Molecular Codes in Biological and Non-Biological Reaction Networks

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Abstract

Can we objectively distinguish chemical systems that are able to process meaningful information from those that are not suitable for information processing? Here, we present a formal method to assess the semantic capacity of a chemical reaction network. The semantic capacity of a network can be measured by analyzing the capability of the network to implement molecular codes. We analyzed models of real chemical systems (Martian atmosphere chemistry and various combustion chemistries), bio-chemical systems (gene expression, gene translation, and phosphorylation signaling cascades), as well as an artificial chemistry and random networks. Our study suggests that different chemical systems possess different semantic capacities. Basically no semantic capacity was found in the atmosphere chemistry of Mars and all studied combustion chemistries, as well as in highly connected random networks, i.e., with these chemistries molecular codes cannot be implemented. High semantic capacity was found in the bio-chemical systems, as well as in random networks where the number of second order reactions is at the number of species. Hypotheses concern the origin and evolution of life. We conclude that our approach can be applied to evaluate the information processing capabilities of a chemical system and may thus be a useful tool to understand the origin and evolution of meaningful information, e.g., at the origin of life.

Introduction

In recent years great advances have been made in understanding the bio-chemical basis of biological information processing. For theoretical analysis of biological information Shannon’s theory of communication \cite{39} has been applied very successfully in various domains, like genomics \cite{38}, bacterial quorum sensing \cite{28}, or signaling in molecular systems \cite{24}. The mathematical theory of communication focuses on uncertainty of events and intentionally neglects semantic aspects of information, because “they are irrelevant for the engineering problem” (Shannon \cite{39}, p. 1). However, in order to obtain a full understanding of biological information, studying also semantic as well as pragmatic aspects would be important, if not necessary \cite{22, 29}. Although syntax, semantics, and pragmatics are interdependent, as detailed in the Co\textsuperscript{3} approach \cite{44}, we concentrate here on semantic aspects of molecular networks in order to keep our formalism and analysis clear and concise.

In general, semantics refers to the relation between a sign and its meaning and can be described by a code. An example is the genetic code, which is a mapping between codons (signs) and amino acids (meanings) \cite{2}. An important property of this mapping or relation is its contingency, that is, the relation could be different and thus is not determined by the signs and meanings alone \cite{2, 29}. We say that the relation between signs and meanings is contingent, if the relation cannot be derived
by applying natural laws to the signs and meanings alone. This implies that by natural laws we can only derive the relation by knowing in addition a context under which the signs are interpreted. Furthermore it implies that there is potentially another context under which the signs are interpreted differently.

It is thus sometimes stressed that the relation between signs and meanings cannot be explained by physical laws \[35\], like the natural laws do not help in understanding the written law or the grammar of a language. However, more often than not this notion of independence from natural laws is the cause for confusion. So, in order to properly use semiotic concepts in biology, we should provide a proper – ideally formal – link from these concepts to the realm of physics. To achieve this we take the following strategy: (Step 1) Select an experimentally grounded and reliable formal description of the targeted biological system. Here, we take the reaction network as this formal description. (Step 2) Provide precise, not necessarily formal, definition of the semiotic concepts that shall be applied to the system. Here, we take the notion of an organic code as reviewed by Barbieri \[2\]. (Step 3) Interpret these definitions by linking them to the formal description of the biological system. Here, this is done by our formal definition of a molecular code. With this a semiotic concept gets – at least partially – operationalized by means of physical experiments. In particular, it allows us to incorporate contingency in a formal model of molecular codes.

To illustrate the basic idea of an explicit modeling of contingency we will briefly discuss an example reaction network, which exhibits a contingency. Figure 1A shows a reaction network containing eight molecular species \{A, B, C, D, E, F, G, H\} and four reactions. Here, we assume that the network contains all possible reactions that can appear when mixing these molecules. The network then is a complete model of the world, i.e., no species and reactions are missing that are physically possible. A mapping in a reaction network relates molecular species. Here, for example, \{A\} can be mapped on \{C\} by reaction \(A + E \rightarrow E + C\). \{E\} is necessary for the reaction to happen and thus we call it a molecular context. The network can implement a molecular code, if there exists a set of molecular species that can be mapped on a second set of molecular species in at least two different ways. In this example network the sets \(S = \{A, B\}\) and \(M = \{C, D\}\) fulfill this property. \(S\) (domain) maps on \(M\) (codomain) by applying the context \{E, H\}. No two elements of the domain \(S\) map to the same element in the codomain \(M\). There exist an alternative molecular context \{F, G\} which realizes a different mapping between domain and codomain. The existence of these two alternative mapping qualifies these as codes, since they emerge from a contingency.

**Materials and Methods**

In this section we provide a formal definition of a molecular code as a contingent mapping that can be realized by a reaction network, then we formally define the semantic capacity of a reaction network based on the number of molecular codes it can realize, and finally describe two algorithms for finding all molecular codes of a reaction network.

**Definition of Molecular Code**

A reaction network \(\langle M, R\rangle\) is defined by a set of molecular species \(M\) and a set of reactions \(R\) occurring among the molecular species \(M\). See Figure 1A for an example. For each reaction \(\rho \in R\), let \(\text{LHS}(\rho)\) and \(\text{RHS}(\rho)\) denote the set of reactant species (left hand side) and product species (right hand side) of reaction \(\rho\), respectively.

A subset of molecular species \(C \subseteq M\) is called **closed**, iff the application of all possible reactions from \(R\) on \(C\) does only produce species from \(C\), i.e., for all \(\rho \in R\) with \(\text{LHS}(\rho) \subseteq C\): \(\text{RHS}(\rho) \subseteq C\).
Figure 1. Two exemplary reaction networks containing molecular codes. Panel A: Chemical reaction network \( \langle M, R \rangle \) with species \( M = \{A, B, C, D, E, F, G, H\} \) and reaction rules \( R = \{A + E \rightarrow C + E, \ldots\} \); panel B: Code pair that can be realized by the network in panel A. The binary molecular codes are characterized by \( S = \{A, B\} \), \( M = \{C, D\} \), and the two codemakers \( C = \{E, H\} \), and \( C' = \{F, G\} \); panel C: Chemical reaction network with species \( M = \{I, J, K, L, M, N, M'\} \). The two species labeled 'M' denote the same species; panel D: Two molecular code pairs can be realized by the network in panel C.

For any set of species \( A \subseteq M \) there exists a smallest closed set \( G_{\text{Cl}}(A) \) containing \( A \) \(^{[10]}\). We say that \( G_{\text{Cl}}(A) \) is the closure of \( A \). Intuitively, the closure of a set of species contains all those species that can be reached by arbitrary long reaction pathways among the species of that set.

**Definition 1:** [molecular mapping] Given a reaction network \( N = \langle M, R \rangle \) and two sets of molecular species \( S, M \subseteq M \), we say that \( f : S \rightarrow M \) is a molecular mapping with respect to the reaction network \( N \), iff there exist a set of species \( C \subseteq M \) (called context), such that for each \( s, s' \in S \) with \( s \neq s' \): \( f(s) \in G_{\text{Cl}}(C \cup \{s\}) \) and \( f(s') \notin G_{\text{Cl}}(C \cup \{s\}) \). If there is a molecular mapping \( f \) with respect to \( N \), we also say that \( N \) can realize the molecular mapping \( f \).

