A Signal Processing Approach to the Analysis of Chemical Networking Protocols

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Academic Year 2009/2010
Abstract

Chemical Networking Protocols (CNPs) are communication protocols, whose design is based on chemical models: distributed networks of reactions and molecule species. The benefits arise from the dynamics analysis of chemical models and thus from the prediction of CNP behaviors. Chemistry already supplies tools for the analysis of reaction network dynamics: the Chemical Master Equation (CME) and Differential Rate Equations (DREs). However, both procedures often lead to complicated solutions.

We propose another deterministic approximation of dynamics, like DREs, but based upon the frequency characterization of chemical models. We used the signal processing background, adapting it into this new scenario and integrating it with analysis methods of other fields (e.g. the Metabolic Control Analysis (MCA)). In linear reaction networks, we identified and frequency characterized elementary building blocks which constitute chemical models. By linking these building blocks with series, parallel and feedback interconnections, we could replace chemical networks with schematics composed by transfer function blocks only. In non-linear networks analysis, we applied the MCA to linearize the model. We showed dynamics of some existing CNPs and gave recommendations for the CNP design (i.e. for possible congestion control CNPs). We also considered chemical links with delays.

Keywords: chemical networking protocols, dynamics analysis, frequency response, metabolic control analysis.
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List of Acronyms

AWGN          additive white Gaussian noise
EU            European Union
i.i.d.        independent and identically distributed
MCA           metabolic control analysis
OSI           open systems interconnection
PSD           power spectral density
SNR           signal-to-noise ratio
CNP           chemical networking protocol
CCN           chemical communication network
LTI           linear time invariant
ODE           ordinary differential equation
DRE           differential rate equation
PDF           probability density function
DDE           delay differential equation
CME           chemical master equation
GPRS          general packet radio service
List of Symbols and Operators

Convolution operator $\otimes$
Absolute value $|\cdot|$
Vector norm $||\cdot||$
Vector $\vdash$
Matrix $\vdash$
Transposition $(\cdot)^T$
Complex conjugate $(\cdot)^*$
Derivative of the function $x \dot{x}$
Real part $\Re\{\cdot\}$
Imaginary part $\Im\{\cdot\}$
Expectation of a random variable $X \mathbb{E}\{X\}$
variance of a random variable $X \text{Var}\{X\}$
Probability of a random variable $X \mathbb{P}\{X\}$
Conditional probability of a random variable $X$ to the event $B \mathbb{P}\{X|B\}$
Mean of a variable $x \langle x \rangle$
Step function $u(\cdot)$
Impulse function $\delta(\cdot)$
The $i^{th}$ reaction coefficient $k_i$
Set of the network vertices (species) $\mathcal{V}$
Set of the network links $\mathcal{E}$
Reaction set $\mathbf{v}$
Product vector of the $i^{th}$ reaction (for general chemical analysis) $\xi_{ri}$
Stoichiometric vector of the $i^{th}$ reaction $\chi_{ri}$
Propensity function $a_r(\cdot)$
Difference between the stoichiometric product vector and the stoichiometric reactant $\psi_{rj}$

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Introduction

The meeting of two different science branches is not always intuitive and often leads to scepticism in parts of the science community. However, sometimes the comparison among distinct fields is useful and even needed. By comparing similar phenomena and processes, known models from a certain field can then be applied in a new different subject.

Probably, the very strong chemistry and biology background of the University of Basel has contributed to the birth of a new type of communication network: Chemical Networking Protocols (CNP). The main goal is to have life-like properties, found in biochemistry (i.e. self-healing), even in CNPs. To satisfy this target, communication models are associated to specific chemical models. This approach is not the first trial in matching these two subjects.

An example of the influence of natural science in communication engineering is the "AntNet", where the routing aspect is based on observations about ant colonies (observations coming from biology). Even congestion control, used in TCP, is based upon life-like properties: the "conservation" of packets and its obvious attempt to reach a "stable state".

CNP try to bring all main rules and laws of chemistry to communication protocol design, leading to exploiting properties observed in nature, difficult to reach otherwise. CNPs represent a new project and thus not so many communication protocols are available nowadays. On the other hand, CNPs may definitely cover a wide research area, that offers a different concept of communication network design. Anyway, CNPs, which deal with important communication network duties, already exist (i.e. self-healing).

The milestone of this new networking approach is changing the starting point of protocol designing: not caring about implementation details like algorithms and program formalisms, but focusing on the behavior of networks. First concerns of engineers will be the dynamics. In relaxed situations or in standard conditions or in critical moments, the network should always grant similar performances and characteristics. With this new perspective, the reference to chemistry is important: chemical models and their tools allow us to predict how communication networks will behave.
Context

The design of CNPs starts from the use of chemical models, composed by molecules, species and reactions. By studying the kinetics of these chemical models, the CNP dynamic can be predicted. However, the analysis of simple chemical models can make the design work very difficult. Mathematical treatments, such as the Chemical Master Equation (CME), often lead to complications even in the study of simple systems. Therefore, simplifications of rigorous solutions are often needed. For instance, differential equations approximate the dynamics of a chemical reaction network, which is instead rigorously found with the CME. Even going through the following pages, terms as ”approximation” will often be used.

Referring to chemistry and biology, CNP designers can rely on centuries of studies and they can use analysis procedures that are now well evaluated. Thus, the first step in designing CNPs is to find chemical models which describe the dynamics as close as possible to our ideal prototypes. Then, CNP designers should probably adapt these models to their needs and, making use of chemical methods, they should try to approximatively evaluate the performance of CNPs.

Basing upon such concepts, we propose to have another point of view. We introduce the use of the frequency analysis. Network behaviors which are nearly impossible to foresee in the time domain, can be easily explained in the frequency world. Portions of chemical reaction networks have different responses depending on the input frequencies. Thus, they can be seen as filters, which have been already deeply treated by the signal processing field.

When the backward procedure is needed, namely when network dynamical behaviors are not as we guessed, the control system theory can help us. It gives methods to calibrate coefficients and to trim network constants in order to get the expected responses, or at least approach them.

We refer to methods that have already been used for long in other sectors and therefore, models that are now well known. However, applying them in a new area (chemistry into communication networks), changes the scenario. There is the need to adapt model characteristics, before its use into the new environment. For instance, we have always been used to treat linear systems only, often even time-invariant. The application of mathematical transformations, such as the Laplace transform, were possible and thus we could easily switch from the time domain to the frequency domain. However, implementable chemical models are far from being linear, even the easiest CNP. There is the need to frequency characterize also non-linear networks.
Dealing with chemical communication networks, another reference to the world of telecommunications engineering could be the information theory field. This is certainly the rigorous way to characterize the flow of information. Information theory would help to quantify key parameters of communication systems, like (maximum) capacity, efficiency and error probability. In chemical networks, the system information is conveyed with signals that have a random component. This randomness leads also to an incomplete (imprecise) forecast of the network dynamics. Unfortunately, the probability study applied to the world of chemistry is not so trivial. Although the origin of such phenomena can be easily explained and treated, the statistical characterization of the process is not simple. There are historical attempts to give a general stochastic representation that fits with the totality of chemical reaction networks. Unfortunately, not many people have succeeded in that purpose. Now some studies exist for special cases, but these academical treatments have complicated backgrounds and often lead also to complicated results. For that reason, we will give an explanation of the stochasticity origin and we will introduce its treatment, without going into further argumentations.

In order to understand the context of the whole project, this thesis has to introduce the basic of chemically-inspired networking. We have analyzed new properties which are reached by the use of this heterogeneous way to design. Note that some parts of this thesis represent only an overview of studies that have been already done by others. Anyway, these introductory subjects are necessary to fully understand further analytical passages and to give a global meaning to the whole project. Of course more space has been left for the chemistry dynamics explanation. Also the background of frequency analysis and signal processing field has been summarized. The application of the frequency analysis to CNPs has been explained more with practical examples. The numerical computation and visualization of the analysis has been performed in MATLAB environment, using optional toolboxes as signal processing and control systems design toolboxes.

Main contributions

This thesis introduces the use of the Laplace transform to fully study frequency responses of simple CNPs. The term "simple" is associated to the fact that, initially, only linear chemical reaction networks will be studied. This does not mean an exclusion of complex analysis of important network typologies, which show even feedbacks and delays. With our contribution, the decomposition of the linear chemical reaction model becomes possible, leading to a system of several elementary entities, which is simple to analyze. We have
characterized these elementary entities with a specific transfer function. The analysis of the overall system can be easily computed by interconnecting these entities together. As in signal processing, we can think about cascades and parallels of blocks and, in order to get control systems, we can put feedbacks in our networks.

We have also been able to characterize, in the frequency domain, networks with delayed links. We have compared the effect of a delayed link with the effect of the respective chemical model, in time and frequency domains. Even if we are now able to plot responses in both domains, we often talk about approximations and not about exact solutions. Anyway, these approximations allow us to predict protocol behaviors and thus to design CNPs looking at stability and efficiency.

We have satisfied also the goal to treat non-linear reaction networks. Even in estimating such a typology of models, there are several ways to find the dynamics, more or less rigorous, rather than more or less simple to use. The Volterra series could represent a possibility, but its complexity has led us in another direction: is there really the need to deal with non-linearity? Is this property really predominant in network behaviors at a specific time? Generally, designers are interested only in particular events that could affect the proper functioning of the system. Designers usually do not care about general solutions that are always valid, but they are often satisfied with a limited number of situations. So, this has been the reason of using of the Metabolic Control Analysis (MCA). The MCA incorporates the study of the system response at equilibrium conditions, and thus only when the system is subjected to small external perturbations around these fixed points. Only after this linearization, we could use the Laplace transform, as well as all other common tools of signal processing area, even for the analysis of non-linear models.

Definitely, finding theoretical formulations of methods and mathematical theorems is much harder than only applying them. So, we would not seem to be comparing the contribution of this thesis with the contribution of works as the MCA. However, it is important to highlight our effort to give an operative way to proceed. We have used the MCA to analyze non-linear chemical models that are actually used in communication protocols. Remember that this means the introduction of a real tool to design new kinds of communication networks.

In the end, it has been compulsory to touch the argument of the noise. We only supply a short introduction to this wide argument of research. Definitely, the stochasticity aspect of chemical model analysis represents an interesting subject for further attentions. Due to time reason and complexity, we have only given an idea of how this noise affects the deterministic signal for a specific practical example. Definitely, a more exhaustive view of this random process would be very useful in designing and developing. However, we should recall that we
still are at the dawn of CNPs and so, any direction may represent an area not completely explored yet.

**Structure of the Thesis**

This thesis is mainly organized in two general parts: an introduction to the chemically inspired networking, Chap. 1 and Chap. 2, and the analysis of dynamics of Chemical Networking Protocols (CNPs), Chap. 3 and Chap. 4. Chapter 1 summarizes the basic theory which CNPs are based on and describes the hierarchical layers of CNPs. Chapter 2 supplies three practical examples of existing chemical models of CNPs: the "Disperser" protocol, the "Quine" structure and the avoidance congestion protocol. Chapter 3 initially summarizes the theory part which is strictly related to the chemical dynamics. The reader will not really need to fully understand and remember Sect. 3.1. The rest of the thesis can be as well appreciated, even going directly to Sect. 3.2, where the frequency domain analysis becomes the main subject. All tools and procedures which are related to that domain and which are useful for CNP dynamics analysis, are explained in Sect. 3.2. Section 3.2.5 explains the metabolic control analysis (MCA), that nowadays, seems to represent the best tool to study non-linear chemical communication networks. Section 3.3 introduces the sophisticated background theory of the intrinsic noise of chemical communication networks. Chapter 4 supplies practical results of the application of the theory described in Chap. 3. Several typologies of linear systems are studied, including the "Disperser" protocol. The possibility of a delayed link is also considered, even including the study of chemical networks with delayed reactions. The MCA is applied in order to analyze the "Quine" structure, in Sect. 4.5.2 and the congestion avoidance protocol, in Sect. 4.5.3. At the end of Chap. 4, an instance of a chemical network model showing band pass behavior is given.

Appendix 6.1 is dedicated completely to the description of MATLAB package tools. Appendix 6.2 gives an important theoretical contribution: it clarifies the difference of a step response and an impulse response applied to a chemical model. Appendix 6.3 reports all studied cases of different types of "loop networks". A similar target has App. 6.4, where two variants of the Disperser CNP are analyzed. Appendix 6.5 studies a linear reaction with the MCA. Appendix 6.6 reports results of the Quine structure, observing its behavior for different model parameters. The whole analytical procedure for the congestion avoidance chemical protocol is left for App. 6.7. Appendix 6.8 supplies important assertions about the stochasticity aspect of the chemical model analysis. The last appendix, App. 6.9, compares results of
the dynamical behavior analysis of three chemical models to the dynamics obtained in the Fraglets implementation of these models. This appendix represents a sort of validation of the theoretical analysis.

All appendixes are specifically recalled in related sections.
Chapter 1

Chemical Communication Networks

In this chapter, we introduce Chemical Networking Protocols (CNPs), giving basic information which we retain are important for understanding the context of the whole project. The first section explains the analogy between chemistry and communication networks. This metaphor, which has been explicated by T. Meyer [1], was used by CNP pioneers to develop this new chemical communication system. Specifically, we give for each communication network element the respective chemical reaction network component. Section 1.1.1 highlights the properties of CNPs, often comparing to traditional communication systems. The conclusion of this section is constituted by Sect. 1.1.2 which describes the chemical model that we use to analyze the dynamical behavior of networks. Then, in Sect. 1.2, the Fraglets simulator is introduced. In order to explain such implementation tool, two points of view are possible. We explain both of them.

Last section, Sect. 1.3, describes the hierarchical layers of CNPs, from the very bottom one of the infrastructure, to the very top and abstract layer, dealing with mathematical tools for the dynamical behavior of CNPs. This top level represents the abstract collocation of our analysis and our contributions.

1.1 Communication Networks and a Chemical Metaphor

The general concept that guides CNP designers is to find new ways of organizing communication systems. The general approach changes: they inspire to biology and chemistry in order to create building blocks of communication protocols. They make use of natural rules to plan the logic of new program algorithms. To lead the reader from traditional networking to chemical networks, a short introduction of packet-based networks and active networks is required.

Packet-based networking is a digital communication method used to share a channel. Information is divided into sized packets. Each of them has a header that contains all the necessary
information for routing. Packets are sent individually through the network and then they are reassembled in the original order at the receiver. These networks allow the simultaneous multiple communications (routes) between sender and receiver. The concept of switching small blocks of data was first explored in the early 1960s. Packet switching optimizes the use of the channel capacity available in digital telecommunication networks such as computer networks. Moreover, this technique minimizes the transmission latency, and improves the robustness of communication. Packet switching is still widespread (i.e. internet and mobile technologies as the GPRS I-mode).

Active networking is a communication model that allows the dynamical modification of the system operation. Active network architecture is composed of active hardware that is capable of routing, switching and executing code within active packets. This communication pattern differs from the traditional network architecture: code as well as user data is conveyed in packets, allowing the data to change its form (code) and thus to match the channel characteristics. Note that, in traditional networking, the stability and the robustness are sought by attempting to remove complexity and flexibility [2].

Chemically-inspired networking has been proposed since the 1980s. The goal was to solve concurrent computations in a natural way [3]. Chemically-inspired computing takes a bottom-up approach. The basics are the natural evolution: selection, recombination or reproduction, mutation and adaptation. Simple rules that, over thousands of years, have produced remarkably complex organisms. Natural evolution can be seen as a decentralized system that often involves a set of simple rules and a set of simple organisms. After several generations of rule application, usually some forms of complex behavior arise. Complexity gets built upon complexity. Often, the final model is completely counterintuitive, but surprisingly efficient and temperate.

A conceptual metaphor from chemistry will characterize the whole thesis as well as it has belonged to the past work of CNP designers [1]. "Packet" becomes a synonym of "molecule". Actually, each molecule is represented by a string of symbols. A generic node of the network can be seen as a chemical vessel. A chemical vessel is an entity which contains a multiset of the set of all possible molecules [4]. Links between network nodes are modeled as specific chemical reactions, that consume molecules in the reactor vessel (sender node) and instantaneously produce molecules in the product vessel (receiver node). Note that, generally, a chemical reaction is an interaction among molecules that leads to the appearance or disappearance of molecules [4]. Being possible the comparisons molecules↔packets and links↔reactions,
1.1 Communication Networks and a Chemical Metaphor

the packet rate can be associated to the rate of these linking reactions. We can anticipate that, under particular conditions and following certain rules, there is a relationship between the reaction rate (or packet rate) and the concentration (number of molecules or number of packets). This concept will be deepened in Chap. 2 and Chap. 3. Concluding the chemical metaphor, a chemical communication network can be seen as a chemical reaction network and thus it can be studied, or even designed, using the tools of the well-known chemistry. The goal of CNP designers still remains the efficient processing of data packets on traditional computing/networking infrastructure. A computer, with a standard CPU where chemical reactions are simulated, represents the chemical virtual machine.

The communication aspect of CNPs is led by the fact that chemical virtual machines make use of the existing network infrastructure (i.e. internet protocol (IP), ethernet medium): the communication between remote nodes, even spatially separated, is possible.

1.1.1 Properties of Chemical Communication Networks

To make the similarity with the natural world stronger, CNPs pioneers have adopted several principles of chemistry, in that way getting profits in the design stage and in the CNP execution.

Molecules can contain code or/and data, thus there is no clear distinction between these two typologies of information. Namely there is no only-read code segment [5]. The chemical reactions in a vessel can involve both and thus CNPs allow changing the running code. This code can also be sent over the network, giving to the latter the properties of active networks.

In the adopted chemical execution model, code is not executed as fast as possible. Unlike in traditional networking protocols, when a code packet is received, it is scheduled for a later time. The reaction timing is defined by an implementation of a chemical rule: the "law of mass action", which will be introduced in Sect. 1.1.2. Moreover, there is no sequential execution of code. The adopted reaction algorithm randomly consumes molecules and thus the execution flow of reactions can not be predicted. This random process is based upon known stochastic principles, that allow the statistical expectation of the dynamical behavior of nodes [5].

Another difference from traditional protocols is how the network treats state information. Usually, state information is symbolically stored in packets as variables. Thus, the knowledge of the system state all over the network requires the transmission of these packets. In CNPs, the packet rate itself represents the state information of the system. The efficiency of the system is improved, since packets are not longer used to communicate the state information. Note that the latter is useless for the CNP user, state information is intrinsically essential
only for the system functioning. Moreover, the packet rate reflects the concentration of the reacting species. This information-coding scheme results in a higher robustness of networking protocols: when a molecule is removed (a packet is lost), the abundance (the concentration) of the respective species only changes by one, leading to a marginal change of the state information instead of a drastic loss of it.

The last benefit which is achieved by the use of chemical principles, deals with the new abstract procedure in design stage of chemical communication networks. Chemical models are exploited in order to have a forecast of the dynamical behavior of networks. Chemical dynamics and kinetics can help designers to find a specific chemical model which shows the desiderate behavior. Then, using the chemical metaphor, designers would reflect this chemical model into a real implementable CNP algorithm. The final result would be a network that performs its tasks as in traditional networking, but additionally, it would perform them in the desiderate and expected way.

1.1.2 Chemical Communication Model

In the same period of the introduction of chemical networking, artificial chemistry was proposed. The goal was to construct models able to describe complex chemical phenomena. Some of these chemistries have helped the evolution of computer programs as well as networking protocols, supplying very powerful computation models [6]. An artificial chemistry is formally defined as the triple $\mathcal{AC} = (\mathcal{S}, \mathcal{R}, \mathcal{A})$, where $\mathcal{S} = \{s_1, s_2, \ldots, s_n\}$ is the set of molecular species, $\mathcal{R} = \{r_1, r_2, \ldots, r_n\}$ is the set of reactions and $\mathcal{A}$ is the algorithm which defines the execution flow of reactions [7]. Each molecule in the vessel is an instance of the molecular species $s_i \in \mathcal{S}$. The $r_j \in \mathcal{R}$ expresses which reactant molecules collide and which ones are consequently generated:

$$r_j : \quad C_i + X_i \rightarrow C_i + X_l$$

The above reaction consumes a $C$-molecule and an $X$-molecule in node $i$, it produces a $C$-molecule again in node $i$ and it "sends" an $X$-molecule to the neighbor node, $l^1$. Of course, the reaction can happen only when at least two molecule are present in the reactant vessel. When this condition is satisfied the reaction is said "active", otherwise it is called "inert". The algorithm $\mathcal{A}$ defines the dynamical behavior of artificial chemistry. Usually, more than one reaction is active contemporaneously. The algorithm decides the number of reactions

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$^1$The reaction $r_j$ produced a $X$-molecule in node $l$. 

---
which are executable in parallel, and the order of their execution. Historically, several solutions have been proposed in order to calculate the timing of artificial chemistry. Most of them approximatively reflect real chemical models by the use of Differential Rate Equations (DREs) and basing upon the important condition of a system with a large number of molecules. The argument is definitely wide and it is still outside targets of this thesis. We only recall that the algorithm adopted in CNPs is a variant of the "Stochastic algorithm”, which has been proposed by Gillespie in 1977. The algorithm is named "Real-time next reaction method”. For details, please refer to [1]. The important feature of this algorithm is that it implements the "law of mass action”. This mathematical model explains and predicts the behavior of solutions in dynamic equilibrium. It can be seen from two points of view: the equilibrium aspect, concerning the composition of a reaction mixture at equilibrium, and the kinetic aspect, concerning the rate equations for elementary reactions. The law of mass action can describe systems only having a large number of molecules. In such systems the stochasticity of single behaviors can be approximatively replaced by a general trend of the species.

1.2 Fraglets Simulator

Fraglets is "a molecular biology inspired execution model for computer communications” [8]. We can see the Fraglets simulator under two points of view: as a string rewriting system and as an artificial chemistry [1].

A fraglet is a fragment of code or data which is represented by a string of symbols (i.e. [exch a b c d]). Fraglets interact among each other being driven by the symbols in the packet headers. Thus, it can be seen as a string rewriting system where the leftmost symbols identifies the rule to apply to the packet. In the previous example, the exch acts as a prefix command for the rest of the word a b c d. Specifically, this prefix changes the order of the original word, creating a string² [a c b d]. Each network node implements a multiset of fraglets, which are selected and processed according to the scheduling algorithm. Two rewriting rules are possible: transformation (rewriting a single fraglet into one or more fraglets) and synchronization (concatenating two fraglets together). Summerizing, fraglets can be seen as passive objects, where active rewriting rules operate, rooting these sized information into the Fraglets system.

Apart from the mere programming language aspect, Fraglets simulator represents an implementation of a distributed artificial chemistry [1]. Differently from the definition given in Sect. 1.1.2, the adjective "distributed” adds in the definition $\mathcal{AC} = (S, R, A)$ the network

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² Strings=Packets=Molecules.
graph $\mathcal{G} = (\mathcal{V}, \mathcal{E})$, leading to the more complete formal definition of a distributed artificial chemistry: $\text{ADC} = (\mathcal{G}, \mathcal{S}, \mathcal{R}, \mathcal{A})$. We can define $\mathcal{G}$ as the graph of network nodes running in Fraglets rewriting system; the set of molecules, $\mathcal{S}$, as the totality of possible words belonging to the symbol alphabet of Fraglets; the reaction set, $\mathcal{R}$, as the production rules of Fraglets simulator together with the fraglets (molecules) inside the network nodes (vessel); the reaction algorithm, $\mathcal{A}$, as the ”Real Time Next Reaction Method”, briefly introduced in the Sect. 1.1.2. We have said that the global distributed chemical network can be completely constituted by the Fraglets structure. Moreover, the nature of the adopted algorithm and its features, make the law of mass action valid even in such a simulation environment. Thus, the Fraglets simulator can be directly used to determine the dynamical behavior of the network, by analyzing the trend of the concentration (abundance) of fraglets\(^3\).

### 1.3 Chemical Network Hierarchical layers

Chemical communication networks convey user data inside molecules as strings of symbols. The information about the system state instead, is not explicitly stored and sent whereby packets, but it is implicitly represented by the reaction rate, namely the packet rate. Historically, network protocol designers have started their work from the tasks of incoming networks, not really caring about how the network would carry out these duties. Differently, in CNPs the chemical metaphor allows designers to start from the dynamical behavior of their future system. Only in a second stage, the designers will focus on the functional part of their protocols. They will attempt to find the right molecules (packets) which would implement the aimed chemical network.

The biggest advantage of the chemical networking theory is that it refers to standard and proper chemical reaction networks, that can be directly described by well-known mathematical tools. Then, this description can be used to study important communication properties like convergence, stability or responsivity. On the other hand, the biggest trouble of such an approach can be the difficulty of applying mathematical methods like the Chemical Master Equation (CME) or the Metabolic Congestion Analysis (MCA). The introduction, as well as the use, of mathematical methods for the dynamical behavior analysis is left for next chapters. Here instead, we provide a clear vision of the hierarchical engineering model: Fig. 1.1. This explanation should make clearer the abstract collocation of the subjects of next chapters.

\(^3\)In next chapters, the relationship between dynamical behavior, reaction rate and concentration will be treated deeply.
1.3 Chemical Network Hierarchical layers

![Diagram of Chemical Network Hierarchical layers]

**Figure 1.1:** The hierarchical engineering model. The light blue area constitutes the macroscopic level, while the blue area indicates the microscopic level. The contributions of this thesis belongs to the very top layer: "Mathematical Tools" for the analysis of CNP dynamics.

#### 1.3.1 Microscopic Model and Macroscopic Model

The most global vision of the chemical networking theory identifies two principal areas: the *microscopic* and the *macroscopic* execution models. Referring to Fig. 1.1, the microscopic model is the bottom blue area, while the macroscopic model is the top light blue area.

