetry-breaking of a homogeneous mixture can be very fast. In our particular case, when the autocatalytic cycle of the glyoxylate production kicks in, all the molecular mass distributed over the large reversible HCN-hydrolysation network (Fig. 3) is re-absorbed into the autocatalytic glyoxylate cycle. The effect of this “flux focusing” is an efficient re-routing of potentially lost raw material towards the downstream primordial metabolic processes.

At this point the question arises if there are alternative cascades of autocatalytic cycles hidden in the chemical space using glyoxylate and oxaloglycolate? In order to search for such alternatives we expanded the chemical space spanned by the hypergraph underlying Fig. 5 and then applied a closure operation, which
Figure 3: A superposition of optimal pathways from the HCN-tetramer to oxaloglycolate, illustrating the combinatorial complexity for even small subspaces.
embeds the reaction network employed for Fig. 5 into its nearby “shadow network”. The resulting chemical space is composed of 415 compounds connected by 1116 reactions (compared to the network of 95 compounds and 133 reactions, which was used as the underlying network for inferring the chemical transformation motif depicted in Fig. 5).

The objective function used for the inference of Fig. 5 was applied to this larger chemical space to search for alternative autocatalytic cascades. A solution is depicted in Fig. 6. The number of reactions in the alternative solution dropped from 24 to 22. Notice that both autocatalytic sub-networks are slightly different from the solution depicted in Fig. 5. The splitting step of the glyoxylate cycle, which generates the two copies of glyoxylate uses only the break-down of a hemiaminal, which can be considered to be a fast reaction. In the oxaloglycolate cycle the addition step of the first glyoxylate goes via a different tautomer of oxoaspartate. Although the oxaloglycolate sub-network was combinatorially modified, the number of reactions stayed the same. However, even one expansion step followed by a closure operation reduced the number of reactions used in the glyoxylate sub-network from 9 to 7.

4 Discussion and Conclusions

The analysis of autocatalytic subsystems of chemical reaction networks has become a lively research area in prebiotic chemistry [23]. Autocatalysis has the potential to canalise reactions in such a way that a relatively small number of well-defined organic molecules can accumulate in substantial concentrations. The key interest is, of course, in the production of the current constituents of living cells and in pathways that
may feed core subsystems of present-day metabolisms such as the reductive citric acid cycle \[29\]. A good example of such hypothetical autocatalytic feeding networks are those that start from the “formal hydrates of CO”, in particular glyoxylate and 2,3-hydroxyfumarate.

The scenario of prebiotic synthesis proposed by Albert Eschenmoser \[17\] is one of the most popular ones of this type. Other hypotheses worth mentioning in this context are, e.g., a model for the cyclic production of HCN \[27\] and a model of sugar chemistry \[31\]. A key feature of Eschenmoser’s model is the idea that the main “food sources”, and hence the main pathways, have evolved in a step-wise manner. Transitions between dominating reaction pathways are triggered whenever intermediate compounds become available that “kick-start” new autocatalytic cycles leading to their own production. In Eschenmoser’s model, first HCN is used as a food source in order to produce glyoxylate. Later-on glyoxylate is consumed as a food source for the autocatalytic production of oxaloglycolate, using itself as an (umpolung-)catalyst.

In order to study the possible chemical pathways in a more systematic manner efficient computational approaches are required to deal with the combinatorial nature and the immense size of chemical spaces. This in turn calls for a modelling approach that has a solid formal background. Here we use a graph-

Figure 5: The autocatalytic production of oxaloglycolate, using glyoxylate as a food source (to the right of the separating green-orange dashed line). This chemical space follows Eschenmoser’s suggestions and integrates a subspace for the autocatalytic production of the glyoxylate (left of the green-orange dashed line) as found in Section \[3.3\].
Figure 6: One of the optimal subspaces within which oxaloglycolate is produced autocatalytically from HCN as food source and the in which the intermediate food source glyoxylate is itself is produced in an autocatalytic manner. To find this solution the space depicted in Fig. 5 was expanded and a closure operation was applied to the expanded space. The two autocatalytic cycles are separated by the green-orange dotted line, cmp. Fig. 5.

Theoretical approach, more precisely graph transformation systems. These make it possible to generate well-defined subsets of chemical spaces. This type of computational modelling indeed allows the exploration of large numbers of alternative pathways, some of which are rather unexpected. In a recent investigation into HCN-polymerisation/-hydrolysis chemistry [2], for example, we found several plausible alternatives to Oró’s prebiotic adenine synthesis [21]. In the computational re-analysis of Eschenmoser’s hypothesis outlined here, we find that autocatalytic pathways can be found abundantly, often involving the same key intermediates.

The formal approach not only recovers the proposals put forward by experienced chemists but also reveals a plethora of alternatives that nevertheless match the same chemical transformation motives as those proposed in Eschenmoser’s work.