Note that in a reaction network there is usually more than one molecular context \( C \) that realizes a particular molecular mapping \( f \). Intuitively, in order to “compute” \( f(s) \) with the reaction network \( N \), we put all molecules from the context \( C \) together with \( s \) in a reaction vessel. Then we repeatedly apply all applicable reaction rules and add the products to the reaction vessel until no novel molecular species can be added anymore. Then we check which molecular species from \( M \) is present, which must be – according to our definition – unique and the result of \( f(s) \).

**Definition 2:** [molecular code] Given a reaction network \( N = \langle M, R \rangle \) and a non-constant\(^1\) mapping \( f : S \rightarrow M \) is called non-constant, iff there exists \( s, s' \in S \) such that \( f(s) \neq f(s') \).

\(^{[10]}\) For any set of species \( A \subseteq M \) there exists a smallest closed set \( G_{\text{Cl}}(A) \) containing \( A \). We say that \( G_{\text{Cl}}(A) \) is the closure of \( A \). Intuitively, the closure of a set of species contains all those species that can be reached by arbitrary long reaction pathways among the species of that set.

\(^1\)A mapping \( f : S \rightarrow M \) is called non-constant, iff there exists \( s, s' \in S \) such that \( f(s) \neq f(s') \).
molecular mapping \( f : S \rightarrow M \), with \( S, M \subseteq \mathcal{M} \) with respect to \( N \), we call the mapping \( f \) a molecular code with respect to \( N \), if all other mappings \( g : S \rightarrow M \) with the same domain \( S \) and codomain \( M \) like \( f \) can also be realized by the reaction network \( N \).

The definition captures the notion of contingency as described above, i.e., the elements of the domain can be mapped to the elements of the codomain in an arbitrary way by changing the mappings that are only partially contingent, here. For binary molecular codes our definition can be reformulated more explicitly:

**Definition 3: [binary molecular code (BMC)]** Given a reaction network \( \langle \mathcal{M}, R \rangle \) and two binary sets of molecular species \( S = \{s_1, s_2\} \subseteq \mathcal{M} \) and \( M = \{m_1, m_2\} \subseteq \mathcal{M} \). The mapping \( f : S \rightarrow M \) is called a binary molecular code, iff there exist two sets \( C \subseteq \mathcal{M} \) (called codemaker) and \( C' \subseteq \mathcal{M} \) (called alternative codemaker) such that the following conditions hold:

\[
\begin{align*}
  f(s_1) &\in G_{CL}(\{s_1\} \cup C), \quad \text{and} \quad f(s_2) \notin G_{CL}(\{s_1\} \cup C), \quad \text{and} \\
  f(s_2) &\in G_{CL}(\{s_2\} \cup C), \quad \text{and} \quad f(s_1) \notin G_{CL}(\{s_2\} \cup C), \quad \text{and} \\
  f(s_2) &\in G_{CL}(\{s_1\} \cup C'), \quad \text{and} \quad f(s_1) \notin G_{CL}(\{s_1\} \cup C'), \quad \text{and} \\
  f(s_1) &\in G_{CL}(\{s_2\} \cup C'), \quad \text{and} \quad f(s_2) \notin G_{CL}(\{s_2\} \cup C').
\end{align*}
\]

As stated in Definition 3 each binary molecular code comes with a second code implementing a different mapping. The alternative code \( g \) is determined by \( g(s_1) = f(s_2) \) and \( g(s_2) = f(s_1) \). \( K = \langle f, g \rangle \) is called code pair. Two simple example networks are shown in Figures 1A and 1C. Both networks appear to be very similar in their structure, but they show different number of codes. While the former network is capable to realize one code pair, the latter network – though being smaller – can realize two code pairs.

**Semantic Capacity**

Now we can measure the semantic capacity of a system as the system’s capacity to realize contingent mappings. Concretely, we count how many different mappings a network can realize. Note that these codes need not be realized at the same time. Further note that we count each mapping only once, even if it can be realized by more than one codemaker.

Because we study only binary codes here and binary codes always come in pairs, it is reasonable to count the number of different code pairs \( CP_N \) of a network \( N \). So, the semantic capacity is defined here as:

\[
\mathcal{SC}(N) = CP_N.
\]

The number of code pairs can be high and can grow exponential with network size, such that we use the logarithm for comparing different network’s semantic capacity. The logarithmic semantic capacity is defined as

\[
\mathcal{SC}_{log}(N) = \log_2(1 + \mathcal{SC}(N)) = \log_2(1 + CP_N).
\]

In \( \mathcal{SC}_{log} \) we apply the transformation \( 1 + x \) to guarantee that the logarithmized semantic capacity is well defined and its smallest value is zero, in case the network cannot realize any molecular code.

The mean semantic capacity of a group of \( n \) networks \( N_i \ (i = 1, \ldots , n) \) is calculated by the arithmetic mean of the respective measure (linear or log).
In future studies, the semantic capacity could be integrated with measures of the code’s quality, fitness, or cost. E.g., two networks with the same number of code pairs could be differentiated with respect to the costs to implement those codes.

**Algorithmic Identification**

The closure-based definition of BMCs (Definition 3) allows us to develop algorithms for automatic detection of codes in reaction networks. The algorithm searches for a network pattern, i.e., a combination of molecular species and reactions, fulfilling the conditions stated above.

Definition 3 leads to an algorithm that first calculates all closed sets and then checks combinations of closed sets for the BMC condition as stated in Definition 3. In particular for the two elements of the domain, and the two elements of the codomain the single molecular closed sets, i.e., the closed sets that are generated by a single molecular species alone \(G_{CL}(m), m \in M\), are used. There exist at most \(|M|\) single molecular closed sets. The closure-based algorithm has a worst-case running time complexity of \(O(|M|^4n_c^2)\) with \(n_c\) as number of all closed sets contained in the system (cf. Supplement Text S1).

Alternatively we can analyze a network in terms of pathways. This makes sense since signs and meanings need always be connected by paths of reactions. The running time complexity of the pathway-based algorithm depends on the number of paths the network contains. For the identification of BMCs all s-t-paths for all pairs of species are identified. Any combination of four paths is checked for the BMC condition. Since the number of paths in a network grows enormously with the density of the network we apply a parameterized algorithm that uses only the \(k\)-shortest paths [27] between every pair of species. The worst case running time then is bounded by \(O(|M|^4k^4)\). If \(k\) is chosen too small the algorithm is not able to find all codes in the system, but gives an approximate measure. Pseudo-code for both algorithms and subroutines is given in the supplement (Supplement Text S1).

The different running time complexities suggests a conditional application of the algorithms. The pathway-based algorithm can be efficiently applied on networks that have a high number of closed sets and a low number of paths, while the closure-based algorithm can be applied in the other case, where the number of paths is high and the number of closed sets in the network is low. Interestingly, systems with high semantic capacity tend to have both, high number of closed sets and many pathways (see below), so that an algorithmic challenge remains for analyzing such systems more efficiently.