The *microscopic* description of the chemical networking theory deals with the look of virtual molecules. Namely, it cares about the features and the syntax structure of molecules (packets), in order to achieve the wanted dynamical behavior. The virtual molecule reaction process is performed on traditional computing infrastructure.

The interest of this thesis is focused on the other level: the *macroscopic* layer. In order to analyze and design chemical communication networks, we need to have a more abstract vision of the microscopic interaction among concrete molecules. We do not care about the specific molecule structure and thus, we are not interested in the specific algorithm which can implement a network. Only the dynamical behavior of networks is important. Our starting point will be the high level description of CNPs, in which the distributed chemical model will be exploited. Note that the use of this abstract chemical model directly supplies tools for the dynamics analysis. However, the difficulty of the solution of methods like the chemical master equation and the differential rate equations, hampers the work of CNPs designers.
A new upper layer in the macroscopic field is needed. This layer involves all mathematical tools useful for the approximation of the dynamics of distributed chemical reaction networks. Making use of the knowledge of other fields of study, we conclude the abstract treatment by the use of procedures as signal processing analysis, control theory or metabolic control analysis.

In order to make clear the analogy between the microscopic layer and the macroscopic model, we cite an example from [1] of this abstraction. Refer to Fig. 1.2 and Fig. 1.3. [X] molecules react with their [matchp X...] counterparts. Each reaction leads to a regeneration of
1.4 Summary

This first chapter is definitely required in order to get into the argument of this thesis. Here, we have briefly explained what a Chemically-inspired networking is and we have cited differences and analogies with other communication techniques (i.e. adaptivity to channel characteristics). Moreover, the communication system-chemical network metaphor should be clear now. In the next chapters of this thesis, we will indistinctly use terms of both telecommunications and chemistry. Molecules $\leftrightarrow$ packets; chemical vessel $\leftrightarrow$ network nodes; chemical reaction $\leftrightarrow$ network link; reaction speed $\leftrightarrow$ packet rate; chemical reaction network $\leftrightarrow$ communication network; chemical virtual machine $\leftrightarrow$ computer, CPU. The reader should have all given definitions clear, before proceeding further.

The chemical model (artificial chemistry) have helped us to give a more formal definition of what has been verbally introduced in Sect. 1.1. Beside the chemical model, the law of mass action has been given. This milestone of chemical kinetics drives the dynamical behavior of distributed chemical models and thus, it drives chemical communication systems themselves. Note that many logical passages that have been done by CNP pioneers, as well as some of our considerations, are based upon the condition of the law of mass action validity.

Actually, CNPs can be implemented using the Fraglets simulator, which has been presented in this chapter. Probably, only the vision of Fraglets simulator as an artificial chemistry implementation is important for our purpose. We use such a tool as a validation of our results. Indeed, we will frequency characterize several networks with new methods and then, starting from the frequency domain analysis, we will give the dynamical time-behavior. We use the Fraglets simulator to observe the dynamical time-behavior of a real implementation of a CNP, which is then compared with theoretical expectations.

An explanation of the hierarchical layers of chemical communication networking, Sect. 1.3, should has made clear the abstract position of network elements, models, rules and mathe-

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4 Transmission chemically means the consumption of a molecule in a vessel and the instant production of a molecule of the same species in another vessel

5 Not all the chemical reactions can be seen as a link between two neighbor nodes in a telecommunication network.
mational tools for the analysis of CNPs. The contribution of this thesis belongs to the very
top layer of the hierarchical structure tower$^6$: the dynamical behavior description of CNPs,
by the use of frequency domain analysis and all its related tools.

$^6$Refer to Fig. 1.1.
Chapter 2

Examples of Chemical Communication Protocols

This chapter helps to better understand the virtual chemical world which has been introduced in Chap. 1. Three actual chemical communication protocols are here introduced. The first is the "Disperser" protocol, in Sect. 2.1. It is an existing protocol which will represent a good example for the frequency analysis in Chap. 3. In Chap. 4, we will demonstrate that the structure of the "Disperser" can be built basing upon almost all the elementary blocks that are specifically described by us (in Chap. 4). The second example is the "Quine", a pilaster of chemical reaction networks. This structure, which is shown in Sect. 2.2, allows chemical networks to have the self-healing feature. The Quine scheme is constituted by a non-linear reaction network, thus it is a new topology of network which will require a special analysis procedure in Chap. 4. Another non-linear reaction network is the realization of a congestion avoidance protocol. Probably, the subject of Sect. 2.3 represents the most interesting protocol for mere Communications. Moreover, this structure is the chemical protocol where this thesis will give the most important contribution (Chap. 4), allowing to opportunely calibrate network coefficients in order to achieve the wanted network responses.

Features and targets of these three protocols are defined. Note that this chapter reports the findings of others [9] [5]. The final goal of this thesis is to supply an analysis tool that helps in the network design stage. The Disperser, the Quine and the congestion control avoidance protocol are actually the expression of our contribute and they represent the purpose of the whole project. Their dynamical behavior will be analyzed in Chap. 4.
2.1 Chemical Disperser Protocol

The general goal was to create a robust chemical networking protocol (CNP), which calculates the average of values in a distributed way. The distribution is important: a centralized scheme requires a complete knowledge of the state system. However, the transmission of such a knowledge to each network nodes could often represent a severe resource waste.

Basing upon such concepts, chemical network designers have implemented the Disperser protocol [5]: Fig. 2.1. Fig. 2.1 shows an undirect graph $G = (V, E)$, where $V$ is the set of nodes and $E$ is the set of links. Each nodes $n \in V$ contains a set of molecule species. The concentration of the $X_i$ molecule species represents the computed average. $X_i$-species concentration is initially set to the local value $x_{i0}$. This means that an initial amount of $X_i$-molecules\(^1\) are injected inside node $i^{th}$. We know that the Fraglets simulator requires only bi-molecular reactions. Thus, in order to implement the chemical network on such a simulator, for each link $(i,k) \in E$ a single molecule of the $C_{ik}$-species is placed. $C_{ik}$-species can be seen as a possibility to have a controllable reaction coefficient. Since the protocol relies on a kind of law of mass action [5], the reaction rate results directly proportional to the reactant concentration. Namely, reaction $r_{ik}$ can be speeded up by increasing the number of $C_{ik}$-molecules.

The general chemical reaction equation can be written as

$$r_i : \quad C_{ik} + X_i \rightarrow C_{ik} + X_k \quad \forall (i,k) \in E$$

(2.1)

Verbally, for each link $(i,k) \in E$ there is a single molecule of the $C_{ik}$-species that reacts with one molecule of the $X_i$-species. In that way, the molecule of the $X_i$-species is sent

\(^1\)Molecules $\equiv$ packets.
2.1 Chemical Disperser Protocol

Figure 2.2: The Disperser protocol for a four-nodes network.

Figure 2.3: Results of a four-nodes Disperser protocol, obtained in Fraglets environment. After the injection of 1000 molecules in node 1, the values of node concentrations asymptotically converge to the average of 250 molecules.
to the neighbor node, \( k \)-node. The global reaction network reaches an equilibrium. In such a stable state, all nodes contain the average concentration of \( X \)-species. The term ”stable” is related to the fact that the system returns back to the fixed point after a small perturbation [5]. Another characteristic that should be explained better, is the robustness of the Disperser protocol. Initially, we have cited such a feature as a goal that has led designers to project this chemical network. Actually, the Disperser protocol is not resilient to packet loss. However, a packet loss in Disperser CNP only decreases the value of the average by one. Since molecule concentrations usually have high values, they are definitely not comparable with such a decrease of the average information. This is the nature of the robustness of the Disperser.

A specific realization of the Disperser protocol is shown in Fig. 2.2. The related set of reactions is

\[
\begin{align*}
r_{12} & : C_{12} + X_1 \rightarrow C_{12} + X_2 \\
r_{21} & : C_{21} + X_2 \rightarrow C_{21} + X_1 \\
r_{23} & : C_{23} + X_2 \rightarrow C_{23} + X_3 \\
r_{32} & : C_{32} + X_3 \rightarrow C_{32} + X_2 \\
r_{24} & : C_{24} + X_2 \rightarrow C_{24} + X_4 \\
r_{42} & : C_{42} + X_4 \rightarrow C_{42} + X_2 \\
r_{34} & : C_{34} + X_3 \rightarrow C_{34} + X_4 \\
r_{43} & : C_{43} + X_4 \rightarrow C_{43} + X_3
\end{align*}
\] (2.2)

As Fig. 2.3 shows, at equilibrium, the values of the concentration in all nodes have reached the average of the initial value, \( x_{10} = 1000 \), over the number of Disperser nodes, \( N_{\text{node}} \):

\[
<x> = \frac{x_{10}}{N_{\text{node}}} = \frac{1000}{4} = 250
\]

### 2.2 A Building Block for Self-Healing Protocols

Chemical communication networks can show a self-healing property [5]. Usually communication networks suffer of packet loss. While the loss of a user data packet effects only in a missing information at the receiver, the loss of a code packets can create irreparable problems that compromise the whole system work. Most of communication networks treat these two typologies of packets in a different way. In chemical networking protocols there is no such a distinction: molecules can contain either code \( or \) and data. Being so, the communication system is less static. It allows changing its behavior and thus its features, depending on the
current condition. The code in a node can be changed or created by a remote vertex in the communication network.

### 2.2.1 The Quine

The basic mechanism of the Quine network is essentially a homeostasis. In Fraglets it is possible to have a set of ”code molecules” that react and actually regenerate themselves [5]. Each regenerating reaction can even produce more copies at a time. Considering the law of mass action, we understand that the number of molecules inside the vessel would increase in time. However, this chemical pot has a maximum number of containable molecules. For that reason, a ”dilution flow” is placed, granting in this way a certain maximum capacity of the vessel. The reaction set related to the chemical network in Fig. 2.4 is

\[
\begin{align*}
    r_1 & : C_1 + C_2 \xrightarrow{k_2} C_3 + \text{Output} \\
    r_2 & : C_3 + C_4 \xrightarrow{k_3} 2C_2 + 2C_4
\end{align*}
\]

(2.3)

Molecules (data and/or code packets) inside the vessel can be lost or, even worse, corrupted. The first event is simply solved by the molecule regeneration which has been described. Note that not all kind of molecule species, inside the vessel, experience this regeneration. When the molecules are modified instead of simply lost, they are not regenerated by the Quine, since they are not part of the self-replicating set of molecules. The number of corrupted molecules of the same species remains the same, while the concentration of the original molecules increases hyperbolically in time [5]. Because of the regenerating reaction, \( r_2 \) in (2.3), produces two
copies of $C_2$-species and two copies of $C_4$-species for each consumed molecule of $C_3$-species and $C_4$-species. The dilution flow is non-selective: when the total number of molecules in the vessel exceeds the maximum capacity, the dilution flow randomly destroys molecules [5]. Doing so, both corrupted and original packets are deleted, but only the latter are regenerated. Like in nature, the surviving population can grow and reach the vessel capacity. This concept represents the self-repairing feature of the Quine network. No external observer is required. The system intrinsically controls itself. When a packet destruction or a packet corruption happens, after a transition time, the system reaches the stable state again.

Note that $C_2$-species, $C_3$-species and $C_4$-species are placed only to grant the self-healing feature. The chemical network in Fig. 2.4 should behave like the "elementary chemical reaction" in Fig. 2.5. However, we should remember that Fraglets simulator requires bimolecular reactions. Thus, the elementary reaction is not directly implementable. The presence of $C_{ij}$-species like in Fig. 2.1 could compromise the self-healing feature of the network: a loss of such a molecule species would modify the reaction rate. Both the "elementary reaction" and the "Quine" will be analyzed in detail in Chap. 4, and specifically in Sect. 4.5.2 we will prove the similarity between these two networks.

### 2.3 Congestion Avoidance

The general goal of a congestion avoidance scheme is to allow more users to share a common channel, always caring about the fairness and the efficiency (Fig. 2.6). Differently from the traditional congestion control algorithms, congestion avoidance mechanisms prevent congested states, instead of repairing them. Anyway, both of these algorithm typologies essentially deal with resource management. Going more deeply inside the argument, we can refer to the graph in Fig. 2.7 [10]. The point at which packets start to get lost is named "cliff". Of course, throughput falls rapidly after this point: the network is congested. "Knee" refers
2.3 Congestion Avoidance

![Diagram](image.png)

**Figure 2.6:** *The general model of a communication system dealing with the congestion avoidance. Note that the CNP in Fig. 2.9 can be found here and isolated (yellow circle).*

...to the point after which the increase of the throughput is small. Note that between the *knee* and the *cliff* there is the maximum growth of time response. Congestion control algorithms work around the *cliff*, while protocols dealing with the avoidance should oscillate around the *knee* [10].

The system should feel its state and then feed this state back to its users, who should review their output rate in order to prevent the congested situation. The state of the network is directly related to the packet rate and thus, the congestion state is determined by the packet rate and the capacity of the communication channel. Note that we are analyzing the sharing by several users of the same communication channel which has a limited bandwidth. In order to implement the congestion avoidance, the "selective feedback" is suggested [10]. This mechanism allows the network to monitor the users and then to enforce them different behaviors (output rates). This should mean working efficiently around the knee.

There are four key criteria to follow when designing a congestion avoidance protocol. Briefly:

- **Efficiency:** the closeness of the total load on the resource to its knee.
- **Fairness:** users in equivalent classes should equally share the same communication channel.
- **Distributiveness:** the centralization requires a complete knowledge of system state and conveying the needed information means to waste resources. The system should do the minimum amount of sufficient feedback.
- **Convergence:** generally measured by the system speed to approach any target state from any starting one. The time to reach this "stable" state is called *responsiveness*, while the size of oscillations around the stable state is named *smoothness*. 
Figure 2.7: Performances of the network as a function of the load. (Dashed curves indicate deterministic service).

Figure 2.8: Responsiveness and smoothness.
In order to guarantee the first criteria, the system should be negative fed back: if the system "asks" users to decrease their traffic, the total load will not increase and viceversa, if it is asked to increase the user rate, the total load will not decrease. Note that the negative feedback guarantees that the system oscillates around the efficiency point, but it does not say anything about the size of the oscillation [10].

A possible chemical network to implement a congestion avoidance protocol is shown in Fig. 2.9. The simplest situation is represented by only two users that are sharing a channel with a limited capacity $R$. The throughput of the first user is $V'_{out}$, while the throughput of the other user is $V_{in}$. (Note that the simplicity of this network does not reduce the generality of this representation: more other users can share the channel, their effect can be considered as a total output rate which equals $V_{in}$.) The effective output rate of the communication system is indicated with $V_{out}$, which must respect the capacity limitation of the channel. The selective feedbacks are constituted by the two back reactions with the ratio terms $V'_{out}$ and $V_{in}/V$. Let us analyze semantically the word "selective feedback". "Feed" because this two reactions are driven by the output rates of the two users and being so, they monitor the system state. (Note that generally for a congestion avoidance protocol, the system state is the packet rate. In this chemical networking protocol the packet rate is the chemical reaction rate and, basing upon the law of mass action, this rate is directly proportional to the concentration of the species.) The term "back" is justified by the fact that these two reactions have an opposite direction comparing to the communication flow. Finally, "selective" because a user is forced to modify its rate by an amount different from the one of the other user. The ratio terms, which characterize these two reactions, are different from a user to the other. For example
referring to the first user, the feedback bases upon the difference between the total demanded resource, \( V \), and the available resource, \( R \), and upon the relative resource request of the user itself, \( \frac{V'}{V} \). Similar observations can be done for the second user.

In Chap. 4, we will be able to trim the reaction coefficients, \( k_1, k_2, k_3 \) and \( k_4 \), of the chemical network in Fig. 2.9, in order to obtain similar graphs to the general one which is shown in Fig. 2.8. This is probably the most important result of this thesis, which can be directly applied to telecommunication purpose (Fig. 2.6).

### 2.4 Summary

This chapter has supplied three examples of CNPs, giving a concrete implementation of the theory in Chap. 1. The "Disperser" protocol and the "Quine" were already implemented and so the reaction coefficients, as well as their network structure, was already fixed [5]. Chapter 4 will help for the frequency analysis of these protocols and it will confirm some suppositions of CNPs designers. On the other hand, the "congestion control" chemical protocol is still in a design stage. Our contribution will be important for the definition of the network structure and for the calibration of the reaction coefficients.

Moreover, the "Disperser" protocol will be meaningful for contributions of this thesis, being a direct application of our first own results. Indeed, it will represent an excellent educative and illustrative model on which the new analysis tools of Chap. 3 could be easily applied.
Chapter 3

Analysis and Mathematical Tools

The first two chapters have been spent to generally approach chemical communication networks and we have understood that this kind of networks allow a new design procedure. The forecast of their dynamical behaviors is the pilaster of this new way of engineering. The basic philosophy of this designing-time analysis is preventing critical events and crucial losses, instead of repairing. Thus, chemical network designers need to know the time (or frequency) behavior of the chemical communication protocol. With this knowledge they would be able to modify the reaction network according to what analysis results suggest.

The first step to do is to evaluate the dynamical behavior of CNPs. There are many methods, more or less complicate, rather than more or less rigorous.

The first section, specifically composed by Sect. 3.1.1 and Sect. 3.1.2, treats the theory part which is strictly related to chemistry. In order to better understand the function and the motivation of the frequency analysis, the Chemical Master Equation (CME) and Differential Rate Equation (DRE) approximation are briefly explained. Due to the complexity of this argumentation, this part occupies several pages which are rich of mathematical equations and definitions. However, the reader will not really need to fully understand and remember Sect. 3.1. The rest of the thesis can be as well appreciated, even going directly to Sect. 3.2. After an introduction and a motivation of the frequency domain analysis (in Sect. 3.2.1), the state-variable description of a linear-time-invariant system is given in Sect. 3.2.2. The mathematical solution of such a description requires the use of the Laplace transform, which is formalized in Sect. 3.2.3. Section 3.2.4 supplies a graphical representation that fully describes the dynamical behavior of a system under the frequency point of view: Bode diagrams. The last part, related to the theory of frequency domain analysis, is Sect. 3.2.5 that explains the metabolic control analysis (MCA). We think that nowadays the MCA represents the best tool to study non-linear chemical communication networks. Even if the MCA gives only an approximation of the dynamical behavior of these complicated systems, it
definitely gains reasonable results with reasonable analysis work. The last section, Sect. 3.3, is
dedicated to introduce the sophisticated background theory of the intrinsic noise of chemical
communication networks. Note that the whole chapter is based on theorems and basics,
which have been already discovered and demonstrated by others. However, we combine such
studies together, supplying a practical and complete knowledge of the theory requested for
CNP analysis.

3.1 Chemical Kinetics and Dynamics

The kinetics is the branch of chemistry that deals with the dynamical evolution of reaction
systems. In chemistry, this knowledge has an extreme importance: the reaction speed gives
the indication to correctly trim the chemical reactors.

First inherent studies were done by F. Wilhelmy, in 1850: the inversion reaction of sucrose
into glucose and fructose. Later, 1863, C. Guldberg and P. Waage highlighted the importance
of the dynamical nature of the chemical equilibrium. Before the end of the 19th century, the
relationship between reaction coefficients, concentrations and reaction speeds was valorized
and partially known\footnote{“Law of mass action”. Please refer to Sect. 3.1.2.}.

Generally, the reaction speed depends upon species concentrations in the reaction system.
Reasonably, in a mono-molecular process, as \( x \rightarrow y + z \), the reaction speed should be
proportional to the reactant concentration: \( kc_x \), where \( k \) is a constant and \( c_x \) is the \( x \)-species concentration. Instead, in a bi-molecular process, as \( x + y \rightarrow a + b \), the reaction speed
should be proportional to the collision frequency of the two species: \( kc_x c_y \), again where \( k \) is
a constant, \( c_x \) is the \( x \)-species concentration and \( c_y \) is the \( y \)-species concentration. We have
now understood what we should expect for higher order reactions.

In scientific literature, studies about dynamical behavior of several chemical systems are
already present. Definitely, rigorous analysis methods are available nowadays. Nevertheless,
we need to understand how to adapt these well-known procedures to chemical networking
protocols. We would like to combine the knowledge from different scientific fields and find a
way to analyze the dynamical behavior of networks. Of course, our intent is to spend as less
time as possible for dynamical analysis, even accepting reasonable approximations.
3.1 Chemical Kinetics and Dynamics

3.1.1 Chemical Master Equation and Markov Process

Chemical communication networks are based upon the distributed artificial chemistry model. This model is driven by the stochastic reaction algorithm and, for this reason, it can be seen as a continuous-time discrete-space Markov jump process [1]. Species concentrations can be seen as the state of the system and thus a vector, $\mathbf{X}(t)$, of these random variables can fully describe the system itself. Concentration $X_{s_i}(t)$ of a species, $s_i$, at a specific time, $t$, are the random elements of this vector.

Other important elements are the stoichiometric reactant and product vectors, $\chi_{r_j}$ and $\xi_{r_j}$, where elements, $\chi_{s_i,r_j}$ and $\xi_{s_i,r_j}$, are the number of consumed and produced molecules respectively in the species $s_i$ with the reaction $r_j$. Note that a generic reaction, $r_j$, can be seen as a transition of the system states:

$$r_j : \sum_{i=1}^{N_s} \chi_{s_i,r_j} \cdot s_i \xrightarrow{k_j} \sum_{i=1}^{N_s} \xi_{s_i,r_j} \cdot s_i.$$  

(3.1)

where $N_s$ is the number of the species and $k_j$ is the reaction coefficient which defines the reaction speed. Let us give an explicative example: for the "basic loop reaction network with no inflows and no outflows" in Fig. 3.1, the elementary set of reactions $r$ can be written as

$$\left( \begin{array}{c} r_1 \\ r_2 \end{array} \right) : \left( \begin{array}{c} s_x \\ s_y \end{array} \right) \xrightarrow{k_1} \left( \begin{array}{c} s_y \\ s_x \end{array} \right) \xrightarrow{k_2} \left( \begin{array}{c} s_x \\ s_y \end{array} \right)$$  

(3.2)

The probability for a reaction, $r_j$, to occur in the next infinitesimal period $[t,t+dt)$, is named propensity function $a_{r_j}(\mathbf{X})$. The propensity function depends on the concentration vector, $\mathbf{X}$, and on the reaction coefficient, $k_j$.

$$a_r(\mathbf{X}) = k_j \prod_{i=1}^{N_s} X_{s_i}^{\chi_{s_i,r_j}}.$$  

(3.3)

Note that this reaction probability can be compared to the transmission rate in a Markov process [1]. A consequence of this Markov property is the chemical master equation (CME).
The CME accurately governs the dynamics of the system probability distribution in a chemical reaction network. Today, this complex chemical equation is the only available tool to fully describe the stochastic dynamical behavior of chemical communication networks. From the propensity vector, the CME can be written [11], [12]:

$$\frac{\partial}{\partial t} P[X(t) = x | X(t_0) = z_0] = \sum_{j=1}^{N_r} \left( a_{r_j} (x - \psi_{r_j}) P[x - \psi_{r_j} | x_0] - a_{r_j} (x) P[x | x_0] \right)$$

(3.4)

where $\psi_{r_j}$ is the difference between the stoichiometric product vector and the stoichiometric reactant one: $\psi_{r_j} = \xi_{r_j} - \chi_{r_j}$. $N_r$ is the number of reactions in the system, $P[X(t) = x | X(t_0) = z_0]$ is the probability to be at the system state $x$ at the time $t$ and the term inside the sum is the difference between the probability to reach the state $x$ with reaction $r_j$ and the probability to leave the state $x$ with reaction $r_j$. Of course everything is referred to specific initial conditions, $z_0$ [13]. Actually, the CME is only exact under the conditions that the system is well-stirred and in thermal equilibrium [12].

Let us understand what these mathematical definitions and tools mean, by applying them to the example in Fig. 3.1: the "basic loop reaction network with no inflows and no outflows". The reaction set, $v$, has two elements: the forward reaction $r_1 = X \xrightarrow{k_1} Y$ and the backward reaction $r_2 = Y \xrightarrow{k_2} X$. This chemical system with no inflows and no outflows is closed (referring to the total number of molecules): the number of molecules is here conserved. Thus, supposing a finite value of initial species population as initial conditions, the Markov jump process is finite. If we fix an initial condition of only 3 molecules in the reaction network, the number of states of the Markov chain will be 4. The CME, which describes this system, results really simplified for this very elementary example:

$$\frac{\partial}{\partial t} p(t) = p(t) \cdot Q$$

(3.5)

where $p(t)$ is a row vector of probabilities which are referred to a specific $i^{th}$ value of all states. It is defined as $p_i(t) = P[X(t) = x_i | X(t_0) = x_{i0}]$. $Q$ is the transition rate matrix of the system, that, generally for $N+1$ states of the system, has dimensions $(N+1) \times (N+1)$.

$^2$This $Q$ form is valid only for such a specific loop reaction system. The generalization is referred to the number of states, $N + 1$, namely the total number of system molecules, $N$. 