In some cases, such as the production of glyoxylate from HCN we found previously undescribed autocatalytic production cycles (see Fig. 4 and Fig. 5). In other parts of the network we found plausible alternative pathways such as the autocatalytic umpolung cycle for the oxaloglycolate production (see Fig. 6). Some of these solutions are particularly appealing since they involve even fewer reaction steps than their previously described alternatives. The superposition of the optimal pathways from the HCN-tetramer to oxaloglycolate, Fig. 3 highlights a product like organisation of a large number of confluent reaction sequences. The analysis of the shadow, i.e., the immediate vicinity of the union of the networks autocatalytic in glyoxylate.
and oxaloglycolate, illuminates a complex interplay of alternative routes.

Of course, the combinatorial analysis of the network only describes possibilities and plausibilities. Considerations of thermodynamic stability and in particular of the kinetics of alternative reactions have to be invoked to further narrow down the scenarios to those that are good candidates for having taken place on Early Earth. Nevertheless, the generation of the chemical spaces remains a necessary first step for any form of more refined and more realistic model of prebiotic chemistry.

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Appendix

Graph Grammar Rules

The following list contains 18 chemical reactions modelled as graph grammar production rules. A production rule in the DPO framework is usually denoted as $r = (L \leftarrow K \rightarrow R)$, where each rule $r$ consists of three graphs (fragments of molecules) $L$, $R$, and $K$ called the left, right, and context graph, respectively. Informally, the left graph $L$ can be replaced by a right graph $R$ if the corresponding subgraphs were found (see section 2.1). The indices of the production rules are consistent to the indices of the production rules as used throughout the paper.

$r_1$, Add HCN

$r_2$, CN to Amide

$r_3$, Amide to Acid

$r_4$, Water to Imine
\( r_5, \text{ Imine to Water} \)

\[
\begin{array}{c}
\text{L} \\
\text{OH} \\
\text{C} \text{---NH} \\
\text{K} \\
\text{OH} \text{ H} \\
\text{C} \text{ N} \\
\text{R} \\
\text{H}_2\text{O} \\
\text{C} \text{===N} \\
\end{array}
\]

\( r_6, \text{ Add Alcohol} \)

\[
\begin{array}{c}
\text{L} \\
\text{N} \text{ C} \text{ H} \text{O} \text{ C} \\
\text{K} \\
\text{N} \text{ H} \\
\text{C} \text{ O} \text{ C} \\
\text{R} \\
\text{NH} \\
\text{C} \text{ ==O} \text{ C} \\
\end{array}
\]

\( r_7, \text{ Aminal} \)

\[
\begin{array}{c}
\text{L} \\
\text{C} \text{ N} \\
\text{OH} \\
\text{K} \\
\text{C} \text{ N} \\
\text{O} \text{ H} \\
\text{R} \\
\text{C} \text{ NH} \\
\text{O} \\
\end{array}
\]

\( r_8, \text{ Aminal (Inverse)} \)

\[
\begin{array}{c}
\text{L} \\
\text{C} \text{ NH} \\
\text{O} \\
\text{K} \\
\text{C} \text{ N} \\
\text{O} \text{ H} \\
\text{R} \\
\text{C} \text{ N} \\
\text{OH} \\
\end{array}
\]

\( r_9, \text{ Keto to Enol} \)

\[
\begin{array}{c}
\text{L} \\
\text{C} \text{==O} \\
\text{CH} \\
\text{K} \\
\text{C} \text{ O} \\
\text{C} \text{ H} \\
\text{R} \\
\text{C} \text{ OH} \\
\text{C} \\
\end{array}
\]

\( r_{10}, \text{ Enol to Keto} \)

\[
\begin{array}{c}
\text{L} \\
\text{C} \text{ OH} \\
\text{C} \\
\text{K} \\
\text{C} \text{ O} \\
\text{C} \text{ H} \\
\text{R} \\
\text{C} \text{==O} \\
\text{CH} \\
\end{array}
\]
$r_{11}$, Ketimine to Enolimine

\[ L \xrightarrow{K} R \]

$\xrightarrow{K}$

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**APPENDIX**

$r_{17}$, Ether Break

![Diagram for Ether Break]

$r_{18}$, Aldol Addition N (Inverse)

![Diagram for Aldol Addition N (Inverse)]

**Double Pushout Diagram for Figure 1**

This section gives an example of using production rule $r_{18}$ for the derivation from an educt (graph $G$) to the two products (graph $H$, which represents two chemical compounds, namely the HCN-tetramer and glyoxylate). The edges changed by the transformation are shown in red and the vertices from $K$ are shown in green. All arrows represent embeddings of one graph into another, formally a subgraph monomorphism.

![Double Pushout Diagram for Figure 1]