**Results**

In the following we survey different kinds of systems for their semantic capacity by the application of the algorithms described above. The analyzed systems are the gene translation chemistry (GC), gene regulatory networks (GRN), phosphorylation cascades (PC), combustion chemistries (CC), the martian atmosphere chemistry, and random networks. A summary of the analysis of the biological systems (GRN, GC, PC) is given in Table 7, while all systems are compared in Table 8. To apply our algorithms we had to construct the reaction networks for some of these systems first. To accomplish this we followed a knowledge-based approach for GC, GRN, PC.
Biological Reaction Networks

Gene Translation

We will show now that the gene translation chemistry can realize molecular codes. In particular, this suggests that the genetic code, i.e., the mapping describing the translation from nucleotide triplets to amino acids, is a molecular code. The fact that there is more than one genetic code is known for a long time \[17, 34\]. Here, we analyze the 17 already known genetic codes, as listed at NCBI \[9\]. The different genetic codes cover nuclear and non-nuclear codes of different genera, e.g., bacterial, archaeal, and plant plastid codes, the vertebrate, invertebrate, and yeast mitochondrial codes, and the alternative yeast nuclear code. In particular, we construct a reaction network containing the codons, the amino acids, and the specific tRNAs, which are necessary for the translation. For all mappings between DNA triplets and amino acids occurring in the 17 codes we add a reaction in the network of the form \(\text{codon} + \text{tRNA} \rightarrow \text{amino acid}\). The obtained reaction network (Supplement Network S2) represents a merge of the 17 genetic codes and contains 234 molecular species and 85 reactions.

Table 1. BMCs found in the merge of the 17 known genetic codes. Here the 16 found BMCs are summarized. If applicable BMCs are grouped. See supplement Text S3 for the code pairs.

| sign (codons) | meanings (amino acids) | #BMC | References |
|--------------|-------------------------|------|------------|
| CTT, CTG, CTA, CTC | L, T | 6 | \[6, 34\] |
| AGG, AGA | G, S, R, Stop | 6 | \[3, 4, 8, 11, 14–16, 20, 32–34, 41, 46\] |
| AGG, TCA | S, Stop | 1 | \[3, 4, 15, 31, 33, 34\] |
| AGA, TCA | S, Stop | 1 | \[3, 4, 15, 31, 33, 34\] |
| TTA, TAG | L, Stop | 1 | \[9, 13, 23, 31, 34\] |
| TAA, TAG | Q, Stop | 1 | \[19, 25, 34, 36, 37\] |

References - Articles reporting the respective alternatives in the genetic code that are part of a BMC in this analysis.

The algorithmic analysis of this network identified 16 binary molecular codes (Supplement Text S3), i.e., a semantic capacity of \(SC_{\log} = 4.09\). The binary codes can partly be assigned to larger molecular codes. CTT, CTG, CTA, and CTC can be mapped on leucin (L) and threonin (T) and give rise to six of the found BMCs. The second group involves the mapping between AGG, AGA and glycin (G), serine (S), arginine (R) and the translation stop. This code can also be decomposed into six BMCs. There does exist four more BMCs that involve the codons TCA, TTA, TAG and the amino acids leucine (L), glutamine (Q) and the stop signal. Table 1 summarizes the BMCs found. The existence of alternative mappings in the genetic translation system suggests that the genetic code qualifies as a molecular code.

We may model the genetic code now by including all potential mappings between codons and amino acids, i.e., the model includes all possible tRNAs such that any codon could be read for any amino acid (Supplement Network S3). In such a system the number of binary molecular codes can easily be calculated. Each pair of codons forms a code pair with each pair or amino acids. Since there exist \(\binom{64}{2}\) pairs of triplets and \(\binom{20}{2}\) pairs of amino acids the number of BMCs in this potential set up is

\[
\binom{64}{2} \cdot \binom{20}{2} = 383,040.
\]

The logarithmic semantic capacity is \(\approx 18.55\).

In the following, we refine the network model by constructing a reaction network containing all possible mappings between the 64 codons and 20 amino acids like described above. Additionally
Figure 2. Subnetwork of the full gene translation network model with synthetases \( (N_{GC}) \). The network (panel A) shows a subnetwork of the gene translation network model containing the translation, and loading reactions for two selected codons (GGA,AGU) and amino acids (Gly,Ser). The semantic analysis shows that four code pairs can be implemented by this network (panel B).

we model the loading step of the tRNAs by inserting the respective amino-acyl-tRNA-synthetases (aaRS) (cf. Figure 2, Supplement Network S5). The reaction network \( N_{GC} = (M_{GC}, R_{GC}) \) describes the core molecular mechanism realizing the standard genetic code and all alternative codes. The set of molecular species \( M_{GC} \) of the network contains all DNA strings of length three (Table 2, Eq. 2), representing the codons. It contains the twenty proteinogenic amino acids in their free form (Table 2, Eq. 3) and the twenty amino acids bound in a protein (Table 2, Eq. 4). To describe the system properly we also need to insert species for all possible tRNAs in their unloaded (Table 2, Eq. 5) and loaded form (Table 2, Eq. 6). In the unloaded form we represent specificity to codon \( n \) with \( n \) as subscript, in the loaded form we represent specificity to \( n \) with a subscript \( n \) and the loaded amino acid \( a \) with a subscript index \( a \), i.e., a tRNA that is loaded with Ser and has specificity to AGU is denoted as \( tRNA_{AGU,Ser} \). The network also contains all possible aaRS (Table 2, Eq. 7), \( Syn_{n,a} \), such that the system is able to load all amino acids to all tRNAs. The specificity of the aaRS to certain combinations of codons and amino acids is represented by two subscript indices \( n,a \), with \( n \) representing the respective codon and \( a \) representing a certain amino acid. The set \( R_{GC} \) contains all reactions loading the amino acids onto the tRNAs (Table 2, Eq. 8) and all reactions inserting an amino acid in the peptide sequence (Table 2, Eq. 9). Figure 2A displays a subnetwork with two codons (GGA,AGU), two amino acids (Gly,Ser) and the respective other elements of the network (tRNA and synthetases).

The analysis of this extended network \( (N_{GC}) \) describing all potential genetic codes with 64 codons and 20 amino acids results in 1,532,160 binary code pairs, i.e., \( SC_{log}(N_{GC}) \approx 20.55 \). This is a different result than for the less detailed model, as calculated by Eq. 3. The extension of the model by aaRS, unloaded tRNAs, and unloaded amino acids increases the semantic capacity. A closer look to the resulting codes shows that not only the codons can be signs, but also the unloaded tRNAs \( (tRNA^{free}) \) can function as signs. These additional signs increase the number of code pairs. The “new” codes differ structurally in their codemakers. While, classically, the codons are mapped to the set of amino acids \( (AA^{prot}) \) using the loaded tRNAs \( (tRNA^{loaded}) \) as codemakers, the new
Table 2. Reaction network formulation of a gene translation system with synthetases