Figure 3.2: The equivalent Markov chain of the "basic loop reaction network with no inflows and no outflows".

and the following form:

\[
Q = \begin{pmatrix}
-N & N & 0 & \ldots & 0 \\
1 & -N & N-1 & \ddots & \\
0 & 2 & \ddots & \ddots & 0 \\
\vdots & \ddots & \ddots & -N & 1 \\
0 & \ldots & 0 & N & -N
\end{pmatrix}
\]

In our example \( Q \) is simply a 4x4 matrix:

\[
Q = \begin{pmatrix}
-3 & 3 & 0 & 0 \\
1 & -3 & 2 & 0 \\
0 & 2 & -3 & 1 \\
0 & 0 & 3 & -3
\end{pmatrix}
\]

The general solution is

\[
p(t) = p_0 e^{Qt}
\]

Note that the stationary probability distribution, \( p_{st} = p(t)|_{t \to 0} \), satisfies \( p_{st} \cdot Q = 0 \), under the condition that the sum of all probabilities (to get into a state or to leave it) must be unitary: \( \sum_{i=1}^{N+1} p_{st}^i = 1 \). Of course, being \( p_{st}^i \) a generic probability, it must have also a positive or null value, \( p_{st}^i \geq 0 \). Under these two conditions, (3.6) has a solution. Completing our example:

\[
\begin{pmatrix}
1/8 \\
3/8 \\
3/8 \\
1/8
\end{pmatrix}
\]
The stationary probability distribution is shown in Fig. 3.3. For any linear first order reaction networks, the CME is solvable [14]. However, (3.4) suffers from the known curse of dimensionality: each species adds one dimension to the problem leading to an exponential growth of the computational complexity. For instance, if we would analyze a system of 3 species, having each between 1 and 100 molecules, the CME will contain $100^3$ coupled ordinary differential equations (ODEs). Actually, one ODE per state. In addition, for reaction systems with higher than second order, the solution of CME is not even possible to achieve.

### 3.1.2 Differential Equation Approximation

Due to the mathematical complexity of finding the CME solution, the common deterministic model of Differential Reaction-rate Equations (DREs) is often exploited. This model only approximates the exact dynamical behavior of a chemical reaction network, but, it extremely reduces the computational work of the analysis. The DREs is a set of D Ordinary Differential Equations (ODEs) that, as we have anticipated, approximate the expected values of the concentrations of the species in the system. The term "deterministic" refers to the loss of the stochasticity in the problem. Moreover, it is related to the fact that initial conditions determine the future evolution of ODEs. When the system parameters are known, for a specific initial condition, the system dynamics can be evaluated. There are several possibilities to proceed: the direct analytical solution of the ODEs (not always possible), the numerical integration based on the Cauchy’s problem, the Euler’s method, the Runge-Kutta method and other implicit or explicit methods.

Deterministic approximations of the ODEs represent a macroscopic approach to chemical
systems. These systems should have high concentrations of involved species in order to show a predictable (evolutonal) behavior. Actually, the approximation is based on the high number of molecules for each species, that dampens the randomness of molecular iterations. This approach deals with the mean of the system time evolution, namely averaging the dynamical behavior of the same network for different trials\(^3\). The empirical "law of mass action" is the basic motivation of such an approximation. This fundamental law of chemical kinetics was enunciated nearly hundred years before the discovery of the CME [15], [16]. It states that, in a free and homogeneous medium, the reaction speed is proportional to the concentration of the involved reactants. The more molecules in the same volume are, the more likely collisions and reactions occur [1].

After this verbal argumentation of the CME solution with the ODEs approximation, let us give some mathematical explanation and definitions. The time derivative of the species concentration vector can be deduced from the CME (3.4) [17]:

\[
\frac{\partial}{\partial t} \mathbb{E} \left[ X_{s_i}(t) \right] = \sum_{j=1}^{N_r} \left( \psi_{s_i,r_j} \cdot a_r(\mathbb{E} [\mathbf{X}]) \right) \quad (\forall i \subset S) \tag{3.8}
\]

where \( S \) is the set of molecule species. Defining the \( N_s \times N_r \) stoichiometric matrix as \( \Psi = [\psi_{s,r_j}] \), we can write (3.8) in matrix notation [1]:

\[
\frac{\partial}{\partial t} \mathbb{E} [\mathbf{X}(t)] = \Psi a_r(\mathbb{E} [\mathbf{X}]) \tag{3.9}
\]

where \( a_{r_j}(\mathbf{X}) \) is the dimensional propensity vector with dimension \( N_r \). The reaction rate equation is yielded by the fact that our interest is the average behavior of the chemical communication network [1]:

\[
\frac{\partial}{\partial t} \mathbb{E} [\mathbf{z}(t)] = \Psi a(\mathbb{E} [\mathbf{z}]) \tag{3.10}
\]

where the species population \( X_i(t) \) is substituted by its expectation: \( c_i(t) = \mathbb{E} [X_i(t)] \) Equation (3.10) can be written in the shorter notation

\[
\dot{\mathbb{E}} = \Psi \cdot a \tag{3.11}
\]

For example, referring to the reaction system in Fig. 3.1, the deterministic approximation which describes its dynamical behavior, consists two coupled ODEs:

\[
\begin{cases}
\dot{c}_x = -k_1 \cdot c_x + k_2 \cdot c_y \\
\dot{c}_y = +k_1 \cdot c_x - k_2 \cdot c_y
\end{cases} \tag{3.12}
\]

\(^3\)The mean should be evaluated on all the realizations of this stochastic process.
The use of the deterministic approximation to describe the dynamical behavior of a (artificial) chemical network, extinguishes the stochasticity of the problem. In the end, the expected number of molecules \(c_s(t)\) of species \(s\) at time \(t\) is known. Note that the exact discrete random variable \(X_s(t)\), which changes according to propensity functions\(^4\), still remains unknown. The advantage of the use of the ODEs is the reduction of the complexity or namely, the computational work: the dimension of the problem grows polynomially, instead of exponentially as it occurs with the rigorous CME method. There are, however, some systems where reaction-rate equations fail to reproduce the actual behavior [18]. Biological systems inside living cells can be an example. Frequently, they consist of species with few instances [19] and therefore, stochastic effects are more pronounced.

### 3.2 Frequency Domain Analysis

The *frequency domain analysis* has been the main and, probably, the new point of view to generally observe basic reactions and complex reaction systems. Furthermore, *signal processing* and *system control* theories are based nearly completely on frequency transforms and transfer functions.

A signal can be seen as a set of frequency components. Depending on what its nature is, a higher or a lower number of these components can be counted. All network entities are characterized by how they behave with signals, specifically their frequency components. This characterization consists of extrapolating the relationship between the input and the output. Of course, a solicitation must be applied to the input and there should be a time variation of the system response.

The main advantage of the use of this theory is the generality of results. As we have said, all signals are defined by a frequency transform and all network blocks are characterized by a transfer function. The prediction of the network behavior does not need always a specific computation for all different possible inputs. Moreover, under specific conditions, the frequency description of the same typology of network portions is always the same and thus, it can be used in all cases where the same network portion is distinguishable.

The use of system control and signal processing theory is not limited to electronics and telecommunications, but it is more and more spread even in biology and chemistry. Analogous to electrical circuits, chemical systems can be thought of having blocks and signals. Signals can be either internally or externally generated. Blocks can count one or more reactions and, with different typologies of interconnections, they globally constitute the whole chemical

\(^4\)State transition.
reaction network. Our goal is to characterize the latter under the frequency point of view, to understand how each "chemical device" treats and elaborates these signals and finally to foresee the effect of the global chemical network on them.

Frequency analysis allows having more "observation dynamic": all signal changes are detectable by specific frequency components. For the same target, referring to the magnitude axis, the logarithmical representation is often used. We should finally remember that frequency domain analysis does not restrict our vision: the time trend of a signal, as well as the time behavior of a system, can always be found by the anti-transformation from one domain to the other.

### 3.2.1 Motivation and Importance of Frequency Analysis

In CNPs, system state information are based upon species concentrations (refer to Chap. 1 and Chap. 2). The concentration of a species is the abundance of molecules (packets) in such a species and, we now know that, the reaction speed is proportional to the concentration itself. The reaction rate in turn, is the speed with which molecules are consumed in the reactant species and instantaneously produced in the neighbor product species. Note the difference between the reaction rate\(^5\), that namely is the concentration, and the variation of the concentration, which actually is the change of the reaction rate. Going through this thesis, the analysis will be oriented to find how frequencies are treated and what components characterize the input, the output and species of the network. The term "frequency" will be referred to the change of concentration and thus to the variation of the reaction (packet) rate, not to the packet rate itself.

The species concentration is the signal which will be analyzed. Let us clarify its features in order to understand what tools are available and how to apply them. The concentration of a species is a time-continuous discrete-valued signal, since the concentration can have only a unitary increment or decrement: just one molecule can be consumed at a time. The consumption of molecules happens according to the law of mass action. Note that even for a chemical communication network this law governs the system [8]. Caring about what CME states, the species concentration should be considered a stochastic process. Nevertheless, we have inquired about the real necessity of such an accurate knowledge. At the moment, the result of ordinary differential rate-equation approximation is sufficient to generally foresee the network behavior and thus to better design it. Since this approximated procedure refers to the mean of the stochastic process, the concentration can be considered a deterministic

---

\(^5\)The concentration equals to the reaction rate, which is equivalent to the packet rate.
continuous signal. The normal frequency transform can be applied. Another interesting application of the signal and/or the information theory could be the characterization of CNPs even caring about the intrinsic stochasticity of involved signals. Making the noise and the deterministic signal both detectable would be a very interesting argument. With the help of the information theory, the amount of information would be valued and the negative noise contribution would be estimated. However, the treatment of such a complex and wide argument requires time and researching work, even going deeply inside mathematical argumentation. In this thesis only an introduction to the problem will be given, leaving an open way for further detailed studies.

3.2.2 State Variables Description of LTI System

We have seen that the dynamical behavior of chemical reaction networks can be approximatively described by a system of ODEs. Under the linearity condition, this system can be expressed using the implicit form

\[
\begin{align*}
\dot{x}(t) &= A \cdot x(t) + B \cdot u(t) \\
y(t) &= O \cdot x(t) + D \cdot u(t)
\end{align*}
\] (3.13)

where \(x(t)\) is the vector of unknown functions that, for our purpose, are expressions of species concentrations of the chemical network. Each element of this vector is named state variable of the system. Note that mathematically, the minimum number of these variables is sufficient to determine the system state at a generic time \(t\), when \(x(t)\) is known at the initial time \(t_0\). Specifically, elements of \(x(t_0)\) are the initial conditions. The vector \(u(t)\) is the solicitation on the system from the outside. Let us define the matrixes:

- \(A\) is the \(n_x \times n_x\) named state matrix
- \(B\) is the \(n_x \times n_u\) named input matrix
$\mathbf{O}$ is the $n_y \times n_x$ named output matrix

$\mathbf{D}$ is the $n_y \times n_u$ named direct transmission matrix

Note that this definition requires the time-invariance of the system: all matrix coefficients must be time independent. Referring to the system state as a time function [20], [21]:

$$\mathbf{x}(t) = e^{\mathbf{A}(t-t_0)} \cdot \mathbf{x}(t_0) + \int_{t_0}^{t} e^{\mathbf{A}(t-k)} \cdot \mathbf{B} \cdot u(k) \, dk \quad (3.14)$$

where the transition matrix of the linear system is recognizable:

$$\phi(t) = e^{\mathbf{A}(t-t_0)} \quad (3.15)$$

The system output is

$$\mathbf{y} = \mathbf{O} e^{\mathbf{A}(t-t_0)} \mathbf{x}(t_0) + \mathbf{O} \int_{t_0}^{t} e^{\mathbf{A}(t-k)} \cdot \mathbf{B} \cdot u(k) \, dk + \mathbf{D} \cdot u(t) \quad (3.16)$$

Steady states\(^6\) of a linear system are solutions to equation $\mathbf{A} \cdot \mathbf{x} + \mathbf{B} \cdot \mathbf{u} = 0$, when the input is constant [21].

If the state matrix, $\mathbf{A}$, is invertible, then the equilibrium state will be unique and it will equal the state and the output:

$$\begin{cases} 
\mathbf{x}_s = -\mathbf{A}^{-1} \mathbf{B} \cdot \mathbf{u} \\
\mathbf{y}_s = (\mathbf{O} \cdot \mathbf{A}^{-1} \mathbf{B} + \mathbf{D}) \mathbf{u}
\end{cases} \quad (3.17)$$

The matrix $(\mathbf{D} - \mathbf{O} \cdot \mathbf{A}^{-1} \mathbf{B})$ is the static gain. It represents the ratio between the input and the output when input, state, and system variables are constant. The difficulty of the system state-variables analysis is the valuation of the transition matrix (3.15). The Laplace transform, which will be explained in the next subsection, helps in such an intent.

### 3.2.3 Laplace Transform Method for Linear Reactions

In mathematics, the Laplace transform is a widely used integral transform, but it has other important applications in mathematics, physics, engineering, and probability theory. The Laplace transform is related to the Fourier transform. The Fourier transform studies vibration modes of a function, while the Laplace transform resolves a function into its mathematics moments. As in general physics and engineering, in this thesis it is used for the analysis of linear time invariant system (LTI). In such a study, the Laplace transform is often interpreted as a transformation from the time-domain, in which inputs and outputs are functions of

\(^6\)State variables at equilibrium.
time, to the frequency-domain, where the same inputs and outputs are functions of complex number/complex angular frequency. Frequency is usually expressed in radians per unit time, $[\text{rad/s}]$. Giving a simple mathematical or functional description of an input or output to a system, the Laplace transform provides an alternative functional description that often simplifies the process of analyzing the behavior of the system.

Denoted $\mathcal{L}\{f(t)\}$, the Laplace transform is a linear operator on an original function $f(t)$, with a real argument $t$. This variable must satisfy $(t \geq 0)$. It transforms the function $f(t)$ into an image function $F(s)$, where the argument $s$ is a complex number: $\sigma + i\omega$ with real numbers $\sigma$ and $\omega$.

$$F(s) = \mathcal{L}\{f(t)\} = \int_{0}^{\infty} e^{-st} f(t) \, dt. \quad (3.18)$$

The respective pairs of $f(t)$ and $F(s)$ are already matched in tables for almost all common functions. As the Fourier transform, the Laplace transform has the useful property that many relationships and operations over the originals $f(t)$ correspond to simpler relationships and operations over the images $F(s)$ [22]. A necessary condition for the existence of the integral is that $f(t)$ must be locally integrable on $[0, \infty)$. This transformation is essentially bijective for the majority of practical uses.

The linearity of such an operation gives important properties. The differentiation theorem is an example:

$$\mathcal{L} \left[ \frac{\partial}{\partial t} f(t) \right] = sF(s) - f(0) \quad (3.19)$$

These annotations allow calculating the characteristic expressions, in the complex variable domain of $s$, of a linear, stable, finite-dimensional and regular representation. The practical application of Laplace transform in dynamical linear systems is the Laplace transformation of (3.13):

$$\left\{ \begin{array}{l}
    sX(s) - x(0) = A \cdot X(s) + B \cdot U(s) \\
    Y(s) = O \cdot X(s) + D \cdot U(s)
\end{array} \right. \quad (3.20)$$

With simple manipulations:

$$X(s) = (sI - A)^{-1} x(0) + (sI - A)^{-1} B \cdot U(s) = \Phi(s)x(0) + G \cdot U(s) \quad (3.21)$$

$$Y(s) = O \left( sI - A \right)^{-1} x(0) + \left( Q \left( sI - A \right)^{-1} R \right) U(s) = \Psi(s)x(0) + H(s) \cdot U(s) \quad (3.22)$$
The matrix, $H(s)$, contains transfer functions referring to where the input and the output is considered applied.

$$H(s) = \frac{Q (I \cdot s - A)^{-1} B + D}{s}$$  \hspace{1cm} (3.23)

$H(s)$ matrix elements are rational functions: polynomial ratios in $s$ variable. The denominator order is $N \geq n$, where the latter is the dynamic matrix dimension.

Note that also the Fourier transform allows switching from ODEs to algebraic equations and from some kinds of Partial Differential Equations (PDEs) to ODEs. However, in ODEs resolution, the Fourier transform is not the best tool for solving a Cauchy problem on the half real axis: its use restricts the search for a solution to tempered distributions, such as the exponential function. The Laplace transform, which is similar to Fourier one as far as formal properties are concerned, allows instead transformations of exponential functions.

### 3.2.4 Transfer Function and Bode Diagrams

Let us consider again the transfer function. In Sect. 3.2.3 the nature of the transfer function has been explained. Proceeding from (3.23), the matrix form of the transfer function can be obtained:

$$H(s) = \begin{bmatrix}
H_{11}(s) & H_{12}(s) & \cdots & H_{1p}(s) \\
H_{21}(s) & H_{22}(s) & \cdots & H_{2p}(s) \\
\vdots & \vdots & \ddots & \vdots \\
H_{q1}(s) & H_{q2}(s) & \cdots & H_{qp}(s)
\end{bmatrix}$$  \hspace{1cm} (3.24)

Observing in the time domain, if we refer to a specific single output, (3.24) will give

$$Y_i = H_{i1}U_1 + H_{i2}U_2 + \cdots + H_{iN}U_N \Rightarrow H_{ij}(s) = \frac{Y_i(s)}{U_j(s)} \bigg|_{U_k=0 \ (k \neq j)}$$  \hspace{1cm} (3.25)

Basing upon observations of Sect. 3.2.3, the transfer function model can be seen as a "black box", where the input, the output and their relationship, $H(s)$, are the only system information known.

Note that, when the input is the unitary impulse, whose transfer function is simply $\mathcal{L}(\delta(t)) = 1$, the time system response is the inverse Laplace transform of the transfer function itself. As well as the transfer function, the impulse response is a behavior model of a LTI system response.

Another due observation is about the initial conditions: generally, analyzing the same system but with different initial conditions, different transfer functions are obtained. Nevertheless, it has been proved that the initial information is removed when the time gets large (i.e. the
network is memoryless, which is as expected for a linear system) \[23\].

The rational form is a different representation of (3.24):

\[ H(s) = \frac{B_0 + B_1 s + \cdots + B_m s^m}{a_0 + a_1 s + \cdots + a_n} \]  

(3.26)

where the generic \(a_i\) is a coefficient and \(B_i\) a matrix of coefficients. Referring to the relationship between only a specific \(i\)th output and a specific \(j\)th input, the \(H_{ij}(s)\) element of the \(H(s)\) matrix is obtained:

\[ H_{ij}(s) = \frac{b_0 + b_1 s + \cdots + b_m s^m}{a_0 + a_1 s + \cdots + a_n} \]  

(3.27)

where \(a_i\) and \(b_i\) are both coefficients. Denominator roots are named "poles", while numerator roots are named "zeros". Note that poles equal eigenvalues associated to the observable and excitable modes of \(H(s)\). They represent motion laws: periodic or pseudo-periodic, converging, constant or diverging. Instead, zeros of the system are \(s\)-values which cancel the system matrix determinant \[24\].

Equation (3.27) can be written in the "zero-poles-gain", \(zpk\), notation:

\[ H(s) = K \prod_{i=1}^{m} \frac{(s - z_i)}{(s - p_i)} \]  

(3.28)

where \(K\) is the gain. Equation (3.28) can be expressed in time constant notation, re-writing all product factors as \((s - 1/z_i)(1 - s/z_i)\) and \((1 - s/p_i)(s - 1/p_i)\):

\[ H(s) = K_{st} \prod_{i=1}^{m} \frac{s \left( -\frac{1}{z_i} \right) + 1}{s \left( -\frac{1}{p_i} \right) + 1} \]  

(3.29)

where \(K_{st}\) is the stationary gain, namely the system behavior for low (null) frequency, and \(1/z_i\) and \(-1/p_i\) represent the time decay constants\(^7\). Note that the transfer function could also show \(g_p\) number of poles or \(g_z\) number of zeros at origin. For such situation, the evaluation of the stationary gain will result \(K_{st} = \lim_{s \to \infty} s^{g_p} H(s)\) in the case of \(g_p\) poles at origin and \(K_{st} = \lim_{s \to \infty} s^{-g_z} H(s)\) in the case of \(g_z\) zeros at origin.

Denoting the generic complex pole\(^8\) \(p_j = \gamma_j + i\omega_j\), the explicit form of the product \((s - p_j)(s - p_j^*)\) can be written as \((s^2 + 2\xi_j \omega_{n,j} s + \omega_{n,j}^2)\), where \(\omega_{n,j} = |p_j|\) is the natural frequency of the root pair \(p_j p_j^*\) and \(\xi_j = -\gamma_j/\omega_{n,j}\) is the damping of the root pair \(p_j p_j^*\).  

\(^7\)If we are dealing with complex numbers, we will indicate \(-1/\Re\{z_i\}\) and \(-1/\Re\{p_i\}\) as time constants.  

\(^8\)Analogous observations can be done for complex zero values.
The most meaningful and clear representation of the transfer function of a system is the Bode diagram. Its magnitude axis is usually expressed as decibels of power, that is 20 times the common logarithm of the amplitude gain: \(20 \log_{10}(\ldots)\). A Bode phase plot is a graph of phase versus frequency, also plotted on a log-frequency axis. It is usually used in conjunction with the magnitude plot to evaluate how much a signal will be phase-shifted. The premise of Bode plot is that products, \(A \prod_{i=1}^{m} (s - z_i)\), once they have been evaluated in decibels, become sum of logarithms: \(\log(A) + \sum_{i=1}^{m} \log(s - z_i)\). Since \(|H(s)| = H(s)H^*(s)\), the exact magnitude plot should be evaluated for each point, especially for frequencies around where the singularity is placed (poles or zeros). However, the magnitude plot can be easily drawn by approximating with straight lines and observing the following general rules:

- at every value of \(s\) where \(\omega = z_i\) (a real zero), increase the slope of the line by 20dB per decade.
- at every value of \(s\) where \(\omega = p_i\) (a real pole), decrease the slope of the line by 20dB per decade.
- The initial value of the graph depends on the boundaries. \(G_{LS} = K_{st}/g_{p} \rightarrow G_{LS}|dB = 20 \log_{10}(K_{st}) + 20 g_{p} \log_{10} |\omega|\).
- The initial slope is +20dB per decade for each zero at origin, or -20dB per decade for each pole at origin.
- at every value of \(s\) where \(\omega = |\Re\{z_i\} + i\Im\{z_i\}|\) (a complex zero), increase the slope of the line by 40dB per decade.
- at every value of \(s\) where \(\omega = |\Re\{p_i\} + i\Im\{p_i\}|\) (a complex pole), decrease the slope of the line by 40dB per decade.

Referring to the cited approximation, the asymptotic graph will differ from the real magnitude plot from:

- Real zero: The maximum error is in \(s = z_i\), around 3dB below the exact line. Refer to Fig. 3.5.
- Real pole: The maximum error is in \(s = p_i\), around 3dB above the exact line.
- For a complex zero or pole, the maximum error depends on the value of the damping factor \(\xi_i\). Refer to Fig. 3.7.

In the time domain a phase-shifting means a delay.
Figure 3.5: Magnitude plot of $H(s) = 1 - \frac{z}{s}$ related to real zero ($\Re\{z\} < 0$).

Figure 3.6: Phase plot of $H(s) = 1 - \frac{z}{s}$ related to real zero ($\Re\{z\} < 0$).
3.2 Frequency Domain Analysis

Figure 3.7: Magnitude plot of \( H(s) = \frac{s^2}{\rho_n^2} + \frac{s\xi}{\rho_n} + 1 \) related to a complex conjugate pair of zeros with frequency \( \rho_n \) and damping factor \( \xi \).

Figure 3.8: Phase plot of \( H(s) = \frac{s^2}{\rho_n^2} + \frac{s\xi}{\rho_n} + 1 \) related to complex a conjugate pair of zeros with frequency \( \rho_n \) and damping factor \( \xi \). x-axis is normalized with respect to the frequency: \( \frac{\omega}{\rho_n} \).
Of course, for $s \gg \omega$ or $s \ll \omega$, asymptotic and exact graph will match.

The actual phase curve is given by $-\arctan \left[ \frac{\Im\{H(s)\}}{\Re\{H(s)\}} \right]$. Again, an asymptotic graph can be drawn, which is composed of straight lines tangent, in the singularity values, to the real phase plots:

- $K_g > 0$: start line (with zero slope) at 0 degrees.
- $K_g < 0$: start line (with zero slope) at 180 degrees.
- For each $s = |z_i|$ with $\Re\{z_i\} < 0$, increase the slope by 45 degrees per decade, beginning one decade before $s = |z_i|$ and ending one decade after $s = |z_i|$. Refer to Fig. 3.6.
- For each $s = |p_i|$ with $\Re\{p_i\} < 0$, decrease the slope by 45 degrees per decade, beginning one decade before $s = |p_i|$ and ending one decade after $s = |p_i|$.
- Singularities with $\Re\{\ldots\} > 0$ have the opposite behavior.
- For complex singularities the phase plot depends on the damping factor $\xi$. Refer to Fig. 3.8.

### 3.2.5 Metabolic Control Analysis for Non-Linear Reaction

We have now tools to analyze the dynamical behavior of linear chemical networks. However, several CNPs like the Quine structure and the congestion control algorithm, show non-linearities. These non-linearities are due to the presence of multiplications between species concentrations in the ODEs.

Some complex methods that treat non-linear systems exist. An example is the Volterra series that represents the match of the Taylor series and linear convolution integrals. With the multi-dimensional Laplace transform, the study of the frequency transform of a non-linear system is possible [25]. However, the use of Volterra series would lead to high dimensionality and complexity of the study of CNPs dynamical behavior, far from our intent.