| Eq. | Definition | Description |
|-----|------------|-------------|
| 1   | $M_{GC} = \text{Codons} \cup AA^{free} \cup AA^{prot} \cup aaRS \cup tRNA^{free} \cup tRNA^{loaded}$ | Definition of the molecular species in the network |
| 2   | $\text{Codons} = \{A,C,G,T\}^3 = \{AAA, AAC, \ldots , TTT\}$ | Set representing the 64 codons of the genetic code |
| 3   | $AA^{free} = \{\text{Ala}^{free}, \text{Arg}^{free}, \text{Asp}^{free}, \ldots , \text{Tyr}^{free}\}$ | Amino acids that are not used in a protein |
| 4   | $AA^{prot} = \{\text{Ala}^{prot}, \text{Arg}^{prot}, \text{Asp}^{prot}, \ldots , \text{Tyr}^{prot}\}$ | Amino acids that have been used in a protein during gene translation |
| 5   | $tRNA^{free} = \{tRNA_n| n \in \text{Codons}\}$ | Unloaded tRNAs specific for codon $n$ |
| 6   | $tRNA^{loaded} = \{tRNA_{n,a}| n \in \text{Codons}, a \in AA^{free}\}$ | tRNAs specific for codon $n$ that have been loaded with amino acid $a$ |
| 7   | $aaRS = \{\text{Syn}_{n,a}| n \in \text{Codons}, a \in AA^{free}\}$ | Amino-acyl-tRNA-synthetases that are specific for amino acid $a$ and codon $n$ |
| 8   | $R_{GC} = \{tRNA_n + a + \text{Syn}_{n,a} \rightarrow tRNA_{a,n} + \text{Syn}_{n,a} | n \in \text{Codons}, a \in AA^{free}\}$ | Loading of the tRNA by suitable synthetasis |
| 9   | $\{n + tRNA_{a,n} \rightarrow n + tRNA_n + a | n \in \text{Codons}, a \in AA^{prot}\}$ | Translation step, i.e., the incorporation of an amino acid into a growing protein |

Signs, i.e., unloaded tRNAs, are mapped to the set of amino acids by using a codemaker that consists of the free amino acid loaded to the free tRNA, the synthetase performing the loading step, and the codon that needs to be recognized by the tRNA. The number of code pairs in this system can be calculated by

$$\left(\frac{n_s}{2} - \frac{n_s}{2}\right) \cdot \left(\frac{n_m}{2}\right),$$

with $n_s$ as number of signs and $n_m$ as number of meanings. For the full gene translation system the number of signs is $n_s = |\text{Codons}| + |tRNA^{free}|$ and $n_m = |AA^{prot}|$. Since there is always one pair of one tRNA and codon belonging together, which therefore can not be combined in an BMC, we have to subtract the number of such pairs $n_s/2$ from the amount of all combinations.

Table 3. Code pairs realized by the subsystem of the gene translation network with synthetases shown in Figure 2

| Signs | Meanings |
|-------|----------|
| Code pair 1 | $\{GGA, AGU\}$ | $\{\text{Gly}^{prot}, \text{Ser}^{prot}\}$ |
| Code pair 2 | $\{GGA, tRNA_{AGU}\}$ | $\{\text{Gly}^{prot}, \text{Ser}^{prot}\}$ |
| Code pair 3 | $\{AGU, tRNA_{GGA}\}$ | $\{\text{Gly}^{prot}, \text{Ser}^{prot}\}$ |
| Code pair 4 | $\{tRNA_{GGA}, tRNA_{AGU}\}$ | $\{\text{Gly}^{prot}, \text{Ser}^{prot}\}$ |

Table 4. Codemakers of the code pairs shown in Table 3

| Code pair | Codemaker | alternative Codemaker |
|-----------|-----------|-----------------------|
| 1         | $\{tRNA_{GGA,\text{Gly}}, tRNA_{AGU,\text{Ser}}\}$ | $\{tRNA_{AGU,\text{Gly}}, tRNA_{GGA,\text{Ser}}\}$ |
| 2         | $\{AGU, \text{Ser}^{free}, \text{Syn}_{AGU,\text{Ser}}, tRNA_{GGA,\text{Gly}}\}$ | $\{AGU, \text{Gly}^{free}, \text{Syn}_{AGU,\text{Gly}}, tRNA_{GGA,\text{Ser}}\}$ |
| 3         | $\{GGA, \text{Ser}^{free}, \text{Syn}_{GGA,\text{Ser}}, tRNA_{AGU,\text{Gly}}\}$ | $\{GGA, \text{Gly}^{free}, \text{Syn}_{GGA,\text{Gly}}, tRNA_{AGU,\text{Ser}}\}$ |
| 4         | $\{GGA, AGU, \text{Gly}^{free}, \text{Ser}^{free}, \text{Syn}_{GGA,\text{Gly}}, \text{Syn}_{AGU,\text{Ser}}\}$ | $\{GGA, AGU, \text{Gly}^{free}, \text{Ser}^{free}, \text{Syn}_{GGA,\text{Ser}}, \text{Syn}_{AGU,\text{Gly}}\}$ |
Gene Regulatory Networks

A gene regulatory network (GRN) is a graph representing the regulation of gene expression of certain genes by the expression of other genes. A node in a GRN stands for a complex process. It represents the gene, the promoter and binding region of that gene, the binding of the transcription factor (TF) plus cofactors and the production of a product by the recruitment of the gene expression machinery.

We will show here that the GRN of a cell is also a highly semantic system. Different sources of signals are integrated into a decision which gene is transcribed and which is repressed. Each of these signals has another meaning which emerges out of the contingency of the system. The system is contingent, because a mapping between signal and gene product can be altered by the exchange of a promoter region of a gene (or vice versa). This may happen enzymatically by the application of nucleases and ligases or by mutations.

To identify the semantic capacity we describe a gene regulatory network as a reaction network $\mathcal{N}_{GRN} = (\mathcal{M}_{GRN}, \mathcal{R}_{GRN})$. $\mathcal{M}_{GRN}$ contains $n$ transcription factors $TF_i$, $m$ products $P_i$, and genes $G_{ij}$. Each gene $G_{ij}$ represents a combination of a promoter site $i$ and a coding region $j$, where the promoter site $i$ is specific to $TF_i$ and the coding region $j$ produces $P_j$. For our model we assume that there exist as many promoter sites and coding regions as transcription factors and products, respectively, such that any gene is possible. The set of all species $\mathcal{M}_{GRN}$ then is

$$\mathcal{M}_{GRN} = \{TF_1, TF_2, \ldots, TF_i, \ldots, TF_n, P_1, P_2, \ldots, P_j, \ldots, P_m, G_{11}, G_{12}, \ldots, G_{ij}, \ldots, G_{nm}\}.$$ 

Assuming that a transcription factor only binds one promoter and that a promoter is bound only by one by one transcription factor the expression of a gene $i, j$ is given by

$$\mathcal{R}_{GRN} = \{TF_i + G_{ij} \rightarrow TF_i + G_{ij} + P_j\}, i = 1, 2, \ldots, n, j = 1, 2, \ldots, m.$$ 

Figure 3 illustrates the network definition. We here do not present a generic model to describe all possible gene regulatory networks, but a model that covers the main properties of regulation important for this study. The analysis of this system shows that the reaction network can implement molecular codes only in one way, i.e., with the transcription factors as signs and the set of products as meanings. The set of genes, i.e., the combination of promoter and coding region, forms the codemaker, because it allows for the contingent implementation of mappings between signs and meanings. Thus, in contrast to the model of the gene translation chemistry described above, the DNA is not the sign, but functions as the codemaker, i.e., it carries the contingency of the system. This shows that a code based analysis can only be done with regards to systems and not to single molecular species.

Signaling Networks: Phosphorylation Cascades

Cells maintain signaling systems of different kinds for signal transmission and integration [21]. The most prominent signaling systems rely on reversible phosphorylation of amino acids side-chains for regulation of signaling protein activity. The direct involvement of such systems in signaling suggest that they may be also semantic systems. If so they should be able to realize molecular codes. We have studied phosphorylation cascades, like the MAP kinase regulatory network, as a typical instance of an intra-cellular signaling system. These systems demonstrate the limitation of our
Figure 3. Gene regulatory network model. Panel A: (left) Simple model of the expression of a gene, (right) reaction network formulation of the same process. Boxes in Panel A indicate the semantic interpretation, i.e., the transcription factors are the signs, the products are the meanings, and the DNA is the codemaker. Panel B: reaction network constructed according to the formalization of gene regulation shown in (A).

closure-based approach. The static approaches described above are not sufficient to detect the codes in a phosphorylation cascade. A more refined approach is necessary that distinguishes between concentrations. It can be derived from our definitions here in a straightforward way.

Applying this more refined approach, i.e., taking concentrations into account, we can see that the activation of a kinase by phosphorylation can generate a molecular mapping, but this mapping is not necessarily a molecular code (Figure 4A). A two-step cascade is able to implement a molecular code (Figure 4B).

The simple one-step phosphorylation model (Figure 4A) contains two kinases; an initial kinase \( S \) which is able to phosphorylate the target kinase \( S^P \rightarrow A \). We also model the dephosphorylation \( A^P \rightarrow A \). For sake of simplicity we do not model the phosphatases, and the phosphate related molecular species (e.g., ATP, ADP, P) involved in the process, but assume a buffered concentration. In the simple, one step, model we may observe a molecular mapping between \( S^P \) and the two states of kinase \( A \). If \( S^P \) has a low concentration the system is in a state where the unphosphorylated state \( A \) has a high concentration and the phosphorylated state \( A^P \) has a low concentration. According to the definition of molecular code given above the system should be able to change the mapping, i.e., be contingent, by the application of a different molecular context to realize a code. Here, no alternative mapping between \( S \) and \( A \) can be realized, such that the system is not able to realize a molecular code.

If we consider a different system where two kinases are inbetween \( S \) and \( A \), we obtain a two-step phosphorylation cascade. \( S^P \) now phosphorylates the inserted species, while these have an effect
Figure 4. Reaction networks describing phosphorylation motifs. Molecular species in these networks represent kinases or phosphatases that may be activated or inactivated. Activated and non-activated forms of a kinase/phosphatase are modeled as different species (e.g., species $A/A^P$). Panel A: (left) Reaction network of a simple phosphorylation motif, which can realize a molecular mapping (right), but not a molecular code; panel B: (left) more complex reaction network that can realize a molecular code. The molecular code is not only specified by the species, but also by their concentrations.

Random reaction networks as null model for code identification

To check whether the motif describing a BMC can be generated by chance we analyzed random reaction networks of different sizes and densities for their semantic capacity. The networks have been generated by random insertion of reaction rules in the empty network. Each random reaction rule is bimolecular, i.e., contains two reactants, and one product.

The analysis showed that the binary code motif can be generated in random networks (see Figure 5), i.e., contingent mappings can be generated randomly. For a fixed network size and varying densities the average semantic capacity shows a unimodal behavior, which suggests that there exist an optimal range of densities for each network size, leading to maximal semantic capacity. This optimal range shifts to higher densities with increasing size of the network (see Figure 6). The optimal interval is bounded on the left (lower densities) by the low complexity of the network, there
Figure 5. Structural properties of random reaction networks. Panel A: Number of paths of the respective combination of species and reactions. Panel B: Number of closed sets. Panel C: Semantic capacity. Panels A, B and C show three important network parameters for five different network sizes and various numbers of reaction rules. Each data point represents the average about random replicates. Error bars indicate the standard error of the mean. Panel A shows the average number of paths in the network. Since we applied the path algorithm which only uses the k-shortest paths between each pair of molecular species the curve shows a sigmoidal behavior, which is saturated at the value $|M| \cdot (|M| - 1) \cdot k$, with $k = 10$. Panel B shows the average number of closed sets. With growing density the number of closed sets decreases. Panel C shows the distributions of the average number of code pairs (log measure with basis 2) in random networks of different sizes. The semantic capacity shows an unimodal distribution, which correlates with the other two shown network parameters. If the number of paths is too low no mappings can be implemented because of the missing links. If the number of closed sets is too low no unique mappings can be implemented.
are not enough reactions to promote the insertion of molecular by chance. On higher densities the network is strongly connected, such that it is harder to obtained closed sets, and therefore it is also harder to implement codes by chance. The optimal interval coincides with two important network properties, i.e., the number of paths, and the number of closed sets. With increasing network density the number of paths grows, while the number of closed sets decreases. High semantic capacity can be found in networks with a high number of pathways and at the same time a high number of closed sets.

Figure 6. Scatter plot of the positions (Molecular Species, Reactions) of the maximal semantic capacity of the unimodal distributions for the random networks analyzed in this study. The linear regression results in the function:

\[ \text{Reactions} = -3.06 + 1.89 \cdot \text{Molecular species}. \]

Non-Biological Reaction Networks

Combustion Chemistries

We analyzed a number of chemical systems, i.e., combustion chemistries of hydrogen [7], methane [45], ethanol [26], dimethyl ether [18]. The original combustion chemistry data (provided in CHEMKIN format [19]) have been processed to obtain the reaction networks describing the respective chemistry. The chemistries are intended to describe all significant processes that can occur in the combustion, i.e., burning, of the respective molecule. In the CHEMKIN files most of the reactions are described as equilibrium reaction with additional thermodynamic parameter. Taking these as basis we obtain reaction networks (see definition above) containing the directed reactions depending on the thermodynamic parameters. The obtained reaction networks (Supplement S7) vary in their size (10 - 79 molecular species) and density (38 - 752 reactions). We found that none of the chemistries is able to realize molecular codes.

We also analyzed the atmosphere chemistry of Mars [30] to check whether other kinds of non-biological chemistries may contain codes. The atmosphere chemistry of mars contains 32 molecular
species, 104 reactions and 5512 closed sets. In particular the network describes the reaction happening on the day side of mars. Therefore, light \((h\nu)\) is modelled explicitly and there exist an inflow reaction for light. The Martian chemistry also is not able to realize molecular codes.

Here, we compare the obtained results with random reaction networks of same size and density. The original hydrogen chemistry could not realize molecular codes. This may be due to the small number of closed sets compared to the number of paths, such that the molecular species are “too connected” and the network is less structured. In random networks of same size and density no molecular code can be identified. The estimated number of closed sets and paths, although differing from the original chemistry, are also marking that the networks are not in the optimal interval (compare section).