A different analysis approach is the *Metabolic Congestion Analysis (MCA)*. The milestone of the MCA is the linearization of non-linear systems [26]. This sensitivity study is focused primarily on the steady state (i.e. asymptotic) response of a system to constant (i.e. step) changes in parameters [27]. An extension to MCA has been formalized in [27], where this sensitivity analysis is not only valid for constant changes, but also for varying perturbations. This is achieved through a frequency domain analysis which describes the response of the non-linear system to a canonical set of inputs.
The key feature of such an analysis is the stoichiometric structure of networks. Under this point of view, the dynamics of a non-linear system can be described by

$$\dot{S}(t) = N \cdot V(S(t), P(t))$$

(3.30)

where $N$ is the stoichiometric $n \times r$-matrix of the reaction network, $S$ is the $n$-vector\(^{10}\) of states, namely the species concentrations, $P$ is the $m$-vector\(^{11}\) of kinetics parameters of reactions and $V$ is the $r$-vector of valued functions which describe the rates for $r$ chemical reactions. Local analysis of the non-linear system described by (3.30) is carried out around steady states $(s_{st}^{st}, p_{st}^{st})$:

$$\dot{X}(t) = N \cdot \frac{\partial V}{\partial S} \cdot X(t) + N \cdot \frac{\partial V}{\partial P} \cdot U(t)$$

(3.31)

where the $n$-vector, $X$, and the $m$-vector, $U$, respectively indicate the deviation from the nominal state and from nominal parameter values of (3.30). Obviously, $V$ depends upon $S$ and $P$. Such a dependance has been omitted in order to simplify the notation. The derivatives in (3.31) are then taken at the fixed point $(s_{st}^{st}, p_{st}^{st})$. The behavior of the original system, (3.30), is approximated by the behavior of the linearized system of (3.31) only near the operative point. It represents the response to just small variations in parameters $U$, but for all kind of perturbations\(^{12}\).

Note that (3.30), as well as (3.31), contains redundant equations because of conservation constraints \(^{28}\). Many treatments of this argument care about this redundancy and thus they simplify the stoichiometric matrix, $N$, before proceeding in the analysis. Such a passage could be computed by using Gauss Jordan elimination with partial pivoting and obtaining $N$ in the reduced row Echelon from. The analysis procedure still remains the same even without such a "complex simplification". In this work, we have proceeded without using the reduced form of the stoichiometric matrix.

The MCA can be entirely reformulated in "control analysis" term \(^{28}\), reducing the problem to find the state, the input, the output and the direct transmission matrixes. Then, from these descriptive mathematical elements, the transfer function of system (3.31) is found. We will directly supply the final procedure, omitting demonstration and intermediate passages. For further information please refer to \(^{23}\) and \(^{27}\).

---

\(^{10}\) $n$ is the number of network species.
\(^{11}\) $m$ is the number of kinetics parameters perturbed, namely the number of input
\(^{12}\) In standard MCA, only constant perturbation are considered.
Referring to (3.13) given in Sect. 3.2.2, we can take as state matrix

$$A = N \cdot \left. \frac{\partial V}{\partial S} \right|_{(s_{st}, p_{st})}$$ (3.32)

and as input matrix

$$B = N \cdot \left. \frac{\partial V}{\partial P} \right|_{(s_{st}, p_{st})}$$ (3.33)

and imposing

$$Y(t) = X(t)$$ (3.34)

The last decision is due to our interest in the deviation of the species concentrations from nominal levels. These deviations are described by the state vector $X(t)$. Equation (3.34) corresponds to valuate (3.13) by imposing $O = I$ and $D = 0$. Once the state variable representation is defined, the transfer function is easily found basing upon contents of Sect. 3.2.2:

$$C(s) = O \left( I \cdot s - A \right)^{-1} B + D$$ (3.35)

The matrix $C$ represents the frequency transforms of species concentration. They are dependent on where the input displacement is considered. In our studies, the input is a small perturbation of one kinetics parameter. The output can be represented directly by the dynamics of species or by the outflow rate of a specific species of the chemical model. In both cases, the description of the network dynamics should be normalized to the input frequency transform, obtaining an actual transfer function.

The approach, in this subsection explained, is a linearization of a more complex system which is driven by non-linearities. We supply again only an approximation of the exact solution. The advantage of such a method is to work on a linear system, meaning the use of common and simple tools (i.e. Laplace transform) and the possibility to make general observations about results basing on this important feature (i.e. superposition effect theorem). Other rigorous analysis would definitely increase the computational work and would supply only specific results hard to generalize.

---

13When we are caring of the deviation of reaction rates to nominal values, (3.34) becomes more complicated. We must chose $O = \frac{\partial V}{\partial S}$ and $D = \frac{\partial V}{\partial P}$. The state matrix $A$ and the input matrix $B$ remain the same.

14Even more kinetics parameters can be solicited at the same time.
3.3 Intrinsic Noise of Chemical Communication Networks

In previous sections of this chapter, we have achieved a deterministic model which can only approximate how CNPs exactly act. We have cited in Sect. 3.1.1 that the CNPs behavior can be rigorously described with a stochastic process which is not easy to study. In the next sections we will introduce a different approach to treat this stochastic process. We will only explain the origins of this stochasticity and the problems of its study.

3.3.1 Origins

The approximation of a stochastic process to a deterministic one arises from the use of DREs. Indeed, while the CME cares even about the randomness of chemical network interactions, the DRE approximation refers only to average trends. Remind that the validity of this approximation is related to the species concentrations of the analyzed system: the more molecules there are, the less the deviation between a certain realization of the stochastic process and its expectation is. Since we start from DREs and we use the frequency analysis to solve the DREs, only the average dynamical behavior of CNPs can be predicted. Actually, the stochasticity is due to the interval time between each reaction and the next one. Until now, according to the law of mass action, we have supposed that a molecule of a certain species was consumed at a deterministic time, which was inversely proportional to the concentration of the species itself. In reality, the exact simulation algorithm in the Fraglets simulator implements the stochastically correct chemical reaction timing: an exponential random variable determines the next reaction time [1]. The parameter of this exponential random time is directly proportional to the concentration of the reactant species\textsuperscript{15}. This chemical point of view of the randomness can be easily explained with the example in Fig. 3.9, where molecules

\textbf{Figure 3.9:} The decay of C-species: molecules of C-species are consumed until no more C-instances are present. The reaction rate is proportional to C-species concentration and the reaction coefficient, $k$.

\textsuperscript{15}The expectation of the exponential random variable equals $\frac{1}{\lambda}$, where $\lambda$ is the exponential variable parameter and thus, taking this parameter $\lambda$ directly proportional to the concentration of the reactant species, the expectation of the exponential time interval equals the deterministic value we have exploited in DREs approximation.
of $C$-species are consumed until no more $C$-instances are present in the vessel. The graph in Fig. 3.10 shows the difference between the exact behavior of a particular realization of the stochastic process (blue line) and the deterministic approximation (red line).

Instead of looking at the randomness of the time, it would be really useful refer to the randomness of concentration, which is actually our signal. The general idea would be to have a continuous-time signal and a stochastic process that corrupts the continuous-valued signal, Fig. 3.11. The continuous-time signal would be the concentration trend, which is obtained assuming the deterministic reaction time\textsuperscript{16}. The random disturbance would stochastically describe the difference of a certain realization of the stochastic process and the ideal continuous-valued signal. Once the stochastic description of this kind of noise is known, CNP designers would have more important data about the accuracy of their network information. The signal-noise ratio (SNR) would be easily calculated and thus, most of the information theory tools would be available.

\textsuperscript{16}The deterministic reaction time is inversely proportional to the concentration itself.
3.3 Intrinsic Noise of Chemical Communication Networks

3.3.2 Complexity in the Mathematical Analysis of Noise

The CNP dynamics can be described by a random process: the concentration of a certain species changes in steps of $\pm$ a certain discrete amount at the exponentially distributed random time whose parameter is directly proportional to the concentration of the species. The similarity to the Poisson process seems quite graspable. A poisson process is a stochastic process in which events occur continuously and independently from each other. This continuous-time process is a set $\{N(t) : t \geq 0\}$ of random variables, where $N(t)$ is the number of events that have occurred until time $t$ (starting from time 0). The number of events between time $t_a$ and time $t_b$ is given as $N(t_b) - N(t_a)$ and has a Poisson distribution. The important common point between our disturbance process and the Poisson process is the exponential probability distribution of the waiting time between two consecutive occurrences. Our aim would be to refer to an already known model of noise (i.e. the shot noise of optical receivers) in order to use the well-known results and to calculate the SNR.

Note that "$\pm"$ does not mean an incertitude, it refers only to a general reaction with $+$ if the reaction generates one or more molecule of the considered species, $-$ if the reaction consumes one or more molecule of the considered species. "$\pm"$ does not indicate a randomness.

The step increse/decrease of the concentration can have amplitude of 1, 2, 3... . It is defined by the reaction: how many molecules produce/consume contemporaneously.

Figure 3.11: The continuous approximation of the concentration.
There are specific requirements to classify a process as a *Homogeneous Poisson*:

- \( N(0) = 0 \)
- No counted occurrences are simultaneous.
- Stationary events (the probability distribution of the number of occurrences counted in any time interval only depends on the length of the interval)
- Independent events (the numbers of occurrences counted in disjoint intervals are independent from each other)
- Parameter of the exponential variable time constant over the time (homogeneous Poisson process)

Unfortunately, the parameter of the random reaction time in CNPs is not constant. This process cannot be a homogeneous Poisson process. Moreover, the number of occurrences, counted in a certain time interval, are defined by the time at which the previous events have been occurred. Since the reaction time is a random variable with the concentration as parameter, the independence of events\(^{19} \) is not directly granted.

Always referring to example in Fig. 3.9, we can write concentration \( c \) at time \( t + \Delta t \), basing upon the previous concentration value, \( c(t) \):

\[
c(t + \Delta t) = c(t) - 1
\]

The stochasticity arises from the time when the next reaction occurs, \( \Delta t \):

\[
\Delta t \in \exp\left\{ \frac{1}{c(t)} \right\}
\]

whose expectation is

\[
\mathbb{E}\{\Delta t\} = \frac{1}{c(t)}
\]

And again at the next occurrence:

\[
c(t + \Delta t + \Delta t') = c(t + \Delta t) - 1
\]

\[
\Delta t' \in \exp\left\{ \frac{1}{c(t+\Delta t)} \right\}
\]

\(^{19}\)The numbers of occurrences counted in disjoint intervals are not independent from each other.
Now, we have caught the point: there seems to be a recursiveness in the dependence of the concentration respect to the exponential random time.

The reference to a Poisson process is not directly possible. There are two still possible ways to proceed: stochastically characterize the random process or find a way to approximate the stochastic process with models which are already known and studied (i.e. Poisson process and shot noise). Both the possibilities result very complicated, even too complicated to be treated in a master thesis. Definitely, the analysis of such a disturbance could be an interesting and wide subject for further studies. Remind that CME mathematically describes a stochastic process. In the appendix, Sect. 6.8, we explicit our approach for a specific chemical model with special features. Anyway, the very general idea we have proposed is to split this stochastic process, isolating a useful and predictable signal from a random noise. However, we have not been able neither to rigorously characterize this disturbance nor to understand when this noise is multiplicative or additive to the signal.

3.4 Summary

In this chapter, the analysis tools for CNP dynamics description have been supplied. The explanation of chemical kinetics basics has required complex description and several detailed equations. Nevertheless, the reader can quickly pass through Sect. 3.1 and directly approach the rest of the thesis. No lacks of knowledge should come out. The concept of Sect. 3.1 is that dynamics are described by complex stochastic systems of equations. A simplification could be obtained by the use of DREs approximation. The obtained behavior description does not suffer of large deviation from the exact one. However, it still remains an approximation. On one hand, the majority of pages of Sect. 3.1 have been reported for completeness, on the other hand, the reader should be able to use the differential rate equation approximation before going further. Indeed, all frequency analysis base upon such a description of CNP dynamics.

In Sect. 3.2.1, we have explained the reason of the use of frequency domain analysis, and then, formal definitions of that domain have been given: Sect. 3.2.4, Sect. 3.2.3 and Sect. 3.2.2. We have englobed more methods and theories (i.e. signal processing and control system theory) in just one general procedure which is valid for all kind of LTI systems. Please note that our contribution has been an adaptation of already existing theories and not a mere statement of new theorems.

The prediction of the dynamical behavior of non-linear networks has required the MCA. Its
extended version allows obtaining directly descriptive elements of the state variable represen-
tation and thus to supply the transfer function of a network and/or frequency transforms of
network species. Remind that, by the use of species frequency transforms, the time-domain
response of the network can always be obtained (i.e. step response).
In last section, Sect. 3.3 we have cared again about the original stochasticity of the problem,
trying to give more rigorousness to our deterministic approach of analysis. The complexity of
involved random processes leads to a difficult treatment of such an argument. For that reason,
only the nature of the model has been supplied, without any formal and final description.
Anyway, the general idea would be to map the problem in terms of information theory: seeing
concentration over the time, as a common continuous signal, and then splitting it in a useful
part \textit{(information)} and a non-wanted part \textit{(noise)}. The critical passage would be finding a
model that correctly describes the stochastic process of the noise. Nevertheless, once this
model would be defined, even the signal-noise ratio could be given, with all its consequences
in the error-probability description field.
Chapter 4

Application of Analysis Tools and Results

In this chapter we finally supply the fruits of all analysis methods which have been introduced in Chap. 3. In Sect. 4.1 the elementary block is individuated. This is a simple chemical reaction which can be found in every chemical communication network. Then, its variant form with more outputs is defined and the general transfer function (or frequency transform of species concentration) is given. In Sect. 4.1.1 the series interconnection of such elementary block is analyzed, while the parallel connection is treated in Sect. 4.1.2. There is also specified when the superposition principle is valid and when instead the link topology does not supply such a principle. Section 4.1.3 represents the glue that collides elementary reactions and interconnections, giving the final frequency analysis procedure for simple communication networks. Section 4.2 introduces a new kind of interconnection: feedback branches. A subsection is dedicated to summarize general results of such a kind of communication network: Sect. 4.2.2. Then, these general results are applied to an existing communication protocol: the Disperser protocol. Delay links are treated in Sect. 4.4. Initially, the link with delay is characterized in the frequency domain, then this model is applied to find the consequences of delay in more complex networks. Non-linear networks are studied in Sect. 4.5. In the beginning, the MCA is used for a linear network only, which results are already known from Sect. 4.2. This propaedeutic section constitutes a sort of validation of the correctness of our use in practical and real cases of the MCA. Then, in Sect. 4.5.2 and Sect. 4.5.3, existing non-linear communication protocols are studied: the Quine and the first idea of protocol which deals with congestion control avoidance. The last section, Sect. 4.6, supplies an example of a chemical model that does not present the usual low pass filter behavior, but it has a band pass filter behavior.
4.1 Basic Reactions

Before starting to give real applications, we would like to remind the reader that this section deals with basic reactions where there are no non-linearities. Thus, in order to solve the differential equation approximation, the use of the Laplace transformation is directly possible. Remember that the solution of the differential equation approximation means the knowledge of the dynamical behavior of chemical communication networks. Our goal is finding the transfer function of the network and frequency transforms of concentration network species.

Let us start the most important chapters of the thesis, warming us up and approaching the argument with the simplest case, Fig. 4.1: a chemical species with an initial concentration that, at the beginning of our observation, shows a decay behavior, "sending away" molecules. Of course, having no input here, sooner or later the species concentration will be null. The speed, with this (stable) condition is reached, is defined by the reaction constant, \( k \). The time-domain trend can be studied starting from the differential equation approximation:

\[
\frac{dc}{dt} = -v_{\text{out}} = -k \cdot c
\]

proceeding in the time domain:

\[
\frac{1}{-k \cdot c(t)} dc = - \int dt \\
\ln(c(t)) = -k \cdot t
\]

the time behavior of this model, \( c(t) \), is found considering the initial concentration, \( c(0) \):

\[
c(t) = c(0) \cdot e^{-k \cdot t} \cdot u(t)
\]

We can also proceed in the frequency domain: always starting from differential equations (4.1) the Laplace transform is calculated:

\[
s \cdot C(s) - C(0) = -k \cdot C(s)
\]

\[
C(s) = \frac{C(0)}{s + k}
\]
Figure 4.2: The time trend decay of C species, model in Fig. 4.1. The analysis is performed in Matlab environment. A unitary reaction constant is considered, $k=1$.

Figure 4.3: The frequency transform, $C(s)$, of the chemical model in Fig. 4.1. The analysis is performed in Matlab environment. A unitary reaction constant is considered, $k=1$. 
Please note that (4.3) is the Laplace transform of (4.2).
This first example of chemical model could represent the input of a more complex chemical network. We have not started yet to talk about transfer functions and blocks with an input and an output. The next spontaneous step in forwarding our treatment is to consider the elementary chemical reaction, Fig. 4.4. Using the same analysis procedure of the previous example, the differential equation is written:

\[ \dot{c} = v_{\text{in}} - v_{\text{out}} = v_{\text{in}} - k \cdot c \]

Solving in Laplace domain, the transfer function of this basic chemical reaction is

\[ H(s) = \frac{V_{\text{out}}(s)}{V_{\text{in}}(s)} = \frac{k \cdot C(s)}{V_{\text{in}}(s)} = \frac{k}{s + k} \]  \hspace{1cm} (4.4)

In (4.4) the initial condition is considered null, \( c(0) = 0 \). By calculating the inverse transform, the impulse response is evaluated:

\[ h(t) = e^{-k \cdot t} \cdot u(t) \]  \hspace{1cm} (4.5)

Equation (4.5) describes the time behavior of this simple chemical reaction when an impulse is applied to the input (this could be seen as a sharp injection of molecules of \( C \)-species). Another interesting plot is the step response of the elementary chemical network: Fig. 4.7. The step response is the time behavior of this chemical reaction when we start to inject a constant amount of molecules in \( C \)-species. The analytical solution of the step response is obtained, in the time domain, by the convolution of the impulse response of the network, (4.5), and the step function, \( u(t) \).

\[ y(t) = u(t) \otimes h(t) \]

In the frequency domain, calculations result easier: the multiplication of the frequency responses of the network, \( H(s) \), and the frequency transform of the step function, \( U(s) = \frac{1}{s} \),

\[ y(t) = Y(s) = \frac{1}{s} \cdot H(s) \]
This chemical model is as simple as important: all our future analysis of any chemical communication network will be based upon this elementary block:

\[ H(s) = \frac{k}{s + k} \]  

(4.6)

The frequency response shows a pole in \(-k\) and no zeros, thus it has a low pass filter behavior: all frequency components higher than the cutoff frequency of this filter are attenuated, while low frequencies (components lower than the cutoff frequency) pass unchanged. Of course, this conclusion can be easily asserted in the frequency domain, but it is not so obvious in the time domain. Let us look at the meaning of a low-pass behavior in the time domain. High frequencies are modified in some way. The sharp increase of the concentration at the input is not matched in a steep growth of the output concentration. The number of molecules needs some time before stabilizing at the equilibrium condition (instead of immediately reaches this desiderate value). The approach to this new stable condition is described by an exponential function.

The knowledge of the chemical model transfer function allows evaluating the response of the model to any kind of perturbation applied to its input. While the time behavior is definitely more intuitive, the frequency analysis conveniently displays how the studied block treats different frequencies of the input signal. Remembering that high frequencies mean
Figure 4.6: The impulse response, \( h(t) \), of an elementary chemical model (network in Fig. 4.4). The simulation is performed in Matlab environment. The initial concentration is considered null, \( c(0) = 0 \), and the reaction constant unitary, \( k=1 \).

Figure 4.7: The step response, \( h_u(t) \), of an elementary chemical model (network in Fig. 4.4). The simulation is performed in Matlab environment. The initial concentration is considered null, \( c(0) = 0 \), and the reaction constant unitary, \( k=1 \).
fast oscillations (changes) in the time domain, while low frequencies are related to slow time oscillations. We can assert that the elementary chemical model better supports "slow" changes. Of course, "slow" is a subjective yardstick. Its accurate definition is related to the reaction constant \( k \).

4.1.1 Cascade of Species

We approach this subsection starting from the example of the series of only two reactions. We report the analytical solution in both time and frequency domains, providing graphs of specific analysis results. In the end, we sum up this basic typology of chemical network by giving a general result for the cascade of \( N \) reactions. Considering the chemical network in Fig. 4.8, the respective system of differential equations in the time domain is

\[
\begin{aligned}
\dot{c}_1 &= V_{in} - k_1 \cdot c_1 \\
\dot{c}_2 &= +k_1 \cdot c_1 - k_2 \cdot c_2
\end{aligned}
\]  

(4.7)

After the transformation in the Laplace domain:

\[
\begin{aligned}
s \cdot C_1(s) - C_1(0) &= V_{in} - k_1 \cdot C_1(s) \\
s \cdot C_2 - C_2(0) &= +k_1 \cdot C_1(s) - k_2 \cdot C_2(s)
\end{aligned}
\]

Considering the initial conditions of both species equal to zero, the transfer function of this block is easily found:

\[
H(s) = \frac{V_{out}(s)}{V_{in}(s)} = \frac{k_2 \cdot C_2(s)}{k_1 \cdot C_1(s)} = \frac{k_1 \cdot k_2}{(s + k_1)(s + k_2)}
\]  

(4.8)

Two observations arise. First, again the chemical network behaves as a low pass filter. It has no zeros and two poles. The higher number of poles\(^1\) means that the model has a higher capability to attenuate high frequencies. In the example, two poles have been placed at different frequencies \((k_1 \neq k_2)\). By assigning the poles in the same cutoff frequency \((k_1 = k_2)\), the side of the transfer function will be steeper. The second observation is more mathematical: the transfer function (4.8) is composed by the multiplication of the transfer functions of elementary blocks, which constitute the global network:

---

\( k \) in \( C_1 \) \( k \) in \( C_2 \)

Figure 4.8: The cascade of two chemical species.
Figure 4.9: The transfer function of a cascade of two chemical species, (network in Fig. 4.8). The initial conditions (concentrations) of both species are null. This analysis has been done with $k_1 = 1$ and $k_2 = 1000$.

Figure 4.10: The impulse response of a cascade of two chemical species, (network in Fig. 4.8). The initial conditions (concentrations) of both species are null. This analysis has been done with $k_1 = 1$ and $k_2 = 1000$. 
4.1 Basic Reactions

Figure 4.11: The step response of a cascade of two chemical species, (network in Fig. 4.8). The initial conditions (concentrations) of both species are null. This analysis has been done with $k_1 = 1$ and $k_2 = 1000$.

\[ H(s) = \frac{k_1}{s + k_1} \cdot \frac{k_2}{s + k_2} \]

As we have done before, we give a meaning of what has been found even in the time domain. The impulse response is plotted in Fig. 4.6, and the step response in Fig. 4.7, for a specific analysis in Matlab environment, where the initial concentrations of both species, $c_1(0)$ and $c_2(0)$, are considered null and $k_1 = 1$ and $k_2 = 4$ are the reaction constants. The impulse response assumes the general form of (4.9):

\[ h(t) = \left( \frac{k_2}{k_2 - k_1} \right) \cdot e^{-k_1 t} + \left( \frac{k_2}{k_1 - k_2} \right) \cdot e^{-k_2 t} \cdot u(t) \]  

(4.9)

Again, we could get same results written in (4.9) directly proceeding in the time domain and thus solving ODEs (4.7).

Let us generalize, supplying the general rule for the series of $N$ species, the chemical model in Fig. 4.12. As in all signal processing problems, the transfer function of the global chemical network can be calculated by the multiplication of all the transfer functions, $H_n(s)$, of the $N$

\footnote{Comparing to the elemental chemical model.}
elementary blocks.

\[ H(s) = \frac{V_{\text{out}}(s)}{V_{\text{in}}(s)} = H_1(s) \cdot H_2(s) \cdots H_N(s) = \prod_{n=1}^{N} H_n(s) \]  

(4.10)

where the \( n \)th transfer function is

\[ H_n(s) = \frac{k_n}{s + k_n} \]

The higher the number of elementary blocks in series is, the higher the high-frequency attenuation is. ”High” is related to the cutoff frequency, which defines which components will pass unchanged and which ones will be attenuated. In the time domain, the impulse response has been evaluated by the convolution of single impulse responses of elementary blocks:

\[ h(t) = h_1(t) \otimes h_2(t) \otimes \cdots \otimes h_N(t) \]

or simpler using the inverse transform of (4.10).

### 4.1.2 Parallelism of Species

Before starting with the analysis of the parallelism of species, we should review what an elementary chemical block is. Indeed, we should consider also models that have more than just one output, as Fig. 4.13 shows. The respective differential equation for an elementary chemical block with \( N \) outputs is

\[ \dot{c} = -k_{\text{out}} c - k_1 c - k_2 c - \cdots - k_N c \]
which means that the general transfer function is

$$H(s) = \frac{k_{\text{out}}}{s + k_{\text{out}} + k_1 + k_2 + \cdots + k_N} = \frac{1}{s + k_{\text{out}} + \sum_{n=1}^{N} k_n} \cdot k_{\text{out}} \quad (4.11)$$

Another step in our treatment of the parallelism of species is to clarify what "parallelism" means. Figure 4.14 reports a chemical reaction which can be easily analyzed by the use of equation (4.11). Indeed the links between $C_1$-species and $C_2$-species can be seen as a unique link with higher reaction coefficient. The value of this reaction coefficient equals the sum of reaction coefficients of each link. Concluding, the transfer function of such a chemical reaction is simply

$$H(s) = \frac{k_{\text{out}}}{s + k_{\text{out}}} \cdot \frac{\sum_{n=1}^{N} k_n}{s + \sum_{n=1}^{N} k_n}$$

To have a real "parallel" of reactions the parallel of species is needed, as Fig. 4.15 shows. As usually, we start from the differential equation approximation and then, moving in the Laplace domain, we will get the species Laplace transforms of the network.

$$\begin{align*}
\dot{c}_1 &= \text{Vin} - (k_{1a} + k_{1b} + \cdots + k_{1N}) \cdot c_1 \\
\dot{c}_a &= +k_{1a} \cdot c_1 - k_a \cdot c_a \\
\dot{c}_b &= +k_{1b} \cdot c_1 - k_b \cdot c_b \\
\vdots \\
\dot{c}_N &= +k_{1N} \cdot c_1 - k_N \cdot c_N \\
\dot{c}_4 &= k_a \cdot c_a + k_b \cdot c_b + \cdots + k_N \cdot c_N - k_{\text{out}} \cdot c_4
\end{align*}$$

$$\begin{align*}
C_1(s) &= \frac{V_{\text{in}}(s)}{s + \sum_{n=1}^{N} k_{1n}} \\
C_a(s) &= \frac{k_{1a}}{s + k_a} \\
C_b(s) &= \frac{k_{1b}}{s + k_b} \\
\vdots \\
C_N(s) &= \frac{V_{\text{in}}(s)}{s + \sum_{n=1}^{N} k_{1n}} \cdot \frac{k_{1N}}{s + k_N} \\
C_4(s) &= \frac{\left(\sum_{n=1}^{N} k_{1n} \cdot k_n\right)}{s + k_{\text{out}}} - \frac{1}{s + k_{\text{out}}}
\end{align*}$$
The parallel of $N$ chemical species: an equivalent scheme is shown in Fig. 4.16 where the superposition principle can be applied.