In the methane combustion chemistry we see that there exist far more paths than closed sets, such that the network is “unstructured”. The according null-model, here, also contains a high number of paths, but also a higher number of closed sets. The algorithmic analysis showed that some null-model networks can realize BMCs, such that the average semantic capacity is \(SC_{\log} = 1.04\). Nevertheless we consider this also as a very low semantic capacity compared with, e.g., the gene translation chemistry. For the other two combustion chemistries (ETH, DME) and the Martian atmosphere chemistry (MARS) the analysis of the random networks is not feasible with our current algorithms, due to the large number of paths and closed sets in these networks.

| Combustion chemistry properties | Null model estimate |
|---------------------------------|---------------------|
| \(|M|\) | \(|R|\) | #closed sets | #paths | \(SC_{\log}\) | est. | #closed sets (s.e.) | est. | #paths (s.e.) | est. | \(SC_{\log}\) (s.e.) |
| HYD 10 | 38 | 16 | \(7.69 \cdot 10^4\) | 0 | 39.8 (0.53) | 878.2 (1.27) | 0 (0.0) |
| MET 37 | 340 | 4,136 | \(> 10^6\) | 0 | 6,521.83 (353.63) | \(> 10^6\) | 1.09 (0.15) |
| ETH 57 | 752 | 5,136 | \(> 10^6\) | 0 | \(> 10^5\) | \(> 10^6\) | n.a. |
| DME 79 | 708 | 8 | \(> 10^6\) | n.a. | \(> 10^6\) | n.a. |

n.a. - not available, s.e. - standard error of the mean, *estimated by performing runs on several networks (or growing values of \(k\) regarding #paths) where not all runs completed due to computational complexity, such that the maximal found value gives the estimate.

**Artificial Chemistry NTOP**

Recall that with increasing density random networks have a vanishing semantic capacity. In the following we show that even a dense network can have a relatively high semantic capacity. For this purpose we analyze an artificial chemistry with 16-species introduced by Banzhaf Banzhaf [1] called NTOP. For each species there is a 4-bit binary representation and the reaction rules are derived with respect to this representation, which is referred to as a structure-to-function mapping (see [1] for details and Supplement S8 for the full network model).

The algorithmic analysis results in six code pairs (for an overview see Figure 7 and Supplement Text S9). One of the code pairs with its respective codemaker is shown in Table 6. Figure 7 illustrates two properties of molecular codes, (1) a meaning can take the role of a sign in another code, and (2) molecular species can function as signs (or meanings) in different codes, i.e., they keep their role in different contexts. Both properties reflect the context dependency of codes, i.e., the molecular species constituting the code depend on the molecular context, the codemaker.

To test the robustness of the network’s ability to realize molecular codes, we randomized it by replacing 1, 2, 5, 10, 15, 200, and 1000 reaction rules randomly. The number of educts and products for each individual reaction is kept constant, only the molecular species are replaced. Increased randomization result in a clear decline of the average semantic capacity. Nevertheless in some cases
Figure 7. Code pairs found in the artificial chemistry NTOP. An edge connects a sign with a meaning if they occur in the same code (indicated by colors). Species belonging to codemakers have been omitted. Note that a sign can be used in different codes and that a meaning might be used as sign in another code (e.g., 10 in \{10,15\}). A vertex represents the closed set generated by the respective species alone.

Table 6. Binary molecular code from the NTOP chemistry. Species are indicated by index. The first line indicates the respective species. The second line contains the closed sets generated by the species alone and the closed sets that form the codemaker.

| species | sign 1 | sign 2 | meaning 1 | meaning 2 | codemaker | alternative codemaker |
|---------|--------|--------|-----------|-----------|-----------|-----------------------|
| closed sets | \{1,2\} | \{13,15\} | \{1,3\} | \{8\} | \{0,5,10,15\} | \{0,1,9\} |

the randomized network is capable to implement more code pairs. The average trend, i.e., loss of code pairs, can be explained by referring to random reaction networks. Random reaction networks with the same number of species and reactions as NTOP also have a very low semantic capacity ($SC_{log} = 0$). Thus the randomization of the NTOP chemistry drives the system towards the mean semantic capacity of random networks.

Conclusion

We introduced a formal criterion for identifying molecular codes in reaction networks and a measure of the semantic capacity of a network, as the number of different code pairs the network can realize. Our notion of contingency, defined as the ability of systems to choose between different mappings, extends the notion of “independence” used by Barbieri.

Applying the new concepts to different networks, our basic finding is that the semantic capacity of biological networks tends to be higher than that of the studied non-biological networks. Thus, an important step during the transition from non-life to life must have been the utilization of a chemistry that allows to implement molecular codes. In our opinion it is an open issue how that first coding chemistry has looked like. But we have now a criterion that can guide us in what we have to look for.

Moreover we can now precisely formulate another hypothesis, namely, that during the course of evolution the semantic capacity of the chemistry employed by the biological systems has a tendency to increase, though not necessarily monotonously. One candidate mechanism is the invention and improvement of compositional adaptors, like proteins with exchangeable domains or genes including their promoter- and coding-regions. Note that also the appearance and evolution of neurons and cognitive systems is in line with the hypothesis of increasing semantic capacity.
Figure 8. Randomization of NTOP. Boxplot showing the $SC$-distribution of the randomized NTOP chemistry. We can observe that on average randomization destroys the semantic capacity (non-logarithmized) of the network. The boxplots show the distribution of the number of code pairs after randomization of NTOP ($n = 100$)

Table 7. Overview of semiotic interpretation of the biological systems surveyed in this paper.

| role      | gene regulatory codes               | genetic codes                                      | phosphorylation cascade codes                                    |
|-----------|-------------------------------------|---------------------------------------------------|------------------------------------------------------------------|
| signs     | transcription factor                | codon or unloaded tRNA                             | high concentration of a kinase or phosphatase                    |
| meanings  | gene product                        | amino acid                                        | high/low concentration of a target molecule                     |
| codemaker | DNA with promoter and coding region | loaded tRNA or mixture of loaded tRNA, aaRS, and codons | a kinase or phosphorylases                                       |

The analysis of a formalization of the genetic code showed that not only the codons are signs for an amino acid, but also tRNAs could be signs. The bio-molecular and evolutionary interpretation of this fact should be left for future studies. Furthermore, we have shown that DNA not only can function as a sign but also as a codemaker, as the modeling of GRNs revealed. The mechanisms in gene regulatory systems and the observation that such systems are highly flexible (i.e. the mapping between TFs and products can easily be changed) leads to the conclusion that the chemistry of GRNs possesses also a high semantic capacity.

The analysis of random networks of different sizes and densities results in a better understanding of the basal rate of code occurrence. We can observe that the distribution of BMCs is unimodal. Random networks with high semantic capacity show at the same time a high number of closures (which decreases with increasing network density) and high number of pathways (which decreases with decreasing network density). The analysis of an artificial chemistry showed that also in dense networks the semantic capacity can be high. We hypothesize that this was caused by the structure-to-function mapping applied in the artificial chemistry.

Future work includes the formal integration of information theory and the integration of pragmatics. Furthermore we can extend this static algebraic approach to a continuous and dynamics approach.

When we address the semantic aspects of biological information, terminology becomes a hot topic of discussion. Although we appreciate this discussion from a philosophical perspective, we believe
a pragmatic focus is necessary to obtain a stronger impact in life sciences. This pragmatic track of the study of meaning for biological information requires at least three ingredients: (1) (semi-)formal definitions, (2) algorithm, tools, and predictions, and (3) links to experimental data (i.e., the physical world). These three ingredients obviously interact with each other and should thus be studied together.

Supporting Information

Text - S1  s1_pseudocode.pdf
Pseudocode of all algorithms and subroutines used in the analysis of the data for this paper.