Figure 4.16: The equivalent of the parallel interconnection, the network in Fig. 4.15.

The transfer function of the total network will be

$$H(s) = \frac{k_{\text{out}} C_4(s)}{V_{\text{in}}(s)} = \frac{k_{\text{out}}}{s + \sum_{n=1}^{N} k_{1n} \cdot k_n} \cdot \left( \sum_{n=1}^{N} k_{1n} \cdot k_n \right) \cdot \frac{1}{s + k_{\text{out}}} \quad (4.12)$$

Note that we could find (4.12) by exploiting the superposition principle and seeing the network as the equivalent shown in Fig. 4.16. For each branch of this equivalent scheme, a first block is distinguishable. The latter represents essentially the elementary chemical model with more outputs, Fig. 4.13. This reaction has been already described by the transfer function (4.11). If we consider the reaction coefficient as an independent module of the scheme, the first transfer function block will not depend on which branch is being analyzed and so this transfer function block will be the same for all branches. In contrast, the second transfer function module has a pole placed according to the respective reaction coefficient of the branch. This transfer function is directly obtained referring to the elementary reaction with only one output.
The general frequency analysis of a network which is composed by the parallel of filters. The filter transfer functions are $H_a(f)$, $H_b(f)$ and $H_c(f)$ and the global frequency behavior of the network is $H_{tot}(f)$. More cases are shown. Case (a): three low pass filters with different cutoff frequencies; case (b): one low pass and two band pass filters, with bandwidth not overlapped; case (c): one low pass and two band pass filters, with bandwidth overlapped; case (d): three low pass filters with the same cutoff frequency.
(4.6). As we have said, in order to get a clearer representation, it is convenient to split up the amplification effect of reaction constants. Even amplification modules depend on which branch of the network we are looking at. Concluding, the last transfer function block is again studied by the use of equation (4.6). The filtering behavior of the global network can be evaluated using the superposition principle and thus summing the effect of each branch.

\[ H(s) = \sum_{n=1}^{N} \left( \frac{1}{s + k_{1a} + k_{1b} + \cdots + k_{1N}} \cdot k_{1n} \cdot \frac{1}{s + k_n} \cdot \frac{1}{s + k_{\text{out}}} \cdot k_{\text{out}} \right) \]  

(4.13)

As we would expect, (4.13) is equivalent to (4.12).

Leaving the mathematical aspect of our result, we focus now on its frequency meaning. Indeed, while the cascade of filters always brings more efficient attenuation of unwanted frequencies, the parallel has a completely different consequence: the global bandwidth is defined by the sum of the effects of each filter. This concept is not easy to explain by words. It is much clearer by representation. We thus summon the reader to look at Fig. 4.17, where different cases are presented.

In chemical communication networks, basically we will deal with low pass filters. Thus, referring to Fig. 4.17, only cases \((a)\) and \((d)\) are really of our interest. Anyway, cases \((b)\) and \((c)\) are treated in order to give an exhaustive explanation.

4.1.3 From a Chemical Reaction to a Schematic Blocks Description

What has been shown at the beginning of this chapter allows simplifying the study of complex networks. Any kind of system can be viewed as basic blocks and modules, which are easier to examine. Indeed, we have not given so much space analyzing the frequency behavior of reactions and definitely we have asserted anything yet about which frequency response we should use to improve our chemical communication networks. Probably, the latter purpose will not be completely exhausted even going through this thesis. However, we now have the knowledge to chance the analysis of every kind of linear chemical communication networks. Starting from the differential equation of species, we can get the transfer function that completely describes them. All species can be associated to the elementary transfer function seen in Sect. 4.1. Thus, considering its effect isolated from the rest of the network, a species represents always a first order low pass filter, where the pole position is defined by the reaction coefficient or coefficients. Indeed, the only decisive thing is the number of outputs that a species has, which will settle the position of the cutoff frequency. The decrease of the frequency response slope is always -20dB/decade.

Now with these analysis elements, we can refer to the interconnection of different low pass
filters: cascades, parallels and loop. The first two typologies of links have been already treated. For the analysis of more complex networks, we should distinguish such blocks as building modules of networks. Then, the global transfer function can be obtained by exploiting module transfer functions and using results obtained in Sect. 4.1.1 and Sect. 4.1.2. This could represent a useful shortcut in studying the global network. Note that the isolation of building blocks is not necessarily required in order to get the right solution.

A general observation is due before proceeding the analysis of linear networks: linear networks are necessary beside mono-molecular reactions. This is in contrast with the requirement of the existing chemical network simulator. As it has been said in Chap. 1, Fraglets simulator works with bi-molecular reactions. This incongruity is dissolved by the possibility to use a "dummy catalyst": Fig. 4.18. The dummy catalyst allows satisfying the bi-molecular necessity of Fraglets simulator, still behaving as an elementary reaction.

\[
\dot{x} = -k \cdot c \cdot x
\]  
(4.14)

Since the concentration of $c$-species is constant, the effect on the reaction of such a species can be englobed in a general reaction constant $k'$. By setting the new reaction coefficient $k' = k \cdot c$, from (4.14) the following equation can be written

\[
\dot{x} = -k' \cdot x
\]  
(4.15)

The non-linearity, shown in Fig. 4.18, can be neglected.

## 4.2 Loops and Feedback

As we have anticipated before, when a linear chemical network is constituted by loops, the analysis becomes more complicated. Definitely, results of previous sections are essential and
they are directly applied in the analysis of such networks. We report several network typologies that we think could clarify and justify the observations that constitute the conclusion of this section: Sect. 4.2.2. Note that, in order to have a sort of validation of extrapolated rules, more other cases have been studied. All other analyzed chemical networks are in App. 6.3.

### 4.2.1 Analytical Solutions for Specific and Representative Networks

This section represents the direct application of what has been found in Sect. 4.1. MATLAB or SIMULINK environment are directly used, in order to deduce frequency responses of complex networks. We have exploited the knowledge of the transfer function of the elementary chemical block (with one or more outputs), which have been seen in Sect. 4.1. The final result has been given by the use of special MATLAB functions: ”tf”, ”zpk”, ”feedback” and ”bode”. Please refer to App. 6.1 to have more details of these MATLAB functions.

Let us start with the simplest reaction that has a feedback branch: Fig. 4.19. Initially, we will also write differential equations and some more didactic passages.

\[
\begin{align*}
\dot{c}_1 &= V_{\text{in}} + k_2 \cdot c_2 - k_1 \cdot c_1 \\
\dot{c}_2 &= k_1 \cdot c_1 - (k_2 + k_{\text{out}}) \cdot c_2
\end{align*}
\]

The transfer function of the total network will be

\[
H(s) = \frac{V_{\text{out}}(s)}{V_{\text{in}}(s)} = \frac{k_{\text{out}} C_2(s)}{s^2 + s(k_1 + k_2 + k_{\text{out}}) + k_1 k_{\text{out}}}
\]

Again, the plot of this transfer function shows a low pass filter behavior. The number of species are two and two is the order of the filter. The exact shape of the transfer function is defined only by the denominator, or more specifically, by its roots. Thus, the nature of the two poles depends on reaction coefficient values.

Studying other more complex networks, a simplification will be necessary: all reaction coefficients have the same value. We do this simplification already for this basic loop. This

---

**Figure 4.19: The simplest chemical network with an input, an output and a feedback branch.**
Figure 4.20: SIMULINK schemes of the simplest chemical network with an input, an output and a feedback branch, Fig. 4.19. (a): The Laplace transform of \( C_1 \)-species is obtained, \( C_1(s) \); (b): The Laplace transform of \( C_2 \)-species is obtained, \( C_2(s) \). The transfer function \( H(s) = \frac{v_{\text{out}}(s)}{v_{\text{in}}(s)} = \frac{k_{\text{out}}C_2(s)}{V_{\text{in}}(s)} \) is directly evaluated from \( C_2(s) \).

will allow us to compare transfer functions in similar conditions and so to make general observations.

\[
H(s) = \frac{k^2}{s^2 + s(3 \cdot k) + k^2} \tag{4.17}
\]

The solution (4.17) equals the one of (4.16) when all reaction coefficients have the same value. For this first example we give also the equivalent SIMULINK scheme. We remind the reader that for next networks which are being studied, we will provide only the final solution. The analysis procedure will be the same.

Figure 4.20 reports the schemes that has been used in order to get the result in (4.17).

Laplace transform of \( C_1 \)-species:

\[
C_1(s) = \frac{s + 2k}{s^2 + s(3 \cdot k) + k^2} \cdot V_{\text{in}}(s)
\]

Laplace transform of \( C_2 \)-species:

\[
C_2(s) = \frac{k}{s^2 + s(3 \cdot k) + k^2} \cdot V_{\text{in}}(s) \tag{4.18}
\]

Note that equation (4.18) gives the result of equation (4.17), multiplying by a factor of \( \frac{k}{V_{\text{in}}(s)} \).

We now briefly report other two cases that are obtained from the just studied basic loop, by adding "loop species". The goal is to find a relationship between the transfer function and the number of species that are involved in the "loop network". We will not argue about it, leaving all observations for Sect. 4.2.2.
"Three species loop with an input and an output": Fig. 4.21.

Laplace transform of $C_1$-species:

$$C_1(s) = \frac{s^2 + s \cdot 3k + k^2}{s^3 + 5k \cdot s^2 + s \cdot 6k^2 + k^3} \cdot V_{in}(s) = \frac{1}{s^2 + 3k)(s + k)} \cdot V_{in}(s)$$

Laplace transform of $C_2$-species:

$$C_2(s) = \frac{k(s \cdot 2k)}{s^3 + 5k \cdot s^2 + s \cdot 6k^2 + k^3} \cdot V_{in}(s)$$

Laplace transform of $C_3$-species:

$$C_3(s) = \frac{k^2}{s^3 + 5k \cdot s^2 + s \cdot 6k^2 + k^3} \cdot V_{in}(s)$$

Transfer function:

$$H(s) = \frac{k}{V_{in}(s)} \cdot C_3(s) = \frac{k^3}{s^3 + 5k \cdot s^2 + s \cdot 6k^2 + k^3}$$ (4.19)

Another chemical network with loops and an outflow has been studied. Please refer to App. 6.3.1.

The next step is to understand the importance of the output in the network. Please note that if a block has no output, there will be no possibility to find explicitly a transfer function. For that reason, in the following examples where no outflow arrows are present, the concentration itself of the specified species will be taken as output.

"Three species loop with no output": Fig. 4.22.

Laplace transform of $C_1$-species:

$$C_1(s) = \frac{s^2 + s \cdot 3k + k^2}{s(s^2 + s \cdot 4k + 3k^2)} \cdot V_{in}(s) = \frac{s^2 + s \cdot 3k + k^2}{s(s + k)(s + 3k)} \cdot V_{in}(s)$$
4.2 Loops and Feedback

![Figure 4.22: A "loop network" composed by three species, without outflow.](image)

Laplace transform of $C_2$-species:

$$C_2(s) = \frac{k(s + k)}{s(s^2 + s \cdot 4k + 3k^2)} \cdot V_{in}(s) = \frac{k(s + k)}{s(s + k)(s + 3k)} \cdot V_{in}(s)$$

Laplace transform of $C_3$-species:

$$C_3(s) = \frac{k^2}{s(s^2 + s \cdot 4k + 3k^2)} \cdot V_{in}(s) = \frac{k^2}{s(s + k)(s + 3k)} \cdot V_{in}(s)$$

The transfer function can be calculated referring to species concentrations:

$$H(s) = \begin{cases} 
\frac{C_1(s)}{V_{in}(s)} & \text{referring to } C_1\text{-species} \\
\frac{C_2(s)}{V_{in}(s)} & \text{referring to } C_2\text{-species} \\
\frac{C_3(s)}{V_{in}(s)} & \text{referring to } C_3\text{-species}
\end{cases}$$

Other chemical loop networks without the outflow have been studied. Please refer to App. 6.3.2.

We now report another instance where we are looking at the contribution of the input position on the transfer function. Note that, chemically, an input is an inflow of molecules which is applied to some species. Of course, to find the transfer function of a network, we can take advantage of the network symmetry\(^2\). For instance, the study of "two species loop networks" where the inflow is placed on $C_2$-species instead of $C_1$-species is completely useless (referring to Fig. 4.19), as well as it is fruitless to move the input in the opposite species of the network and then study it again.

"Three species loop with an inflow in the center of the network": Fig. 4.23.

Laplace transform of $C_1$-species:

$$C_1(s) = \frac{k(s + k)}{s(s^2 + s \cdot 4k + 3k^2)} \cdot V_{in}(s) = \frac{k(s + k)}{s(s + 3k)(s + k)} \cdot V_{in}(s)$$

Laplace transform of $C_2$-species:

$$C_2(s) = \frac{(s + k)^2}{s(s^2 + s \cdot 4k + 3k^2)} \cdot V_{in}(s) = \frac{(s + k)^2}{s(s + 3k)(s + k)} \cdot V_{in}(s)$$

\(^2\)The term "symmetry" is referred to the inflow position itself.
For the known symmetry reason, the Laplace transform of $C_3$-species equals the Laplace transform of $C_1$-species. The transfer function can be calculated referring to species concentrations:

$$H(s) = \begin{cases} \frac{C_1(s)}{V_{in}(s)} & \text{referring to } C_1\text{-species} \\ \frac{C_2(s)}{V_{in}(s)} & \text{referring to } C_2\text{-species} \\ \frac{C_3(s)}{V_{in}(s)} & \text{referring to } C_3\text{-species} \end{cases}$$

In order to clarify the importance of the input displacement, other chemical loop networks have been studied. Please refer to App. 6.3.2.

At the end of this long subsection we leave this type of networks, which could be intuitively named as "birth-dead chains", and we focus on the last representative case of chemical networks with feedback branches. Namely, we will sum up the influence of the number of feedback reactions on the frequency behavior of the network.

"Five species network with six feedback reactions (no output)": Fig. 4.24.

The term "feedback reaction" means a two-ways link that joins two neighbor species.

Laplace transform of $C_1$-species:

$$C_1(s) = \frac{s^4 + s^3 \cdot 11k + s^2 \cdot 39k^2 + s \cdot 46k^3 + 8k^4}{s(s^4 + s^3 \cdot 16k + s^2 \cdot 94^2 + s \cdot 78k^3 + 40k^4)} \cdot V_{in}(s) = \frac{(s^2 + s \cdot 5k + k^2)(s + 4k)(s + 2k)}{s(s + 5k)(s + k)(s + k)(s + 2k)} \cdot V_{in}(s)$$

Laplace transform of $C_2$-species:

$$C_2(s) = \frac{k(s^3 + s^2 \cdot 8k + s \cdot 19k^2 + 12k^3)}{s(s^4 + s^3 \cdot 16k + s^2 \cdot 94^2 + s \cdot 78k^3 + 40k^4)} \cdot V_{in}(s) = \frac{k(s + k)(s + 4k)(s + 2k)}{s(s + 5k)(s + k)(s + 4k)(s + 2k)} \cdot V_{in}(s)$$
4.2 Loops and Feedback

Figure 4.24: A "loop network" composed by five species and six feedback reactions. There is no outflow.

Laplace transform of $C_3$-species:

$$C_3(s) = \frac{k^2(s^2 + s \cdot 6k + 8k^2)}{s(s^4 + s^3 \cdot 16k + s^2 \cdot 94^2 + s \cdot 78k^3 + 40k^4)} \cdot V_{in}(s) = \frac{k^2(s + 4k)(s + 2k)}{s(s + 5k)(s + k)(s + 4k)(s + 2k)} \cdot V_{in}(s)$$

The Laplace transform of $C_4$-species and $C_5$-species can be seen directly from the previous one. Indeed, looking where the input is applied, $C_4$-species and $C_5$-species are symmetric to $C_3$-species.

The transfer function can be calculated referring to species concentrations:

$$H(s) = \begin{cases} 
\frac{C_1(s)}{V_{in}(s)} & \text{referring to } C_1\text{-species} \\
\frac{C_2(s)}{V_{in}(s)} & \text{referring to } C_2\text{-species} \\
\frac{C_3(s)}{V_{in}(s)} & \text{referring to } C_3\text{-species} \\
\frac{C_4(s)}{V_{in}(s)} & \text{referring to } C_4\text{-species} \\
\frac{C_5(s)}{V_{in}(s)} & \text{referring to } C_5\text{-species} 
\end{cases}$$

4.2.2 Observations

Once these network typologies have been reported, we now try to summarize the general rules that characterize a linear network with feedback reactions. In order to reassure the correctness of our beliefs, we have studied networks with even more species, with a higher number of reactions, with different displacements of the input and with or without the output. All analyzed networks are reported in App. 6.3. We believe that the span of results in Sect. 4.2 is enough to understand the next observations and rules.
As in all chemical communication networks, we are interested in the dynamical behavior of species concentrations. Usually the solution in the time domain is definitely not suggested: soon, the system of differential equations assumes huge dimension and even equations become quite long. Instead, we look at the filtering behavior of network modules. The frequency transforms of each species can be found and, by comparing them, a general characterization can be chanced. Proceeding with the same order as we have presented models in Sect. 4.2.1, ”networks with an input and an output” are treated. For such models, we can talk about ”transfer function” of the network as well as ”frequency transforms” of species concentrations. Note that the first differs from the second, referring to the species where the output is taken, only for the term \( \frac{k}{V_{m(s)}} \). The network structure defines the denominator of frequency transforms, which is always the same for all species. Moreover, the denominator does not depend on where the perturbation (the input) is applied. Generally, the denominator has the following form:

\[
\begin{align*}
2 \text{ species:} & \quad s^2 + s \cdot \xi k + \xi k^2 \\
3 \text{ species:} & \quad s^3 + s^2 \cdot \xi k + s \cdot \xi k^2 + \xi k^3 \\
\vdots \\
N \text{ species:} & \quad s^N + s^{N-1} \cdot \xi k + s^{N-2} \cdot \xi k^2 + \cdots + s^2 \cdot \xi k^{N-2} + s \cdot \xi k^{N-1} + \xi k^N
\end{align*}
\] (4.20)

Where \( k \) are the reaction coefficients\(^4\) and \( \xi \) are constants with different values each\(^5\).

Another property of the denominator is clear: the number of species defines the order of the denominator and thus the number of poles. Moreover, according to the sensible choice of only positive reaction coefficients, poles always present a non-negative real part and so the network should not have problems of stability.

The numerator of the frequency transform of a species instead, depends on the position where the inflow is applied. Placing the output in the perturbed species (where the input is applied), the numerator will be

\[
s^{N-1} + s^{N-2} \cdot \xi k + s^{N-3} \cdot \xi k^2 + \cdots + s^2 \cdot \xi k^{N-2} + s \cdot \xi k^{N-1} + \xi k^{N-1}
\]

\(^3\)This is valid only when the output reaction coefficient is \( k \). Remind that we are simplifying the notation, assuming all reaction coefficients equal to \( k \).

\(^4\)All reaction coefficients, \( k \), have the same value.

\(^5\)Each constant has a different value from the other. They should have a subscript each. This mathematical indication has been removed in order to get a clearer representation.
As before, analogous considerations about constants $\xi$ are valid. The numerator order is one less than the denominator order, namely one less the number of species. Placing the output moving away from the perturbed species, the numerator will have the form

$$
\epsilon_1(s^{N-2} + s^{N-3} \cdot \xi k + s^{N-4} \cdot \xi k^2 + \ldots + s \cdot \xi k^{N-3} + \xi k^{N-2})
$$

$$
\epsilon_2(s^{N-3} + s^{N-4} \cdot \xi k + s^{N-5} \cdot \xi k^2 + \ldots + s \cdot \xi k^{N-4} + \xi k^{N-3})
$$

$$
\vdots
$$

$$
\epsilon_M(s^{N-(M+1)} + s^{N-(M+2)} \cdot \xi k + \ldots + s \cdot \xi k^{N-(M+2)} + \xi k^{N-(M+1)})
$$

where $k$ and $\xi$ have the same meaning of in (4.20), while $\epsilon_i$ are constants that usually have values of $k$ power of the number of steps away from the perturbation displacement.

We can assert that the general transfer function of such a kind of chemical networks depends on the structure of the network itself, on where the input is applied and on where the output is taken. However, it always represents a low pass filter behavior: always, the order of numerator is lower than the order of denominator and there is a pole value lower than the lowest zero value. The filtering capacity of the network is defined by the number of species and the distance between the input and the output: the farther the outflow is taken from the inflow, the more severe the low pass filter is. Thus, the furthest species presents the most severe filtering behavior, while the directly perturbed species has the weakest one, but it still remains a low pass filter.

Similar conclusions can be asserted for chemical networks which have no output. As we have said in Sect. 4.2.1, no output condition means that the transfer function is referring to the specific frequency transform of a species concentration: $H(s) = \frac{C_i(s)}{V_{in}(s)}$ because $C_i(s) \equiv V_{out}(s)$. Moreover, having this kind of chemical system an inflow but not an outflow, it presents no stable condition and thus its transfer function has always a pole at origin. Obviously, the form of the denominator is not exactly the same as the one shown in (4.20):

$$
\begin{align*}
3 \text{ species:} & \quad s(s^2 + s \cdot \xi k + \xi k^2) \\
4 \text{ species:} & \quad s(s^3 + s^2 \cdot \xi k + s \cdot \xi k^2 + \xi k^3) \\
\vdots \\
N \text{ species:} & \quad s(s^{N-1} + s^{N-2} \cdot \xi k + s^{N-3} \cdot \xi k^2 + \ldots + s^2 \cdot \xi k^{N-3} + s \cdot \xi k^{N-2} + \xi k^{N-1})
\end{align*}
$$

Specific observations are left for networks which have been named as "birth-dead networks". The name refers to the obvious likeness with the better known "birth-dead chains".

*Perturbation in one of the ends:*
The numerator (of the frequency transforms of species concentrations) shows as many zeros as the number of species to reach the other end without the perturbation. The gain value is $k^{N_{step}}$, where $k$ is the reaction coefficient and $N_{step}$ is the number of step away from the perturbation. (i.e. in a network composed by a total of 5 species, the perturbed species will have 4 zeros and no gain, the next species 3 zeros and a gain of $k$ and so on. The last species at the end of the chain, opposite to the perturbation, has no zeros. The last species shows a very severe low pass behavior and it has the highest gain of $k^4$.)

Perturbation in the middle of the chain:

Perturbed species has (in its frequency transform) as many second order zeros as the number of species to reach one end. Transfer functions of the rest of species have as many zeros as the number of species to reach the end (moving in the opposite direction of where the perturbation is), adding by the number of species between the end and the middle of the chain (where the perturbation is applied). The gain value is again $k^{N_{step}}$, where $k$ is the reaction coefficient and $N_{step}$ is the number of step away from the perturbation. (i.e. in a network composed by a total of 5 species and where the perturbed species is the 3rd, the latter will have 2 second order zeros and no gain, its neighbors will have 3 zeros and a gain of $k$, and other species will have 2 zeros and gain of $k^2$)

We could now attempt a sort of general rule:

Perturbed species have as many zeros as the number of species to reach the right end, adding the number of species to reach the left end of the network. The rest of species has as many zeros as the number of species to reach the end (moving in the opposite direction of where the perturbation is), added by the number of species between the other end and where the perturbation is applied. The gain value is $k^{N_{step}}$, where $k$ is the reaction coefficient and $N_{step}$ is the number of step away from the perturbation. (i.e. in a network composed by a total of 5 species and where the perturbed species is the 2nd, the frequency transform of the latter will have 4 zeros (3+1) and no gain, of the first species will have 3 zeros (0+3) and a gain of $k$, of the third species will have 3 zeros (2+1) and gain $k^2$, of the fourth species will have 2 zeros (1+1) and gain $k^3$, and for the last species will have 1 zeros (0+1) and gain $k^4$.)

The denominator follows the general rules that have been written at the beginning of this
4.3 CNPs analysis: the Disperser

The analysis of chemical communication network with feedback branches allows us to describe an already existing protocol used by network designers, [1]: the "Disperser" protocol. In Chap. 2, this CNP has been introduced and its role in chemical communications has been explained. Now, a frequency characterization and the respective dynamical behavior are supplied. As we can see from Fig. 4.26, the Disperser has no output. Thus, according to Sect. 1.3.1 and Sect. 4.1, the Disperser network will have a pole at origin. Talking about the time domain, observations about its response to an impulse signal will be more interesting rather than to a step. Note that the application of an impulse perturbation to the network, means an instantaneous injection of molecules in the node where the perturbation is considered placed. On the contrary, the step response of a Disperser node, would show a continuous increase of the concentration, never leading to a stable condition.