Network - S2  s2_gc_merge.zip
Network file in rea- and sbml-format containing the description the merge of the 17 genetic codes listed at NCBI.

Text - S3  s3_gc_merge_codes.pdf
Text file containing the result of the computational analysis of the network from S2.

Network - S4  s4_gcfull_64_20.zip
Network file in rea- and sbml-format containing the description of the full gene translation chemistry (without synthetases).

Network - S5  s5_gcsynth_Gly_Ser.zip
Network file in rea- and sbml-format containing the description of a subnetwork (Gly,Ser) of the full gene translation chemistry (with synthetases).

Text - S6  s6_gcsynth_Gly_Ser_codes.pdf
Text file containing the result of the computational analysis of the network from S5.

Network - S7  s7_non-biological-networks.zip
Network files of the analyzed combustion chemistries and the Martian atmosphere chemistry.

Network - S8  s8_ntop.zip
Network files of the artificial chemistry NTOP.

Text - S9  s9_ntop_codes.pdf
Text file containing the result of the algorithmic analysis of NTOP.

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References

1. W. Banzhaf. Self-replicating sequences of binary numbers. *Comput Math Appl*, 26:1–8, 1993.
2. M. Barbieri. Biosemiotics: a new understanding of life. *Naturwissenschaften*, 95(7):577–599, 2008.
3. B. Batuecas, R. Garesse, M. Calleja, J. R. Valverde, and R. Marco. Genome organization of Artemia mitochondrial DNA. *Nucleic Acids Res*, 16(14A):6515–6529, 1988.
4. J. L. Boore and W. M. Brown. Complete DNA sequence of the mitochondrial genome of the black chiton, Katharina tunicata. *Genetics*, 138(2):423–443, 1994.
5. E. Bornberg-Bauer, A. K. Huylmans, and T. Sikosek. How do new proteins arise? *Curr Opin Struct Biol*, 20:390–396, 2010.
6. G. D. Clark-Walker and G. F. Weiller. The structure of the small mitochondrial DNA of Kluyveromyces thermotolerans is likely to reflect the ancestral gene order in fungi. *J Mol Evol*, 38(6):593–601, 1994.
7. M. O. Conaire, H. J. Curran, J. M. Simmie, W. J. Pitz, and C. Westbrook. A comprehensive modeling study of hydrogen oxidation. *Int J Chem Kinet*, 36(11):603–622, 2004.
8. G. A. Durrheim, V. A. Corfield, E. H. Harley, and M. H. Ricketts. Nucleotide sequence of cytochrome oxidase (subunit III) from the mitochondrion of the tunicate Pyura stolonifera: evidence that AGR encodes glycine. *Nucleic Acids Res*, 21(15):3587–3588, 1993.
9. A. Elzanowski and J. Ostell. The genetic code, 2010. http://www.ncbi.nlm.nih.gov/Taxonomy/Utils/wprintgc.cgi, version 3.9, July 07, 2010, retrieved: Feb 20, 2011.
10. W. Fontana and L. Buss. The arrival of the fittest: Toward a theory of biological organization. *Bulletin of Mathematical Biology*, 56:1–64, 1994.
11. J. R. Garey and D. R. Wolstenholme. Platyhelminth mitochondrial DNA: evidence for early evolutionary origin of a tRNA(serAGN) that contains a dihydrouridine arm replacement loop, and of serine-specifying AGA and AGG codons. *J Mol Evol*, 28(5):374–387, 1989.
12. D. Görlich and P. Dittrich. Identifying molecular organic codes in reaction networks. In G. Kampis and E. Szathmáry, editors, *10th European Conference on Artificial Life*, volume 5777 of *LNCS*, pages 305–312. Springer, 2011. Sep 13 - Sep 16, 2009, Budapest.
13. Y. Hayashi-Ishimaru, T. Ohama, Y. Kawatsu, K. Nakamura, and S. Osawa. UAG is a sense codon in several chlorophycean mitochondria. *Curr Genet*, 30(1):29–33, 1996.
14. H. Himeno, H. Masaki, T. Kawai, T. Ohta, I. Kumagai, K. Miura, and K. Watanabe. Unusual genetic codes and a novel gene structure for tRNA(AGYSer) in starfish mitochondrial DNA. *Gene*, 56(2-3):219–230, 1987.
15. R. J. Hoffmann, J. L. Boore, and W. M. Brown. A novel mitochondrial genome organization for the blue mussel, Mytilus edulis. *Genetics*, 131(2):397–412, 1992.
16. H. T. Jacobs, D. J. Elliott, V. B. Math, and A. Farquharson. Nucleotide sequence and gene organization of sea urchin mitochondrial DNA. *J Mol Biol*, 202(2):185–217, 1988.

17. T. H. Jukes and S. Osawa. Evolutionary changes in the genetic code. *Comp Biochem Physiol B*, 106(3):489–494, 1993.

18. E. Kaiser, T. Wallington, M. D. Hurley, J. Platz, H. J. Curran, W. J. Pitz, and C. K. Westbrook. Experimental and modeling study of premixed atmospheric-pressure dimethyl ether-air flames. *Journal of Physical Chemistry*, 104(35):8194–8206, 2000.

19. P. J. Keeling and W. F. Doolittle. A non-canonical genetic code in an early diverging eukaryotic lineage. *EMBO J*, 15(9):2285–2290, 1996.

20. A. Kondow, T. Suzuki, S. Yokobori, T. Ueda, and K. Watanabe. An extra tRNA_Gly(U*CU) found in ascidian mitochondria responsible for decoding non-universal codons AGA/AGG as glycine. *Nucleic Acids Res*, 27(12):2554–9, 1999.

21. G. Krauss. *Biochemistry of Signal Transduction and Regulation*. Wiley-VCH, Weinheim, 4 edition, 2008.

22. B.-O. Küppers. *Information and the origin of life*. MIT Press, Cambridge/MA, 1990. (Originally published 1986).

23. M. J. Laforest, I. Roewer, and B. F. Lang. Mitochondrial tRNAs in the lower fungus *Spizellomyces punctatus*: tRNA editing and UAG 'stop' codons recognized as leucine. *Nucleic Acids Res*, 25(3):626–632, 1997.

24. T. Lenaerts, J. Ferkinghoff-Borg, F. Stricher, L. Serrano, J. W. H. Schymkowitz, and F. Rousseau. Quantifying information transfer by protein domains: analysis of the Fyn SH2 domain structure. *BMC Struct Biol*, 8:43, 2008. [http://dx.doi.org/10.1186/1472-6807-8-43](http://dx.doi.org/10.1186/1472-6807-8-43)

25. A. Liang and K. Heckmann. Blepharisma uses UAA as a termination codon. *Naturwissenschaften*, 80(5):225–226, 1993.

26. N. M. Marinov. A detailed chemical kinetic model for high temperature ethanol oxidation. *Int. J. Chem. Kinet.*, 31:183–220, 1999.

27. E. Q. V. Martins and M. M. B. Pascoal. A new implementation of yen’s ranking loopless paths algorithm. *4OR: A Quarterly Journal of Operations Research*, 1:121–133, 2003. ISSN 1619-4500. [http://dx.doi.org/10.1007/s10288-002-0010-2](http://dx.doi.org/10.1007/s10288-002-0010-2)

28. P. Mehta, S. Goyal, T. Long, B. L. Bassler, and N. S. Wingreen. Information processing and signal integration in bacterial quorum sensing. *Mol Syst Biol*, 5:325, 2009.