Using the usual procedure and tools, we get the frequency response of the node (species) and the plots of the frequency behavior (Fig. 4.27) and time behavior (Fig. 4.28) of Disperser nodes (species). The perturbation is applied to $C_1$-species.

Laplace transform of $C_1$-species:

$$C_1(s) = \frac{s^3 + s^2 \cdot 7k + s \cdot 11k^2 + 3k^3}{s(s^2 + 2k + 3k^2 + 12k^3)} \cdot V_{\text{in}}(s) =$$

$$= \frac{(s^2 + s \cdot 4k + k^2)(s + 3k)}{s(s + 4k)(s + k)(s + 3k)} \cdot V_{\text{in}}(s)$$

\[\text{In the Disperser chemical model, network nodes are equivalent to species. The chemical vessel includes only a single species.}\]
Figure 4.27: The frequency behaviors of Disperser CNP species, when molecules are injected in node $C_1$. Reaction coefficients are all equal to 10.

Figure 4.28: The time behaviors of Disperser CNP species, when 1000 molecules are injected in node $C_1$. Reaction coefficients are all equal to 10. Analysis results ($C_i$) are overlayed to Fraglets simulation results ($C_i^s$).
Laplace transform of $C_2$-species:

\[
C_2(s) = \frac{k(s^2 + s \cdot 3k + k^2)}{s(s^3 + s^2 \cdot 8k + s \cdot 19k^2 + 12k^3)} \cdot \frac{C_1(s)}{V_{in}(s)} = \]

\[
\frac{k(s + k)(s + 3k)}{s(s + 4k)(s + k)(s + 3k)} \cdot V_{in}(s)
\]

Laplace transform of $C_3$-species:

\[
C_3(s) = \frac{k^2(s + 3k)}{s(s^3 + s^2 \cdot 8k + s \cdot 19k^2 + 12k^3)} \cdot \frac{C_1(s)}{V_{in}(s)} = \]

\[
\frac{k^2(s + 3k)}{s(s + 4k)(s + k)(s + 3k)} \cdot V_{in}(s)
\]

The Laplace transform of $C_4$-species can be seen directly from the previous one. Indeed, looking where the input is applied, $C_4$-species is symmetric to $C_3$-species.

The transfer function can be calculated referring to the species concentrations:

\[
H(s) = \begin{cases} 
\frac{C_1(s)}{V_{in}(s)} & \text{referring to } C_1\text{-species} \\
\frac{C_2(s)}{V_{in}(s)} & \text{referring to } C_2\text{-species} \\
\frac{C_3(s)}{V_{in}(s)} & \text{referring to } C_3\text{-species} \\
\frac{C_4(s)}{V_{in}(s)} & \text{referring to } C_4\text{-species}
\end{cases}
\]

Again, all species present a low pass filter behavior, the most severe ones are constituted by $C_3$-species and $C_4$-species. In the time domain, this network is characterized only by slow changes of species concentrations. As we have seen in Chap. 2.1, this CNP leads to the distributed computation of the average concentration of network nodes, refer to Fig. 4.28.

We have reported also Fraglets simulation results of this four-node Disperser. Using the same model parameters, all plotted functions in Fig. 4.28 are very close to the ones obtained in a Fraglets simulation. The deviation between curves of simulation and curves of analysis is due to the stochasticity of the exact process. We have repeated analysis and simulation in the case of an injection of 10000 molecules. The difference between the curves is even no more appreciable. Remind that, when the number of molecules gets higher, the stochasticity effect decreases.

Our analysis approach supplies the same results as the ones obtained by CNP pioneers in [9] and [5].
4.4 Delay Link and Respective Chemical Model

Until now, we have always assumed that links show no delay in forwarding packets from one species to another of the communication network. Thus, what happens when consumed molecules are not immediately created in the neighbor species? Kinetics deals with this situation with delay differential equations (DDEs), differential equations that specify the dependence of a species concentration to the time and to the involved delay. However, the solution to DDEs is not so easy to find, even when delays are fixed and discrete. Chemical designers [29] have partially avoided the problem, conducting their studies at equilibrium, when the total number of molecules in the network equals the number of molecules in network species. Namely, no molecules (or packets) are traveling and thus no molecules are contained inside links. On one hand this approach gives an immediate vision of the network state at equilibrium, on the other hand it will be useless in most of projecting stages. The usual goal of a communication network designer is to analyze dynamical behaviors and to make as rare as possible transitions of the network state, which could result critical for its functionality. Other forwarding step has been to speculate a chemical model, that shows analogous properties as the original delayed model: packets (molecules) should be delayed for $\tau$, where $\tau$ is the delay, it should have the same input rate, $v_{\text{in}}$, and the same output rate, $v_{\text{out}}$, and an amount of $c = v_{\text{in}}\tau$ of packets (molecules) should be stored within the link. The depicted chemical model could be the elementary chemical model with one output in Fig. 4.29, which has been analyzed in Sect. 4.1.1. The reaction coefficient would be inversely proportional to the delay, $\tau$: $k = \frac{1}{\tau}$.

This chemical model of a delayed link is rigorously valid only when stable conditions are reached, while can not depict the exact transition behavior. To study the effect of a delayed link we have to introduce a model based on a block with a discrete delay $\tau$. Such a model differs from the real situation, only for the fact that the delay is not continuously distributed. This mismatch has completely no effect on the analysis. Figure 4.30 shows the new model.

---

\(^7\)Informatics packages are chemical molecules.
Comparison between transfer functions of delay block model, Fig. 4.30, and of an equivalent chemical model, Fig. 4.29. $k = \frac{1}{T} = 3$.

Comparison between step responses of delay block model, Fig. 4.30, and of an equivalent chemical model, Fig. 4.29. $k = \frac{1}{T} = 3$.

Following, the transfer function and the impulse response of this new model are reported, (4.23). The two models are also mathematically and graphically compared, Fig. 4.31 and
Fig. 4.32. For the delay block model:

$$H(s) = e^{-\frac{t}{\tau}} = e^{-1/s} \Rightarrow h(t) = \delta \left( t - \frac{1}{k} \right) = \delta(t - \tau)$$ (4.23)

While, rewriting results of Sect. 4.1.1, a chemical model of a delayed link would have the following transfer function:

$$H(s) = \frac{k}{s + k} = \frac{1}{s + 1/\tau} \Rightarrow h(t) = e^{-kt} = e^{-\frac{t}{\tau}}$$ (4.24)

It is now clear that the chemical model can only approximate the real dynamical behavior of a link with delay, while it has same features at equilibrium. Moreover in the frequency domain, the chemical model of a link with delay shows a low pass behavior and thus it has a bandwidth limitation that the equivalent real link does not present. Note that this limitation of frequencies is not directly referred to the input packet rate (molecule concentration), instead, this low pass filter effects on the variation of the packet rate.

### 4.4.1 Communication Network with Feedbacks and Delay Links

With analysis based upon delay block models, the prediction of the real dynamical behavior is possible even for complex chemical communication networks. The following is an example of results obtained through the use of introduced models in a communication network with feedback branches: Fig. 4.33 and Fig. 4.34. The two-species loop network with output has been reconsidered. Its study is reported in Sect. 4.2.1. Links have been supposed introducing a delay of $\tau = \frac{1}{k_d}$.

Figure 4.33: Delayed model in a two-species loop network with output and link delay of $\tau = \frac{1}{k_d}$. It is a review of already analyzed network in Fig. 4.19.

Figure 4.34: Chemical model of a two-species loop network with output and link delay of $\tau = \frac{1}{k_d}$. It is a review of already analyzed network in Fig. 4.19.
Figure 4.35: Comparison of transfer functions of two-species loop network (network in Fig. 4.33/Fig. 4.34) obtained by using block delay model and chemical one. $k = \frac{1}{\tau} = 3$.

Figure 4.36: Comparison of step responses of two-species loop network (network in Fig. 4.33/Sect. 4.34) obtained by using block delay model and chemical one. $k = \frac{1}{\tau} = 3$.

and step responses of the two models. As we can see from the step response graph, the simpler chemical model does not lead always to a large deviation between the approximated and the effective transition behavior. Nevertheless, this is true only for not so high delays.
When delay values become significant, the approximation is not so far acceptable, especially for network stability studies. Concluding, the analysis of chemical communication networks should include link delays. However, the rigorous solution of ODEs is complicated even for simple networks. Also, the use of chemical delay model introduced by T. Meyer in [29], can not describe the exact dynamical behavior of the network, but sometimes this model can give a good approximation of it. Anyway, calculations in such a procedure are the simplest ones. Probably, this model is the tool to prefer to study the network at equilibrium and to have a rough view of its behavior even for transition stages. The deviation from approximation and the actual behavior depends on delay values, comparing to the value of other network constants. If there is the need to analyze distresses of networks, as the study of stability of distributed reactions, the exploiting of introduced frequency model will be requested. With the side effect of more complicated calculations, such a model allows plotting the actual response of delayed network in the frequency and the time domain.

4.5 Non-Linear Reaction

The last big step is the analysis of non-linear chemical networks. As well as links with delays, non-linearity is a characteristic of a real communication network almost always present. Thus, its modeling and treatment are indispensable. In Sect. 3.2.5, this new complicated method has been introduced. In this section, its effective application is shown.

4.5.1 Metabolic Control Analysis for Studied Linear Reactions

Let us start with an already analyzed network which is linear and whose behaviors, in the frequency and the time domains, are now known: the two-species network with feedback (network in Fig. 4.19). This study has been reported for propaedeutic purposes. We start with not too complicated systems. As for the case in App. 6.5, this study can also represent a sort of validation of the whole section. The first step is finding differential equations which describe the behavior of the network.

\[
\begin{align*}
\dot{c}_1 &= k_{in} - k_1 c_1 + k_2 c_2 + 0 \\
\dot{c}_2 &= 0 + k_1 c_1 - k_2 c_2 - k_{out} c_2
\end{align*}
\] (4.25)
The rate vector, $V$, and the state vector, $C$, are respectively

$$V = \begin{pmatrix} k_{\text{in}} \\ k_1 \cdot c_1 \\ k_2 \cdot c_2 \\ k_{\text{out}} \cdot c_2 \end{pmatrix}$$

$$C = \begin{pmatrix} c_1 \\ c_2 \end{pmatrix}$$

while the stoichiometric matrix, $N$, is no more than an indicator of reaction signs. $N$ is directly found from ODEs (4.25):

$$N = \begin{pmatrix} 1 & -1 & 1 & 0 \\ 0 & 1 & -1 & -1 \end{pmatrix}$$

The perturbation must be considered applied to the reaction coefficient of the inflow, $k_{\text{in}}$, and thus

$$P = \begin{pmatrix} k_{\text{in}} \\ 0 \\ 0 \\ 0 \end{pmatrix}$$

leading to getting derivatives, which are expression of the validity of our study, only for small changes of the system parameters:

$$\frac{\partial V}{\partial C} = \begin{pmatrix} 0 & 0 & k_1 & 0 \\ 0 & k_2 & 0 & k_{\text{out}} \end{pmatrix}$$

$$\frac{\partial V}{\partial P} = \begin{pmatrix} 1 & 0 \\ 0 & 0 \\ 0 & 0 \end{pmatrix}$$

Other important step of the MCA is evaluating steady states of the network by solving differential equations at equilibrium, $N \cdot V = 0$. This is the implicit description of $C = C(P)$. Note that steady state represents the fixed point around which the system response will be evaluated.

$$C_s = \begin{cases} c_{1s} = k_{\text{in}} - k_1 c_1 - k_2 c_2 + 0 = 0 \\ c_{2s} = 0 + k_1 c_1 - k_2 c_2 - k_{\text{out}} c_2 = 0 \end{cases} \rightarrow \begin{cases} c_{1s} = \frac{-k_2}{k_{\text{out}}} \\ c_{2s} = \frac{k_{\text{in}}}{k_{\text{out}}} \end{cases}$$
Using previous elements evaluated around steady states, it is possible to find the system
matrix, \( A \), and the input matrix, \( B \):
\[
A = N \cdot \left. \frac{\partial V}{\partial C} \right|_{(C_s, P_s)} = \begin{pmatrix} -k_1 & k_2 \\ k_1 & -k_2 - k_{out} \end{pmatrix}
\]
\[
B = N \cdot \left. \frac{\partial V}{\partial P} \right|_{(C_s, P_s)} = \begin{pmatrix} 1 \\ 0 \end{pmatrix}
\]

Being here the main goal of the MCA the species concentration, the output matrix is the
identity matrix, \( O = I \), while the direct transmission matrix is null, \( D = 0 \). In the end, we
can linearize the system dynamics, \( \dot{S} = N \cdot V(S, P) \), around the point \((C_s, P_s)\):
\[
\dot{x}(t) = \left[ N \cdot \frac{\partial V}{\partial C} \right] x(t) + \left[ N \cdot \frac{\partial V}{\partial P} \right] u(t) \tag{4.28}
\]

where \( x(t) = c(t) - c_s \) and \( x(t) = p(t) - p_s \).

The transfer function of a system that is approximatively described by (4.28), can be found
as following:
\[
C(s) = \left( O (sI - A)^{-1} B + D \right) \cdot V_{in}(s) = \begin{pmatrix} C_1(s) \\ C_2(s) \end{pmatrix} = \begin{pmatrix} \frac{k_{out} s + k_2 + k_{out}}{s^2 + s(k_1 + k_2 + k_{out}) + k_1 k_{out}} \cdot V_{in}(s) \\ \frac{k_1 k_{out}}{s^2 + s(k_1 + k_2 + k_{out}) + k_1 k_{out}} \cdot V_{in}(s) \end{pmatrix}
\]
\[
H(s) = \frac{V_{out}(s)}{V_{in}(s)} = \frac{k_{out} C_2(s)}{V_{in}(s)} = \frac{k_1 k_{out}}{s^2 + s(k_1 + k_2 + k_{out}) + k_1 k_{out}} \tag{4.29}
\]

Our goal is satisfied: equation (4.29) is the same as the already found equation (4.16). The
reader has probably already noted the bizarreness of linearizing a linear system. Note that
steady states have never been used, because the derivatives, \( \frac{\partial V}{\partial C} \) and \( \frac{\partial V}{\partial P} \), do not depend on
states, see (4.26) and (4.27). This situation is due to the linearity of the studied system.
Obviously the solution of linear models is not related to the evaluation only around the fixed
point. Things will change in the MCA of non-linear networks.

### 4.5.2 Quine Analysis

After the previous warm-up subsection, the MCA is now applied in order to get the dynamical
behavior of "the Quine". This chemical network has been introduced in Sect. 2.2.1. The
reaction network is represented in Fig. 2.4. The goal here is the transfer function of the
network, \( H(s) \). From Fig. 2.4, it is clear that the output is the multiplication of concen-
trations of \( C_1 \)-species and \( C_2 \)-species. Thus, in evaluating the transfer function, \( H(s) \), the
convolution of frequency transforms of these two species is needed. A solution that avoid such a mathematical complication is to add a species to the network. This new species should be placed where there is the wanted output, Fig. 4.37. The transfer function of the new network is obtained by taking as output of the network, the output from the last added block. Of course, the so obtained transfer function will differ from the original one, because of the effect of the added species. However, the latter can be seen as a block in series to the original network. Specifically, it can be seen as a low-pass filter. In this way, its effect on the global transfer function of the network can be easily isolated. Resuming, the following MCA will be refereed to network in Fig. 4.37 instead of the one in Fig. 2.4. Nevertheless, by considering the effect of $C_{out}$-species, the transfer function of the real Quine scheme will be supplied.

As for the previous linear network, the analysis starts from differential equations. This time, non-linearities are present. They are constituted by the multiplication between species concentrations:

$$
\begin{align*}
\dot{c}_1 &= k_1 - k_2 c_1 c_2 + 0 - c_1 \phi + 0 \\
\dot{c}_2 &= 0 - k_2 c_1 c_2 + 2k_3 c_3 c_4 - c_2 \phi + 0 \\
\dot{c}_3 &= 0 + k_2 c_1 c_2 - k_3 c_3 c_4 - c_3 \phi + 0 \\
\dot{c}_4 &= 0 + 0 + k_3 c_3 c_4 - c_4 \phi + 0 \\
\dot{c}_5 &= 0 + k_2 c_1 c_2 + 0 - 0 - k_{out} c_{out}
\end{align*}
$$

Some words have to be spent on what $\phi$ is. $\phi$ is a function, named ”the dilution flow” [30], which depends on reaction coefficients and species concentrations. The role of $\phi$ is related to the capacity of the vessel [5] (refer to Sect. 2.2.1). Specifically, $\phi$ guarantees that the population (number of molecules)

$^8$It is equivalent to talk of the number of packets in a communication system.
Outflows from species which are related to the dilution flow, are not governed by the usual law of mass action. The demonstration of the way to mathematically obtain $\phi$ is not our goal. Its final form is just reported and used: $\phi = k_1 - k_2 c_1 c_2 + 2k_3 c_3 c_4$. Reactions which are involved in the dilution flow, are not reported in Fig. 4.37. Anyway, they have to be considered in differential equations. Note that the last added species is actually outside from the "flow reactor" and for that reason, it can be not considered in the dilution flow.

The rate vector, $V$, and the state vector, $C$, are respectively

$$V = \begin{pmatrix} k_1 & k_2 c_1 c_2 & k_3 c_3 c_4 & k_1 c_1 & k_1 c_2 & k_1 c_3 & k_1 c_4 & k_2 c_1 c_2 & k_2 c_1 c_2^2 \\ k_2 c_1 c_2 & k_2 c_1 c_2 & k_2 c_1 c_2 & k_3 c_3 c_4 & k_3 c_3 c_4 & k_3 c_3 c_4^2 & k_3 c_3 c_4^2 & k_3 c_3 c_4^2 & k_3 c_3 c_4^2 & k_3 c_3 c_4^2 \end{pmatrix}^\top$$

while the stoichiometric matrix, $N$, directly found from ODEs (4.30), is

$$N = \begin{pmatrix} 1 & -1 & 0 & -1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & -2 & 0 & 0 & 0 & 0 \\ 0 & -1 & 2 & 0 & -1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & -2 & 0 & 0 & 0 \\ 0 & 1 & -1 & 0 & 0 & -1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & -2 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & -2 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 \end{pmatrix}$$

The perturbation is considered applied to the inflow reaction coefficient, $k_1$, and thus

$$P = \begin{pmatrix} k_1 & 0 & \cdots & 0 \end{pmatrix}^\top$$

leading to getting derivatives, which are expression of the validity of our study only for small
4.5 Non-Linear Reaction

changes of the system parameters:

\[
\frac{\partial V}{\partial C} = \begin{pmatrix}
0 & 0 & 0 & 0 & 0 \\
k_2 s_2 & k_2 c_1 & 0 & 0 & 0 \\
0 & 0 & k_3 c_4 & k_3 c_3 & 0 \\
k_1 & 0 & 0 & 0 & 0 \\
0 & k_1 & 0 & 0 & 0 \\
0 & 0 & k_1 & 0 & 0 \\
0 & 0 & 0 & k_1 & 0 \\
2 k_2 c_1 c_2 & k_2 c_1^2 & 0 & 0 & 0 \\
k_2^2 & 2 k_2 c_1 c_2 & 0 & 0 & 0 \\
k_2 c_2 c_3 & k_2 c_1 c_3 & k_2 c_1 c_2 & 0 & 0 \\
k_2 c_2 c_4 & k_2 c_1 c_4 & 0 & k_2 c_1 c_2 & 0 \\
k_3 c_3 c_4 & 0 & k_3 c_1 c_4 & k_3 c_1 c_3 & 0 \\
0 & k_3 c_3 c_4 & k_3 c_2 c_4 & k_3 c_2 c_3 & 0 \\
0 & 0 & 2 k_3 c_2 c_4 & k_3 c_3^2 & 0 \\
0 & 0 & k_3 c_4^2 & 2 k_3 c_3 c_4 & 0 \\
0 & 0 & 0 & 0 & k_{out}
\end{pmatrix}
\] (4.31)

\[
\frac{\partial V}{\partial P} = \begin{pmatrix}
1 & 0 & 0 & c_1 & c_2 & c_3 & c_4 & 0 & \ldots & 0
\end{pmatrix}^T
\] (4.32)

As we have anticipated in the previous subsection, in the Quine analysis there is really the
need to evaluate the behavior around a fixed point. Indeed, the Quine is an actual non-
linear model and so there is a dependence from network states, refer to (4.31) and (4.32).
The evaluation of steady states of the Quine requires a lot of simple calculations. They are
accomplished by solving differential equations at equilibrium, \( \mathbf{N} \cdot \dot{\mathbf{V}} = 0 \)

\[
C_s = \begin{cases}
  c_{1_s} = \frac{1}{2} \left( 1 - \sqrt{1 - k_1} \right) \\
  c_{2_s} = \frac{1}{4} \left( 3 - c_{1_s} - \sqrt{1 + 2 k_1 + 2 c_{1_s} + c_{1_s}^2} \right) \\
  c_{3_s} = \frac{1}{2} \left( 1 - \sqrt{1 - 2 c_{2_s} - c_{1_s}} \right) \\
  c_{4_s} = \frac{1}{2} (1 - c_{1_s}) \\
  c_{out_s} = \frac{k_{out}}{k_2} c_{1_s} c_{2_s}
\end{cases}
\] (4.33)
The system matrix, \( A \), and the input matrix, \( B \), result respectively

\[
A = \frac{N \cdot \partial V}{\partial C}(C_e, P_e) = \begin{pmatrix}
2k_2c_1c_2 - k_2c_2 - k_1 - 2k_3c_3c_4, & k_2c_1^2 - k_2c_1,
2k_2c_1c_2 - k_2c_1 - k_1 - 2k_3c_3c_4, & k_2c_1 + k_2c_3
k_2c_2c_4, & k_2c_1c_4,
-k_2c_2, & k_2c_1,
-2k_3c_1c_4, & -2k_3c_1c_3,
0, & 0
\end{pmatrix}
\]

\[
B = \frac{N \cdot \partial V}{\partial P}(C_e, P_e) = \begin{pmatrix}
1 - c_1, & -c_2, & -c_3, & -c_4, & 0
\end{pmatrix}^T
\]

Being here the main goal of the MCA the species concentration, the output matrix is the identity matrix, \( O = I \), while the direct transmission matrix is null, \( D = 0 \). In the end, we can linearize the system dynamics, \( \dot{S} = N \cdot V(S, P) \), around the point \((C_e, P_e)\). This means that all involved vectors and matrices have to be evaluated around such a point, using steady states of (4.33):

\[
\dot{x}(t) = \left[ \frac{N \cdot \partial V}{\partial C} \right] x(t) + \left[ \frac{N \cdot \partial V}{\partial P} \right] u(t) \tag{4.34}
\]

where \( x(t) = c(t) - c_s \) and \( x(t) = p(t) - p_s \).

The frequency transform of network species can be found as following

\[
C(s) = \left( O \left( sI - A \right)^{-1} B + D \right) \cdot V_{in}(s) = \begin{pmatrix}
C_1(s),
C_2(s),
C_3(s),
C_4(s),
C_{out}(s)
\end{pmatrix}
\]

The reaction coefficient of the original Quine are unitary, \( k_2 = k_3 = 1 \). Note that the value of the reaction coefficient of the last species will have no influence on the transfer function of the original Quine in Fig. 2.4. The input is a small perturbation of reaction coefficient \( k_1 \). 

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and our interest is to evaluate the transfer function for different values of such a coefficient, when small perturbations are applied: $k_1 = 0.0 \div 0.125$

$$H(s) = \frac{V_{\text{out}}^{\text{wanted}}(s)}{V_{\text{in}}(s)} = \frac{C_{\text{out}}(s)}{V_{\text{in}}(s)} \cdot (s + k_{\text{out}})$$

As we would expect, the frequency transform of the last added species, $C_{\text{out}}(s)$, shows a pole defined by $k_{\text{out}} = 10^3$. In order to get the transfer function of the original Quine in Fig. 2.4, this pole must be compensated.

The transfer function, as well as the step response, of the original Quine is plotted respectively in Fig. 4.39 and Fig. 4.40. All frequency transforms show a low pass filter behavior. As almost always in chemistry world, time domain behaviors are dictated by slow changes, since low frequencies are advantaged over high ones. The final frequency response of the Quine is a low pass filter of the first order. The pole, or the cutoff frequency, is related to the choice of reaction coefficients, obviously not $k_{\text{out}}$. In the analysis with $k_2 = k_3 = 1$ the cutoff frequency results:

$$f_{\text{cutoff}} = \begin{cases} 0.5000 & (k_1 = 0.000) \\ 0.5125 & (k_1 = 0.025) \\ 0.5249 & (k_1 = 0.050) \\ 0.5373 & (k_1 = 0.075) \\ 0.5497 & (k_1 = 0.100) \\ 0.5620 & (k_1 = 0.125) \end{cases} [\text{rad/s}]$$

In this case, only the reaction coefficient $k_2$ mainly defines the pole: when $k_1 = 0$ then $f_{\text{cutoff}} = \frac{k_2}{2}$, for other values of $k_1$ things get complicated and all coefficients contribute to shape the transfer function and frequency transforms of species concentrations. Appendix 6.6 reports graphical results related to different choices of reaction coefficients. The result that the Quine shows a first order low pass filter behavior is great: in design stages, when the dynamics of a network behavior is studied, the Quine can be simply replaced by the elementary reaction in Fig. 4.4 of Sect. 4.1.1, since the latter and the Quine have finally the same transfer function. This assertion leads to enormous simplification in network analysis and it reassures those who have designed this chemical networking protocol. Note that the Fraglets simulator, which is currently the only possible implementation of such networking philosophy, mandatorily requires bimolecular reactions. The Quine architecture derives from that need. Indeed, in order to achieve a self-healing protocol [5], it would have been sufficient the elementary reaction in Fig. 4.4. However, the exigency of bimolecularity of the Fraglets

\[\text{rad/s}\]
Figure 4.38: Species of network in Fig. 4.37 for different values of $k_1$: frequency transforms normalized respecting to $V_{in}(s)$ and concentrations when a step perturbation is applied on $k_1$. 
Figure 4.39: The transfer function of the original Quine in Fig. 2.4 for different values of $k_1$.