29. J. Monod. *Chance and necessity*. Alfred Knopf, New York/NY, 1971. (Originally published 1970).

30. H. Nair, M. Allen, A. D. Anbar, and Y. L. Yung. A photochemical model of the martian atmosphere. *Icarus*, 111:124–150, 1994.
31. A. M. Nedelcu, R. W. Lee, C. Lemieux, M. W. Gray, and G. Burger. The complete mitochondrial DNA sequence of Scenedesmus obliquus reflects an intermediate stage in the evolution of the green algal mitochondrial genome. *Genome Res*, 10(6):819–831, 2000.

32. T. Ohama, S. Osawa, K. Watanabe, and T. H. Jukes. Evolution of the mitochondrial genetic code. IV. AAA as an asparagine codon in some animal mitochondria. *J Mol Evol*, 30(4): 329–332, 1990.

33. S. Osawa, T. Ohama, T. H. Jukes, and K. Watanabe. Evolution of the mitochondrial genetic code. I. origin of AGR serine and stop codons in metazoan mitochondria. *J Mol Evol*, 29(3): 202–207, 1989.

34. S. Osawa, T. H. Jukes, K. Watanabe, and A. Muto. Recent evidence for evolution of the genetic code. *Microbiol Rev*, 56(1):229–264, 1992.

35. H. H. Pattee. The physics of symbols: bridging the epistemic cut. *Biosystems*, 60(1-3):5–21, 2001.

36. S. U. Schneider and E. J. de Groot. Sequences of two rbcS cDNA clones of Batophora oerstedii: structural and evolutionary considerations. *Curr Genet*, 20(1-2):173–175, 1991.

37. S. U. Schneider, M. B. Leible, and X. P. Yang. Strong homology between the small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase of two species of Acetabularia and the occurrence of unusual codon usage. *Mol Gen Genet*, 218(3):445–452, 1989.

38. T. D. Schneider and R. M. Stephens. Sequence logos: a new way to display consensus sequences. *Nucleic Acids Res*, 18(20):6097–6100, 1990.

39. C. E. Shannon. A mathematical theory of communication. *The Bell Systems Technical Journal*, 27:379–423, 623–656, 1948.

40. P. Speroni di Fenizio, P. Dittrich, J. Ziegler, and W. Banzhaf. Towards a theory of organizations. In *German Workshop on Artificial Life (GWAL 2000), in print*, Bayreuth, 5.-7. April, 2000, 2000. available online: http://di.ttri.ch/p/SDZB2001gwal.ps.gz.

41. M. J. Telford, E. A. Herniou, R. B. Russell, and D. T. Littlewood. Changes in mitochondrial genetic codes as phylogenetic characters: two examples from the flatworms. *Proc Natl Acad Sci U S A*, 97(21):11359–11364, 2000. [http://dx.doi.org/10.1073/pnas.97.21.11359](http://dx.doi.org/10.1073/pnas.97.21.11359)

42. T. Tlusty. Casting polymer nets to optimize noisy molecular codes. *Proc Natl Acad Sci U S A*, 105(24):8238–8243, 2008.

43. T. Tlusty. Rate-distortion scenario for the emergence and evolution of noisy molecular codes. *Phys Rev Lett*, 100(4):048101, 2008.

44. S. Tsuda, S. Artmann, and K.-P. Zauner. The Phi-Bot. In A. Adamatzky and M. Komosinski, editors, *Artificial Life models in hardware*, pages 213–232. Springer, Dordrecht, 2009.

45. T. Turnyi, K. Hughes, M. Pilling, and A. Tomlin. The Leeds methane oxidation mechanism. online, 2001. Version 1.5, available at http://www.chem.leeds.ac.uk/Combustion/methane.htm.
46. S. Yokobori, Y. Watanabe, and T. Oshima. Mitochondrial genome of Ciona savignyi (Urochordata, Asciacea, Enterogona): comparison of gene arrangement and tRNA genes with Halocynthia roretzi mitochondrial genome. *J Mol Evol*, 57(5):574–587, 2003. [http://dx.doi.org/10.1007/s00239-003-2511-9](http://dx.doi.org/10.1007/s00239-003-2511-9)
Table 8. List of all analyzed systems stating their size, density, semantic capacity, the reference of the system, and the method used for analysis.

| Abbrev      | #species | #reactions | #closed sets | #paths | SC$_{log}$ | Method  | Reference | Description                                                                                   |
|-------------|----------|------------|--------------|--------|------------|---------|-----------|------------------------------------------------------------------------------------------------|
| FIG1A       | 8        | 4          | 161          | 12     | 1.58       | c & p   | this study | Network from Figure 1A.                                                                         |
| FIG1C       | 6        | 4          | 41           | 16     | 4.09       | p       | this study | Network from Figure 1C.                                                                          |
| GCMERGE     | 234      | 85         | n.a.         | 170    | 18.55      | t       | this study | Network reconstructed from the genetic codes reported at [8]                                    |
| GCFULL      | 1364     | 1280       | n.a.         | n.a.   | 20.55      | t       | this study | Theoretical estimate of SC$_{log}$ of a network, based on GCMERGE, generated by inserting all possible mappings between codons and amino acids |
| GCFULLSYNTSMALL | 16     | 8          | n.a.         | 200    | 2.32       | p       | this study | Network with two codons, two amino acids, and synthetases.                                       |
| GCFULLSYNT  | 2,728    | 2,560      | n.a.         | n.a.   | 20.55      | t       | this study | Theoretical evaluation of a reaction network containing all possible mappings between the 64 codons and 20 amino acids with synthetases. |
| MARS        | 32       | 104        | 5,512        | > 10$^6$ | 0          | c       | [30]     | Chemical processes occurring in the Martian atmosphere during the daylight phase                 |
| HYD         | 10       | 38         | 16           | 7.69 $\cdot$ 10$^4$ | 0          | c       | [7]      | Combustion chemistry of hydrogen                                                               |
| MET         | 37       | 340        | 4,136        | > 10$^6$ | 0          | c       | [45]     | Combustion chemistry of methane                                                                |
| ETH         | 57       | 752        | 5,136        | n.a.   | 0          | c       | [26]     | Combustion chemistry of ethanol                                                                |
| DME         | 79       | 708        | 8            | > 10$^6$ | 0          | c       | [18]     | Combustion chemistry of dimethyl ether                                                           |
| NTOP        | 16       | 207        | 244          | 474, 218 | 2.81       | c & p   | [4]      | Artificial chemistry based on binary strings operations                                          |
| R_NTOP      | 16       | 207        | 244          | 474, 218 | 2.81       | c         | this study | Average on 1000 random networks of the same size and density as the NTOP network.                 |
| RANDOM      | varies   | varies     | varies       | varies | varies     | c & p   | this study | Analysis of different random networks.                                                          |

Abbrev.: c - closure based algorithm, p - pathway-based algorithm, t - theoretical analysis, SC$_{log}$ - logarithmized semantic capacity, n.a. - not available, s.e. - standard error of the mean.

*: determined with $k = 10000$. 

22