Figure 4.40: The step response of the original Quine in Fig. 2.4 for different values of $k_1$. 
simulator, has led to designing a complex structure. Its final form is shown in Fig. 2.4. In other words, this subsection widely supports and reiterates the logic of Quine designers [5], which have been introduced in Chap. 2.

Another due observation refers to the time domain: the step response of the network has been plotted. As we know, it means an immediate perturbation with unitary amplitude on the reaction coefficient $k_1$. This could arise concerns, since the intrinsic low value of such a coefficient. However, the system has been linearized and thus this problem can be considered only an amplification matter. Namely, we should find the response of the Quine to a step perturbation which is commensurate to the nature of $k_1$. To do that, the division of the given step response by an opportune coefficient is sufficient. We have preferred to show a step response with unitary amplitude, because it is normally defined in this way.

A brief consideration about the mere analytic method:
There would be also another procedure that leads to obtaining the same transfer function, without the use of the trick of the additive species. This method is still based upon the MCA, but it considers the normal Quine structure in Fig. 2.4. This time, the MCA focuses directly on rate variations, instead of on species concentrations. In that case, according to [27], the output matrix and the direct transmission matrix are respectively $O = \frac{\partial V}{\partial S}$ and $D = \frac{\partial V}{\partial P}$ (not as usual $O = I$ and $D = 0$). The vector of the frequency transform of normalized reaction rate variations, $R(s)$, would count fourteen elements, as the number of reactions (also considering the dilution flow). The normalization is referred to the variation of $k_1$, where the perturbation is applied (the input). The transfer function of our interest would be just the one related to the output reaction, $v_1 = k_2c_1c_2$, namely the second element of the vector obtained with

$$R(s) = O (I \cdot s - A)^{-1} B + D$$  \hspace{1cm} (4.35)

Both methods lead to the identical result. The difference is in the computation complexity. The procedure which has been explained in details in this subsection, has easier calculations. Nevertheless, it has required the shrewdness of the additive species.

4.5.3 Analysis of the Congestion Avoidance CNP

The last analyzed chemical network with non-linearities is oriented to study performances of a protocol dealing with congestion avoidance. The behavior of the network in Fig. 4.41 is described by the following differential equations:

$$\left\{ \begin{array}{l}
\dot{w} = k_1 + 0 - k_3 \cdot w \cdot l + 0 \\
\dot{l} = 0 + k_2 \cdot w - R \frac{k_2 w}{k_2 w + v_{in}} + 0 - k_4 \cdot l
\end{array} \right.$$  \hspace{1cm} (4.36)
Note the ratio term in (4.36), \(-R\frac{k_2 w}{w + v_{in}}\). The latter leads to an extreme complication of the whole analysis. For that reason, we have preferred to report the entire analytic treatment in App. 6.7, giving in this subsection, only general steps of the analysis and final results of it. Obviously, the used procedure is similar to the one of previous cases: from ODEs (4.36), rate vector \(V\), state vectors \(C\) and stoichiometric matrix \(N\) are found. Once we have defined \(v_{in}\) variable as input, namely once fixed \(P\) vector, the derivatives are obtained: \(\frac{\partial V}{\partial C}\) and \(\frac{\partial V}{\partial P}\).

Then, the values of steady states\(^9\) have to be replaced in last calculated elements. For already known reasons, the output matrix and direct transmission matrix are directly taken as \(O = I\) and \(D = 0\), while the system and the input matrix have to be found:

\[
\begin{align*}
A &= N \cdot \frac{\partial V}{\partial C} \\
B &= N \cdot \frac{\partial V}{\partial P}
\end{align*}
\]

At this point, the linearization is possible:

\[
\dot{x}(t) = \left[ N \cdot \frac{\partial V}{\partial C} \right] x(t) + \left[ N \cdot \frac{\partial V}{\partial P} \right] u(t) \tag{4.37}
\]

where \(x(t) = c(t) - c_s\) and \(x(t) = p(t) - p_s\).

The frequency behaviors of the species is calculated using linearization (4.37):

\[
C(s) = \left( O \left( sI - A \right)^{-1} B + D \right) \cdot V_{in}(s)
\]

Note that the transfer function of this complex chemical network is obtained from the frequency transform of \(W\)-species:

\[
H(s) = \frac{V'_{out}(s)}{V_{in}(s)} = \frac{k_2 W(s)}{V_{in}(s)} \tag{4.38}
\]

\(^9\)Steady states are solutions of ODEs (4.36) at equilibrium.
Indeed, we care about the packet flow through $W$-species. Note that the traffic which is constituted by $v_{\text{in}}$, is the additive service which is sharing the total available channel with the flow through $W$-species. This chemical networking protocol should manage the sharing of the same channel by more users. Namely, the system has to be able to adapt to new situations as soon as possible and in the best way. We are interested in the network behavior at the output of $W$-species, when instantaneously a new traffic $v_{\text{in}}$ starts to send packets. Once again, we underline that the whole rigorous analysis is postponed to App. 6.7. In this subsection instead, the plots of the results gained from (4.38) are directly shown: Fig. 4.42 and Fig. 4.43. Graph 4.43 specifically shows such a network adaptation: how the concentration of $W$-species changes, consequently to the start of a new service. The new service is using the same channel and thus, it is limiting the maximum packets rate through $W$-species. Note that a physical channel has an intrinsic bandwidth. In order to have an error probability lower than a prefixed maximum value, this bandwidth binds the maximum rate allowed in it. Thus, when a new packet flow starts to occupy the channel, the already present service has to reduce its rate.

The time domain graph 4.43 shows the same shape and the same features in Fig. 2.8. The theoretical predictions about a general congestion avoidance protocol in Chap. 2 have been satisfied. Let us benefit of such a new representation: by the "calibration" of reaction coefficients $k_3$ and $k_4$, the adjustment of dynamical behavior of $W$-species is possible. Roughly, values of $k_4$ too small lead to fast changes in the concentration. However, the behavior of the network in $W$-species remains non-stable for long time. To make this time shorter, $k_4$ has to be increased in value. Oscillations still remain. Taking small value of $k_3$ moves instead the result in the opposite direction: less oscillations, but slower time to have an answer from $W$-species.

Our target here can be appreciated: we would a transfer function with two real negative poles, instead of a complex conjugate pair of poles. For instance referring to Fig. 4.42, the black line trace is defined by a frequency response with no zeros and two real poles: $p_1 = -1.129$ and $p_2 = -8.871$ (damping=1). The black line trace was obtained with $k_3 = 0.01$ and $k_4 = 10$. Instead, the yellow line was dictated by no zeros and the complex conjugate pair of poles $p_{1/2} = -0.1 \pm 31.6i$, with the damping value equals 0.0158. This analysis was obtained using $k_3 = 1$ and $k_4 = 1$.

\(^{10}\)The channel bandwidth is defined by several elements, like the nature of the channel itself, the environment where it is used or the communication technics that have been adopted.

\(^{11}\)The error probability is related to the probability to lost packets.
Figure 4.42: The frequency response of the congestion control protocol. Note that Fig. (b) is a zoom in Fig. (a).
Figure 4.43: The step response of the congestion control protocol. Note that Fig. (b) is a zoom in Fig. (a).
4.6 Band Pass Chemical Reaction Network

Almost all chemical networks show the frequency behavior of a low pass filter: lower frequency components are advantaged respect to higher ones. Following instead, an example of a chemical network which has a band pass behavior. The non-linear reaction system in Fig. 4.44 has been used. The MCA is required. Note that the non-linearity is due to what has been taken as input: reaction coefficient \( k_2 \). A perturbation of this network parameter can be obtained taking advantage of the structure in Fig. 4.44. Note that such an input can be implemented on the Fraglets simulator by the use of a bimolecular reaction where another extra species reacts with \( C_1 \)-molecule, generating a \( C_2 \)-molecule. The reaction rate would be driven by the concentration of both reactant species. Thus, modifying the abundance of molecule of the extra species, the reaction rate itself would be controllable.

Differential equations, which describe the behavior of this chemical network, are

Figure 4.44: The chemical network with a band pass behavior. It is obtained starting from the "loop network" in Fig. 4.19.
\[
\begin{aligned}
    \dot{c}_1 &= k_1 - k_2 \cdot c_1 + k_3 \cdot c_2 \\
    \dot{c}_2 &= k_2 \cdot c_1 - k_3 \cdot c_2 - k_{\text{out}} \cdot c_2
\end{aligned}
\] (4.39)

The rate vector, \( V \), and the state vector, \( C \), are respectively

\[
V = \begin{pmatrix}
    k_1 \\
    k_2 \cdot c_1 \\
    k_3 \cdot c_2 \\
    k_{\text{out}} \cdot c_2
\end{pmatrix}
\]

\[
C = \begin{pmatrix}
    c_1 \\
    c_2
\end{pmatrix}
\]

The stoichiometric matrix, \( N \), is directly found from ODEs (4.39):

\[
N = \begin{pmatrix}
    1 & -1 & 1 & 0 \\
    0 & 1 & -1 & -1
\end{pmatrix}
\]

As we have said, the perturbation must be considered applied to reaction coefficient \( k_2 \) (such an input leads to have the non-linearity), and thus

\[
P = k_2
\]

leading to getting derivatives, which are expression of the validity of our study, only for small changes of the system parameters:

\[
\frac{\partial V}{\partial C} = \begin{pmatrix}
    0 & 0 \\
    k_2 & 0 \\
    0 & -k_3 \\
    0 & k_{\text{out}}
\end{pmatrix}
\]

\[
\frac{\partial V}{\partial P} = \begin{pmatrix}
    0 \\
    c_1 \\
    0 \\
    0
\end{pmatrix}
\]

Steady states of the network are found by solving differential equations (4.39) at equilibrium, \( N \cdot V = 0 \). This is the implicit description of \( C = C(P) \). Note that the steady state represents the fixed point around which the system response will be evaluated.

\[
C_s = \begin{cases}
    c_{1s} = \frac{k_1 k_3 + k_1 k_{\text{out}}}{k_2 k_{\text{out}}} \\
    c_{2s} = \frac{k_1}{k_{\text{out}}}
\end{cases}
\]
Using previous elements evaluated around steady states, the system matrix, $A$, and the input matrix, $B$, can be found:

$$
A = \left. N \cdot \frac{\partial V}{\partial C} \right|_{(C_s, P_s)} = \begin{pmatrix}
-k_2 & -k_3 \\
2 & k_3 - k_{\text{out}}
\end{pmatrix}
$$

$$
B = \left. N \cdot \frac{\partial V}{\partial P} \right|_{(C_s, P_s)} = \begin{pmatrix}
-k_1 k_2 + k_1 k_{\text{out}} \\
k_2 k_{\text{out}} - k_1 k_{\text{out}}
\end{pmatrix}
$$

Being here the main goal of the MCA the species concentration, the output matrix is the identity matrix, $O = I$, while the direct transmission matrix is null, $D = 0$. In the end, we can linearize the system dynamics, $\dot{S} = N \cdot V(S, P)$, around the point $(C_s, P_s)$:

$$
\dot{x}(t) = \left[ N \cdot \frac{\partial V}{\partial C} \right] x(t) + \left[ N \cdot \frac{\partial V}{\partial P} \right] u(t)
$$

where $x(t) = c(t) - c_s$ and $x(t) = p(t) - p_s$.

The transfer function of the linearized system can be found as following:

$$
C(s) = \left( s I - A \right)^{-1} B + D \cdot V_{\text{in}}(s) = \begin{pmatrix} C_1(s) \\ C_2(s) \end{pmatrix}
$$

$$
C_1(s) = \frac{-(k_1 k_3 + k_1 k_{\text{out}})(k_{\text{out}} - k_3 + s)}{k_2 k_{\text{out}}(k_2 k_{\text{out}} + s(k_2 - k_3 + k_{\text{out}}) + s^2)} \cdot V_{\text{in}}(s)
$$

$$
C_2(s) = \frac{(k_2 + s)(k_1 k_3 + k_1 k_{\text{out}})}{k_2 k_{\text{out}}(k_2 k_{\text{out}} + k_2 s - k_3 s + k_{\text{out}} s + s^2)} \cdot V_{\text{in}}(s)
$$

$$
H(s) = \frac{V_{\text{out}}(s)}{V_{\text{in}}(s)} = \frac{C_2(s)}{C_1(s)}
$$

As we have planned, the transfer function advantages the frequency components around the magnitude of the pair of complex conjugate poles. This band pass behavior is definitely not so selective. Our goal here was only to find a frequency behavior different from the usual low pass filter. If the initial target was to achieve a specific filter shape, the design of chemical network should be done following the backward-procedure. First the frequency transform would be defined, in order to get the wanted filter shape. Then system, input, output and direct transmissions matrixes would be found. In the end, differential equations would be extrapolated and thus the chemical reaction network could be planned. However, the mere project of such a "chemical protocol filter" is not in this thesis ambition. We have limited to supply an example of a chemical network which has a different frequency behavior than the standard low pass one.
Figure 4.45: The frequency (a) and the time (b) behavior of $C_1$-species and $c_2$-species network in Fig. 4.44. The frequency plot is normalized respecting to the $V_{in}(s)$. Analysis with $k_1 = 6$, $k_2 = 2$, $k_3 = 1$ and $k_{out}$. 
4.7 Summary

In this chapter, we have given the frequency characterization of several typologies of chemical communication networks. Beside the frequency analysis, the dynamical network behavior in the time domain has been supplied. We have shown the transfer function of the "elementary block" in all its variants. Different kind of interconnections have been analyzed and then general rules have been extrapolated. These rules have been used in all the rest of network study. After series and parallel connections, feedbacks have been considered. Note that identifying elementary building blocks which constitute chemical networks and linking these building blocks with series, parallel and feedback interconnections, we could replace chemical networks with schematics composed by only transfer function blocks. Doing so, we could easily analyze linear system dynamics. We have analyzed more cases\(^\text{12}\) of similar topologies of networks with feedbacks (i.e."birth-dead networks" with or without output or with different displacements of the input). The large amount of studied cases should be sufficient to motivate the observations in Sect. 4.2.2. We have tried to generalize networks with feedbacks. The transfer function form have been generally defined: once denoted a specific network, we are able to predict the form of the frequency transforms of molecule species without any calculations. Nevertheless, the estimation of the specific pole/zero position still requires all computations, that anyway are easily performed in MATLAB environment. The loop network topology also includes a real CNP: the Disperser. Even such a protocol shows a low pass filter behavior and thus the usual exponential dynamical time behavior.

The chemical model of a delayed link, that was introduced by T.Meyer [29], has been compared with the standard model of delayed link, which shows an exponential term in frequency transform. This complication has been easily bypassed, by the use of MATLAB Control System toolbox. Then, the effects of the delay have been evaluated on more complex model. The result is that the basic model that was introduced in [29], can well approximate the exact dynamical behavior of a link with delays. Of course, for more complex networks the approximation can deviate more from the real model, thus the network designer should reach a compromise between analysis complexity and solution rigorousness.

The extended MCA [28] has led to characterizing another existing CNP: the self-healing protocol and the Quine chemical network. Again, results support the original idea in [5], which satisfies both the desire of self-healing features of networks and the need of bimolecular processes in the Fraglets environment. The extended MCA has been also applied to foresee

\(^{12}\)Several analysis have been done, but only important ones are in this section reported. Refer to the appendix in order to have all studied chemical communication networks.
the dynamics of CNP that still not really defined yet. Indeed, with this new analysis tool, we have confirmed CNP designers’s work in the congestion control avoidance. The calibration of reaction coefficients have allowed us to approach the wanted dynamical behavior. Of course, this represents only an example of what can be done using the new acquired methods of analysis. These procedures can be deeply applied to the design of many other CNPs. Probably, CNP designers should consider again the most interesting cases which have been already analyzed in this work. Now that designers can rely on frequency domain analysis, they should enhance the studies of these chemical models with a more ”communication target”. Remind that, this thesis has given tools and procedures to design CNPs, not chemical network solution directly useful in communication purpose.
Chapter 5

Conclusions and Perspectives

Chemically inspired networking may represent an innovative approach to communications. The advantageous feature of Chemical Networking Protocols (CNPs) is the design stage that, basing upon the system behavior, allows the prediction of stressed situations and thus their avoidance. Since the possibility to relate a communication protocol to a distributed chemical reaction network, the analysis of the chemical kinetics catches the CNP designers’s attention. The direct use of known chemical models, like the Chemical Master Equation (CME) or the Differential Rate Equation (DRE) approximation, often leads to very complicated solutions in the time domain\(^1\). This thesis has combined different analysis methods, mainly focussing on the frequency characterization of CNPs, or better, of their distributed chemical model. Often, by exploiting the frequency description, many relationships and operations over the original time-models correspond to simpler relationships and operations over the frequency-images. Note that, since the analysis with DREs is an approximation of the exact stochastic solution of the CME, also the frequency characterization refers to the deterministic approximation solution. CNPs dynamics can experience particular realizations of their random behavior which differ from the average behavior described with the frequency analysis.

Our contribution makes possible the analysis of CNPs based on both linear and non-linear chemical models. We propose to see linear networks as interconnections of elementary building blocks. Then, each of these elements is characterizable with its specific transfer function. The latter can be found without the solution of DREs, but directly looking at the simple structure of the elementary building block (number of outflows and respective reaction coefficients). We have defined mainly three elementary interconnections: series, parallels and feedbacks. We have described and formalized the effect of all of them. The benefits of such a perspective is that a linear network can be substituted with the respective "analysis scheme", which is composed by interconnected "black boxes". All black boxes are

\(^1\)Some times the rigorous solution is not possible at all.
described by a particular transfer function. In the end, the analysis scheme allows the fast computation\(^2\) of the chemical model behavior in both frequency and time domains. Moreover, since the linearity of the system, the superposition principle is valid. This observation can further simplify the treatment of the analysis. Once the transfer function of a more complex block\(^3\) is found, it could be directly used in all systems which would show the same portion of network. In the analysis scheme, the related black box could be directly applied, reducing the amount of needed computations.

Note that nowadays, all CNPs are implementable only on Fraglets simulator, which requires a chemical model with bimolecular reactions. This feature leads to the non-linearity of the chemical model. However, we have seen that this non-linearity often does not effect on the kinetics analysis and thus it can be bypassed. Actually, linear chemical reaction networks are implementable on Fraglets simulator.

Non-linear networks can also be analyzed. The non-linearity is due to the dependance of reactions on the multiplication of two species concentrations. For this type of networks, the computational complexity of the analysis would increase enormously. The Metabolic Control Analysis (MCA) provides a way to linearize the system and that helps to extremely reduce the effort in the analysis. Two more consequences of the use of the MCA must be highlighted. First, the linearization leads to an ulterior approximation\(^4\) of the description of system dynamics. Second, because of the linearity of the system, all methods described for linear networks are useable again. Moreover, the exact treatment of non-linear systems often leads to very specific results. Introducing small changes to parameters or to the network structure would mean the necessity of a complete new analysis. Instead, making use of the MCA, the modification of network parameters does not require a remake of the whole study. Even considering different inputs or outputs, the MCA remains the same.

Anyway, comparing to the treatment of real linear networks, the non-linear network solution still shows a high complexity. For that reason observations, as the one about the Quine structure, gain importance. Some complex non-linear networks could be characterized by the same transfer function\(^5\) as the one of very simple linear networks. In these cases, the whole

\(^2\)By the use of software packages, as MATLAB.

\(^3\)A block which is composed by more elementary building blocks, that are linked by elementary interconnections.

\(^4\)The first approximation comes from the use of DREs instead of the CME.

\(^5\)Having the same transfer function means showing the same frequency behavior and thus the same time dynamics.
non-linear network can be replaced by the analogous linear one. Referring to the analysis, the non-linear network can be directly substituted by a black box, which is characterized by the frequency transform only. Note that the replacement of a non-linear system with a linear one is not a linearization. Since the frequency transform would have the same form, there would be no approximation.

Dealing again with linear networks, a completely different aspect has been studied: links with delays. A delay could be chemically interpreted as a reaction that consumes a molecule of a certain reactant species but that does not immediately produce a molecule of the product species. The chemical model of a chemical networking link with delay was proposed by T. Meyer, [29]. We have compared the rigorous analysis of the exact behavior of a link with delay and the one found with the chemical model in [29]. The conclusion is that the latter approximates the rigorous link with delay. Considering the effect of a delayed link even inside a network, the deviation between the results can be acceptable or not, depending on the reaction coefficient dimensioning. The exact analysis which considers delayed links is simple for networks without a feedback reaction. Thus, when only parallels and series interconnections are present, the exact analysis is to prefer. However, the presence of a feedback complicates the treatment and so, the analysis with the use of the delayed link chemical model is definitely advantageous. Remind that in this case, the analysis could show differences from the rigorous one.

All analyzed CNPs show a low pass filter behavior. The only exception is the one we have supplied in order to get a band-pass behavior. Note that we have not followed the "backward-procedure": we do not start from the desiderate network dynamics and then we find the chemical model with the wanted behavior. We have simply given an example of a model with different behavior. We have always used the same analysis method that starts from the chemical model. However, the backward-procedure could be another tool that would help CNP design. At the moment, this probably represents more a curiosity rather than a real necessity. Anyway, it could be an argument for further studies.

Another wider unexplored field to be deepened is the rigorous treatment of the stochasticity aspect of the dynamics. Of course, since the exact CNP behavior in a particular realization can differ from the approximated average one, CNP designers should base their work upon the supplied deterministic dynamics, always caring about this difference. A very interesting project could be to statistically describe the deviation between exact and deterministic solutions and then to give the margins that should be respected in the design
stage, in order to work with specific probabilities and guarantees. We would propose to still use the deterministic analysis deepened in this thesis, but including the randomness effect. The extension of our analysis could involve a stochastic disturbance that influences a deterministic signal. The latter would be represented by the concentration over the time, while the disturbance would be the stochastic process which describes the deviation between the exact solution of the CME and the approximation of DREs. The complexity of such an argumentation lies in the statistical description of the disturbance. Definitely, this complexity is aggravated when the target is a general characterization. The next interesting step could be to study CNPs under the mere information theory point of view. In order to quantify the information flow, parameters as signal noise ratio or entropy may be calculated even in CNPs.

Summarizing, we have proposed new procedures to study the dynamics of chemical models used in chemically-inspired networking. We have also shown how to use these dynamics analysis "glasses". Our approach is still based upon a deterministic approximation of the exact time evolution of the stochastic process, but it offers a general analysis method useful for all linear models and for a broad range of non-linear models.
Chapter 6

Appendix

6.1 MATLAB and SIMULINK

MATLAB, developed by MathWorks Inc., is a software package for high performance numerical computation and visualization. It is definitely one of the most used software package in general engineering. MATLAB provides an interactive environment with hundreds of reliable and accurate built-in mathematical functions. These functions provide solutions to a broad range of mathematical problems including matrix algebra, linear systems, signal processing, and many other types of scientific computations. There are several optional toolboxes written for special applications. Some of these optional packets, such as signal processing and control systems design, have been widely used for the solution of both kind of networks: simple, easily analyzable even without such powerful tools, and much more complex architecture, where instead the use of such functions has resulted essential. We have also based conclusive numerical studies on SIMULINK program. SIMULINK is a graphical mouse-driven program for the simulation of dynamic systems, which enables to deal with linear, as well as nonlinear, systems easily and efficiently. In this thesis, SIMULINK has been used only for linear model solution with no delay. We have been able to directly replace a chemical network schematic, having species, chemical reactions and reaction coefficients, with a SIMULINK schematic, showing instead interconnections and simple frequency transform blocks. We have demonstrated that all species can be replaced with its frequency transform, which depends only on the number of outflows and the respective reaction coefficients (Sect. 4.1). The chemical network is equivalently built by placing these transfer function blocks in series, parallels and feedback loops. With delayed communication systems, the use MATLAB has been preferred, since the simplicity of the involved schematics. Anyway, specific functions of the mentioned toolboxes, such as ”zpk”, ”tf” or ”feedback”, have been always exploited. For more complex reaction networks, the potential of MATLAB has been experienced only for the matrix algebra of the metabolic control analysis.
Several used MATLAB functions are briefly here introduced. For a more specific description or for all other commands and useful features, please refer to the MATLAB Users Guide.

"tf" function: Included in "Control System Toolbox". "tf" is used to create real- or complex-valued transfer function models or to convert state-space or zero-pole-gain models to the transfer function form. The standard form of a "tf" object is:

\[
H(s) = \frac{s^N \cdot k_N + s^{N-1} \cdot k_{N-1} + \cdots + s^2 \cdot k_2 + s \cdot k_1 + k_0}{s^N \cdot c_N + s^{N-1} \cdot c_{N-1} + \cdots + s^2 \cdot c_2 + s \cdot c_1 + c_0}
\]

where \(k_i\) and \(c_i\) are generic constant.

"zpk" function: Included in "Control System Toolbox". "zpk" is used to create zero-pole-gain models or to convert "tf" and "ss" models to the zero-pole-gain form. The standard form of a "zpk" object is:

\[
H(s) = \frac{(s - k_0)(s - k_1)\ldots(s - k_N)}{(s - c_0)(s - c_1)\ldots(s - c_N)}
\]

where \(k_i\) and \(c_i\) are generic constant.

"ss" function: Included in "Control System Toolbox". "ss" is used to create real- or complex-valued, state-space models or to convert transfer function or zero-pole-gain models to state space. "sys = ss(A,B,O,D)" creates a "ss" object representing the continuous-time state-space model. Namely, using "ss" the transfer function of the system is directly obtained, by providing the system matrix, \(A\), the input matrix, \(B\), the output matrix, \(O\), and the direct transmission matrix, \(D\).

"feedback" function: Included in "Control System Toolbox". It returns a linear time invariant model which has been obtained from the feedback interconnection. Either positive or negative feedback are possible.

\[
H(s) = \frac{V_{\text{out}}(s)}{V_{\text{in}}(s)} \leftrightarrow \text{feedback}(H_1(s), H_2(s))
\]

Particular attention must be paid when systems present delays.

"bode" function: Included in "Control System Toolbox". It computes the magnitude and the phase of the frequency response of linear time invariant models. The magnitude is
directly plotted in decibels (dB), and the phase in degrees. The decibel calculation for the magnitude is computed as $20\log_{10}(|H(s)|)$, where $H(s)$ is the system’s frequency response. Bode plots can be used to analyze system properties such as the gain margin, phase margin, DC gain, bandwidth, disturbance rejection, and stability.

"step" and "impulse" function: Included in "Control System Toolbox". They computes the step response and the impulse response of a time invariant linear system. For the state space case, zero initial state is assumed. The duration of simulation is determined automatically, based on the system poles and zeros. The impulse response is the response to a Dirac input for continuous-time systems and to a unit pulse at for discrete-time systems.

Operations, like "addition", and commands, like "feedback", operate on more than one linear time invariant model at time. If these models are represented as MATLAB objects of different types (for example, the first operand is "tf" and the second operand is "ss"), it is not obvious what type (for example, "tf" or "ss") the resulting model should be. Such type of conflicts is solved by precedence rules. Specifically, "tf", "zpk", and "ss" objects are ranked according to the precedence hierarchy: $ss > zpk > tf$. Thus "zpk" takes precedence over "tf", "ss" takes precedence over both "tf" and "zpk".
6.2 The Meaning of the Time Response

We must clarify the meaning of the time response of a transfer function. The easiest and quickest way to do that, it is a simple example. Let us consider the well-known chemical network in Fig. 6.2, by reporting the results arise by the MCA considering two different cases.

First, the input (the dotted-line in Fig. 6.2) is the input rate. In that case, a step response means that, at time $t = 0$ when it is applied, we start to continuously inject a certain amount of molecules (i.e. a unitary step amplitude means the continuous injection of one molecule, while a step amplitude of 100 means the continuous injection of hundred molecules). On the other hand the impulse response means that, at time $t = 0$, we inject a certain amount of molecules.

Referring to Fig. 6.2, the transform function vector is composed by:

$$H(s) = H_1(s) = -\frac{k_1}{s(s+k_1+k_2)}$$
$$H_2(s) = \frac{k_1}{s(s+k_1+k_2)}$$

(6.1)

Second, we consider the concentration of $C_1$-species itself as input. In that case, a step response means that, at time $t = 0$ when it is applied, we inject a certain amount of molecules (i.e. a unitary step amplitude means the injection of only one molecule, a step amplitude of 100 means the injection of hundred molecules). In that case, the impulse response has not much sense: it would be an instantaneous injection and deletion of a certain amount of molecules.

The transform function vector is:

$$H(s) = H_1(s) = -\frac{s k_1}{s(s+k_1+k_2)}$$
$$H_2(s) = \frac{k_1}{s(s+k_1+k_2)}$$

(6.2)

In order to compare the two results of (6.1) and (6.2), we should consider the impulse response of (6.1) with the impulse response of (6.2). Indeed, being the transfer function of a time-step $u(t) \leftrightarrow 1/s$ and the transfer function of a time-impulse $\delta(t) \leftrightarrow 1$, we obtain

$$\text{Impulse}_1(s)^\text{Rate} = -\frac{k_1}{s(s+k_1+k_2)} \cdot 1 = -\frac{k_1}{s(s+k_1+k_2)}$$
$$\text{Impulse}_2(s)^\text{Rate} = \frac{k_1}{s(s+k_1+k_2)} \cdot 1 = \frac{k_1}{s(s+k_1+k_2)}$$

$$\text{Step}_1(s)^\text{Conc} = -\frac{k_1 s}{s(s+k_1+k_2)} \cdot \frac{1}{s} = -\frac{k_1}{s(s+k_1+k_2)}$$
$$\text{Step}_2(s)^\text{Conc} = \frac{k_1 s}{s(s+k_1+k_2)} \cdot \frac{1}{s} = \frac{k_1}{s(s+k_1+k_2)}$$

We have demonstrated that both solutions of MCAs lead to the same result. Moreover, we should have understood the difference between impulse and step responses and the difference
between transfer functions, which are gained considering as input the rate, and transfer functions, which are gained considering as input the concentration.
6.3 Chemical Networks with Loop

6.3.1 Loop Networks with Outflow

The chemical network in Fig. 6.3 is comparable to chemical networks in Fig. 4.19 and Fig. 4.21 in Sect. 4.2.

"Four species loop with an input and an output": Fig. 6.3.

![Figure 6.3: A "loop network" composed by four species with an input and an output.](image)

Laplace transform of $C_1$-species:

$$C_1(s) = \frac{s^3 + s^2 \cdot 6k + s \cdot 10k^2 + 4k^3}{s^4 + s^3 \cdot k + s^2 \cdot 7k^2 + s \cdot 10k^3 + k^4} \cdot V_{\text{in}}(s)$$

Laplace transform of $C_2$-species:

$$C_2(s) = \frac{k(s^2 + s \cdot 4k + 9k^2)}{s^4 + s^3 \cdot k + s^2 \cdot 7k^2 + s \cdot 10k^3 + k^4} \cdot V_{\text{in}}(s)$$

$$C_2(s) = \frac{k(s + k)(s + 3k)}{s^4 + s^3 \cdot k + s^2 \cdot 7k^2 + s \cdot 10k^3 + k^4} \cdot V_{\text{in}}(s)$$

Laplace transform of $C_3$-species:

$$C_3(s) = \frac{k^2(s + 2k)}{s^4 + s^3 \cdot k + s^2 \cdot 7k^2 + s \cdot 10k^3 + k^4} \cdot V_{\text{in}}(s)$$

Laplace transform of $C_4$-species:

$$C_4(s) = \frac{k^3}{s^4 + s^3 \cdot k + s^2 \cdot 7k^2 + s \cdot 10k^3 + k^4} \cdot V_{\text{in}}(s)$$

Transfer function:

$$H(s) = \frac{k}{V_{\text{in}}(s)} \cdot C_4(s) = \frac{k^4}{s^4 + s^3 \cdot k + s^2 \cdot 7k^2 + s \cdot 10k^3 + k^4} \quad (6.3)$$
6.3 Chemical Networks with Loop

6.3.2 Loop Networks without Outflow

Chemical networks in Fig. 6.4 and Fig. 6.5, are comparable to the chemical network in Fig. 4.22 in Sect. 4.2.

"Four species loop with no output": Fig. 6.4.

![Figure 6.4](image)

Figure 6.4: A "loop network" composed by four species, without outflow.

Laplace transform of $C_1$-species:

$$ C_1(s) = \frac{s^3 + s^2 \cdot 5k + s \cdot 6k^2 + k^3}{s(s^3 + s^2 \cdot 6k + s \cdot 10k^2 + 4k^3)} \cdot V_{in}(s) $$

Laplace transform of $C_2$-species:

$$ C_2(s) = \frac{k(s^2 + s \cdot 3k + k^2)}{s(s^3 + s^2 \cdot 6k + s \cdot 10k^2 + 4k^3)} \cdot V_{in}(s) $$

Laplace transform of $C_3$-species:

$$ C_3(s) = \frac{k^2(s + k)}{s(s^3 + s^2 \cdot 6k + s \cdot 10k^2 + 4k^3)} \cdot V_{in}(s) $$

Laplace transform of $C_4$-species:

$$ C_4(s) = \frac{k^3}{s(s^3 + s^2 \cdot 6k + s \cdot 10k^2 + 4k^3)} \cdot V_{in}(s) $$

The transfer function can be calculated referring to species concentrations:

$$ H(s) = \begin{cases} 
C_1(s) & \text{referring to } C_1\text{-species} \\
\frac{V_{in}(s)}{C_1(s)} & \text{referring to } C_2\text{-species} \\
\frac{V_{in}(s)}{C_2(s)} & \text{referring to } C_3\text{-species} \\
\frac{V_{in}(s)}{C_3(s)} & \text{referring to } C_4\text{-species} 
\end{cases} $$
"Five species loop with no output": Fig. 6.5.

Figure 6.5: A “loop network” composed by five species, without outflow.

Laplace transform of $C_1$-species:

$$C_1(s) = \frac{s^4 + s^3 \cdot 7k + s^2 \cdot 15k^2 + s \cdot 10k^3 + k^4}{s(s^4 + s^3 \cdot 8k + s^2 \cdot 21k^2 + s \cdot 20k^3 + 5k^4)} \cdot V_{in}(s)$$

Laplace transform of $C_2$-species:

$$C_2(s) = \frac{k(s^3 + s^2 \cdot 5k + s \cdot 6k^2 + k^3)}{s(s^4 + s^3 \cdot 8k + s^2 \cdot 21k^2 + s \cdot 20k^3 + 5k^4)} \cdot V_{in}(s)$$

Laplace transform of $C_3$-species:

$$C_3(s) = \frac{k^2(s^2 + s \cdot 3k + k^2)}{s(s^4 + s^3 \cdot 8k + s^2 \cdot 21k^2 + s \cdot 20k^3 + 5k^4)} \cdot V_{in}(s)$$

Laplace transform of $C_4$-species:

$$C_4(s) = \frac{k^3(s + k)}{s(s^4 + s^3 \cdot 8k + s^2 \cdot 21k^2 + s \cdot 20k^3 + 5k^4)} \cdot V_{in}(s)$$

Laplace transform of $C_5$-species:

$$C_5(s) = \frac{k^4}{s(s^4 + s^3 \cdot 8k + s^2 \cdot 21k^2 + s \cdot 20k^3 + 5k^4)} \cdot V_{in}(s)$$

The transfer function can be calculated referring to species concentrations:

$$H(s) = \begin{cases} 
C_1(s) & \text{referring to } C_1\text{-species} \\
V_{in}(s) & \\
C_2(s) & \text{referring to } C_2\text{-species} \\
V_{in}(s) & \\
C_3(s) & \text{referring to } C_3\text{-species} \\
V_{in}(s) & \\
C_4(s) & \text{referring to } C_4\text{-species} \\
V_{in}(s) & \\
C_5(s) & \text{referring to } C_5\text{-species} \\
V_{in}(s) & 
\end{cases}$$
6.3 Chemical Networks with Loop

6.3.3 The Importance of the Inflow Position

Chemical networks in Fig. 6.6 and Fig. 6.7 are comparable to the chemical network in Fig. 6.5 in App. 6.3.2 and Fig. 4.23 in Sect. 4.2

"Five species loop with an inflow in the center of the network": Fig. 6.6.

**Figure 6.6:** A "loop network" composed by five species, without outflow and the inflow is placed in a central position.

Laplace transform of $C_1$-species:

$$C_1(s) = \frac{k^2(s^2 + s \cdot 3k + k^2)}{s(s^4 + s^3 \cdot 8k + s^2 \cdot 21k^2 + s \cdot 20k^3 + 5k^4)} \cdot V_{in}(s)$$

Laplace transform of $C_2$-species:

$$C_2(s) = \frac{k(s^3 + s^2 \cdot 4k + s \cdot 4k^2 + k^3)}{s(s^4 + s^3 \cdot 8k + s^2 \cdot 21k^2 + s \cdot 20k^3 + 5k^4)} \cdot V_{in}(s)$$

Laplace transform of $C_3$-species:

$$C_3(s) = \frac{s^4 + s^3 \cdot 6k + s^2 \cdot 11k^2 + s \cdot 6k^3 + k^4}{s(s^4 + s^3 \cdot 8k + s^2 \cdot 21k^2 + s \cdot 20k^3 + 5k^4)} \cdot V_{in}(s)$$

for symmetry, the Laplace transform of $C_4$-species equals the Laplace transform of $C_2$-species, as well as what happen with $C_5$-species and $C_1$-species. The transfer function can be calculated referring to species concentrations:

$$H(s) = \begin{bmatrix}
C_1(s) & \text{referring to } C_1\text{-species} \\
V_{in}(s) & \\
C_2(s) & \text{referring to } C_2\text{-species} \\
V_{in}(s) & \\
C_3(s) & \text{referring to } C_3\text{-species} \\
V_{in}(s) & \\
C_4(s) & \text{referring to } C_4\text{-species} \\
V_{in}(s) & \\
C_5(s) & \text{referring to } C_5\text{-species} \\
V_{in}(s) & 
\end{bmatrix}$$
"Five species loop with an unbalanced position of the inflow": Fig. 6.7.

Figure 6.7: A "loop network" composed by five species, without outflow. The inflow position is unbalanced, referring to the network symmetry.

Laplace transform of $C_1$-species:

$$C_1(s) = \frac{k(s^3 + s^2 \cdot 5k + s \cdot 6k^2 + k^3)}{s(s^4 + s^3 \cdot 8k + s^2 \cdot 21k^2 + s \cdot 20k^3 + 5k^4)} \cdot V_{in}(s)$$

Laplace transform of $C_2$-species:

$$C_2(s) = \frac{s^4 + s^3 \cdot 6k + s^2 \cdot 11k^2 + s \cdot 7k^3 + k^4}{s(s^4 + s^3 \cdot 8k + s^2 \cdot 21k^2 + s \cdot 20k^3 + 5k^4)} \cdot V_{in}(s)$$

Laplace transform of $C_3$-species:

$$C_3(s) = \frac{k(s^3 + s^2 \cdot 4k + s \cdot 4k^2 + k^3)}{s(s^4 + s^3 \cdot 8k + s^2 \cdot 21k^2 + s \cdot 20k^3 + 5k^4)} \cdot V_{in}(s)$$

Laplace transform of $C_4$-species:

$$C_4(s) = \frac{k^2(s^2 + s \cdot 2k + k^2)}{s(s^4 + s^3 \cdot 8k + s^2 \cdot 21k^2 + s \cdot 20k^3 + 5k^4)} \cdot V_{in}(s)$$

$$= \frac{k^2(s + k)^2}{s(s^4 + s^3 \cdot 8k + s^2 \cdot 21k^2 + s \cdot 20k^3 + 5k^4)} \cdot V_{in}(s)$$

Laplace transform of $C_5$-species:

$$C_5(s) = \frac{k^3(s + k)}{s(s^4 + s^3 \cdot 8k + s^2 \cdot 21k^2 + s \cdot 20k^3 + 5k^4)} \cdot V_{in}(s)$$

The transfer function can be calculated referring to species concentrations:

$$H(s) = \begin{cases} 
    \frac{C_1(s)}{V_{in}(s)} & \text{referring to } C_1\text{-species} \\
    \frac{C_2(s)}{V_{in}(s)} & \text{referring to } C_2\text{-species} \\
    \frac{C_3(s)}{V_{in}(s)} & \text{referring to } C_3\text{-species} \\
    \frac{C_4(s)}{V_{in}(s)} & \text{referring to } C_4\text{-species} \\
    \frac{C_5(s)}{V_{in}(s)} & \text{referring to } C_5\text{-species} 
\end{cases}$$
6.3 Chemical Networks with Loop

6.3.4 More Complicated Loop Networks without Outflow

Chemical networks in Fig. 6.8, Fig. 6.9, Fig. 6.10, Fig. 6.11 and Fig. 6.12 are comparable to the chemical network in Fig. 4.24 in Sect. 4.2.

"Five species network with four feedback reactions (no output)"; Fig. 6.8.

![Chemical Network Diagram](image)

**Figure 6.8**: A "loop network" composed by five species and four feedback reactions. There is no outflow.

Laplace transform of $C_1$-species:

$$C_1(s) = \frac{s^4 + s^3 \cdot 7k + s^2 \cdot 11k^2 + s \cdot 7k^3 + 3k^4}{s(s^4 + s^3 \cdot 8k + s^2 \cdot 18k^2 + s \cdot 16k^3 + 5k^4)} \cdot V_{in}(s) = \frac{(s^2 + s \cdot 5k + k^2)(s + k)^2}{s(s + 5k)(s + k)^3} \cdot V_{in}(s)$$

Laplace transform of $C_2$-species:

$$C_2(s) = \frac{k(s^3 + s^2 \cdot 3k + s \cdot 3k^2 + k^3)}{s(s^4 + s^3 \cdot 8k + s^2 \cdot 18k^2 + s \cdot 16k^3 + 5k^4)} \cdot V_{in}(s) = \frac{k(s + k)^3}{s(s + 5k)(s + k)^3} \cdot V_{in}(s)$$

Laplace transform of $C_3$-species:

$$C_3(s) = \frac{k^2(s^2 + s \cdot 2k + k^2)}{s(s^4 + s^3 \cdot 8k + s^2 \cdot 18k^2 + s \cdot 16k^3 + 5k^4)} \cdot V_{in}(s) = \frac{k^2(s + k)^2}{s(s + 5k)(s + k)^3} \cdot V_{in}(s)$$

The Laplace transform of $C_4$-species and $C_6$-species can be seen directly from the previous one. Indeed, looking where the input is applied, $C_4$-species and $C_6$-species are symmetric to $C_3$-species.

The transfer function can be calculated referring to species concentrations:

$$H(s) = \begin{cases} 
C_1(s) & \text{referring to } C_1\text{-species} \\
V_{in}(s) & \\
C_2(s) & \text{referring to } C_2\text{-species} \\
V_{in}(s) & \\
C_3(s) & \text{referring to } C_3\text{-species} \\
V_{in}(s) & \\
C_4(s) & \text{referring to } C_4\text{-species} \\
V_{in}(s) & \\
C_5(s) & \text{referring to } C_5\text{-species} \\
V_{in}(s) & 
\end{cases}$$
"Five species network with five feedback reactions (no output)" : Fig. 6.9.

\[
\begin{align*}
\text{Laplace transform of } C_1 \text{-species:} & \quad C_1(s) = \frac{s^4 + s^3 \cdot 9k + s^2 \cdot 26k^2 + s \cdot 26k^3 + 4k^4}{s(s^4 + s^3 \cdot 10k + s^2 \cdot 34k^2 + s \cdot 46k^3 + 20k^4)} \cdot V_{\text{in}}(s) \\
\text{Laplace transform of } C_2 \text{-species:} & \quad C_2(s) = \frac{k(s^3 + s^2 \cdot 30k + s \cdot 10k^2 + 4k^3)}{s(s^4 + s^3 \cdot 10k + s^2 \cdot 34k^2 + s \cdot 46k^3 + 20k^4)} \cdot V_{\text{in}}(s) \\
\text{Laplace transform of } C_3 \text{-species:} & \quad C_3(s) = \frac{k^2(s^2 + s \cdot 4k + 4k^2)}{s(s^4 + s^3 \cdot 10k + s^2 \cdot 34k^2 + s \cdot 46k^3 + 20k^4)} \cdot V_{\text{in}}(s) \\
& \quad = \frac{k^2(s + 2k)^2}{s(s^4 + s^3 \cdot 10k + s^2 \cdot 34k^2 + s \cdot 46k^3 + 20k^4)} \cdot V_{\text{in}}(s) \\
\text{Laplace transform of } C_4 \text{-species:} & \quad C_4(s) = 2k^3(s + 2k) \\
& \quad \qquad s(s^4 + s^3 \cdot 10k + s^2 \cdot 34k^2 + s \cdot 46k^3 + 20k^4) \cdot V_{\text{in}}(s) \\
\text{Laplace transform of } C_5 \text{-species:} & \quad C_5(s) = \frac{2k^3(s + 2k)}{s(s^4 + s^3 \cdot 10k + s^2 \cdot 34k^2 + s \cdot 46k^3 + 20k^4)} \cdot V_{\text{in}}(s)
\end{align*}
\]

The Laplace transform of \( C_4 \text{-species} \) can be seen directly from the previous one. Indeed, looking where the input is applied, \( C_4 \text{-species} \) is symmetric to \( C_3 \text{-species} \).

The transfer function can be calculated referring to species concentrations:

\[
H(s) = \left\{ \begin{array}{l}
\frac{C_1(s)}{V_{\text{in}}(s)} \quad \text{referring to } C_1 \text{-species} \\
\frac{C_2(s)}{V_{\text{in}}(s)} \quad \text{referring to } C_2 \text{-species} \\
\frac{C_3(s)}{V_{\text{in}}(s)} \quad \text{referring to } C_3 \text{-species} \\
\frac{C_4(s)}{V_{\text{in}}(s)} \quad \text{referring to } C_4 \text{-species} \\
\frac{C_5(s)}{V_{\text{in}}(s)} \quad \text{referring to } C_5 \text{-species}
\end{array} \right.
\]
"Five species network with seven feedback reactions (no output)" : Fig. 6.10.

Figure 6.10: A "loop network" composed by five species and seven feedback reactions. There is no outflow.

Laplace transform of $C_1$-species:

$$C_1(s) = \frac{s^4 + s^3 \cdot 13k + s^2 \cdot 58k^2 + s \cdot 99k^3 + 45k^4}{s(s^4 + s^3 \cdot 16k + s^2 \cdot 942 + s \cdot 240k^3 + 225k^4)} \cdot V_{in}(s) = \frac{(s^2 + s \cdot 5k + k^2)(s + 3k)(s + 5k)}{s(s + 5k)^2(s + 3k)^2} \cdot V_{in}(s)$$

Laplace transform of $C_2$-species:

$$C_2(s) = \frac{k(s^3 + s^2 \cdot 7k + s \cdot 16k^2 + 12k^3)}{s(s^4 + s^3 \cdot 16k + s^2 \cdot 942 + s \cdot 240k^3 + 225k^4)} \cdot V_{in}(s) = \frac{k(s + 5k)(s + 3k)^2}{s(s + 5k)^2(s + 3k)^2} \cdot V_{in}(s)$$

The Laplace transform of $C_3$-species and $C_4$-species can be seen directly from the previous one. Indeed, looking where the input is applied, $C_3$-species and $C_4$-species are symmetric to $C_2$-species.

Laplace transform of $C_2$-species:

$$C_5(s) = \frac{2k^2(s^2 + s \cdot 8k + 15k^2)}{s(s^4 + s^3 \cdot 16k + s^2 \cdot 942 + s \cdot 240k^3 + 225k^4)} \cdot V_{in}(s) = \frac{k(s + 5k)(s + 3k)}{s(s + 5k)^2(s + 3k)^2} \cdot V_{in}(s)$$

The transfer function can be calculated referring to species concentrations:

$$H(s) = \left\{ \begin{array}{ll}
\frac{C_1(s)}{V_{in}(s)} & \text{referring to } C_1\text{-species} \\
\frac{C_2(s)}{V_{in}(s)} & \text{referring to } C_2\text{-species} \\
\frac{C_3(s)}{V_{in}(s)} & \text{referring to } C_3\text{-species} \\
\frac{C_4(s)}{V_{in}(s)} & \text{referring to } C_4\text{-species} \\
\frac{C_5(s)}{V_{in}(s)} & \text{referring to } C_5\text{-species}
\end{array} \right.$$
"Five species network with seven feedback reactions with an output flow":

\[
\begin{align*}
C_1(s) &= \frac{s^4 + s^3 \cdot 13k + s^2 \cdot 47k^2 + s \cdot 64k^3 + 20k^4}{s^5 + s^4 \cdot 13k + s^2 \cdot 58k^2 + s^2 \cdot 103k^3 + s \cdot 66k^4 + 8k^5} \cdot V_{in}(s) \\
C_2(s) &= \frac{k(s^3 + s^2 \cdot 8k + s \cdot 18k^2 + 12k^3)}{s^5 + s^4 \cdot 13k + s^2 \cdot 58k^2 + s^2 \cdot 103k^3 + s \cdot 66k^4 + 8k^5} \cdot V_{in}(s) \\
C_3(s) &= \frac{k^2(s^2 + s \cdot 7k + 10k^2)}{s^5 + s^4 \cdot 13k + s^2 \cdot 58k^2 + s^2 \cdot 103k^3 + s \cdot 66k^4 + 8k^5} \cdot V_{in}(s) = \\
&= \frac{k^2(s + 5k)(s + 2k)}{s^5 + s^4 \cdot 13k + s^2 \cdot 58k^2 + s^2 \cdot 103k^3 + s \cdot 66k^4 + 8k^5} \cdot V_{in}(s) \\
C_4(s) &= \frac{k^2(s^2 + s \cdot 6k + 8k^2)}{s^5 + s^4 \cdot 13k + s^2 \cdot 58k^2 + s^2 \cdot 103k^3 + s \cdot 66k^4 + 8k^5} \cdot V_{in}(s) = \\
&= \frac{k^2(s + 4k)(s + 2k)}{s^5 + s^4 \cdot 13k + s^2 \cdot 58k^2 + s^2 \cdot 103k^3 + s \cdot 66k^4 + 8k^5} \cdot V_{in}(s) \\
H(s) &= \frac{C_5(s) k}{V_{in}(s)} = \frac{k^3(s + 4k)(s + 2k)}{s^5 + s^4 \cdot 13k + s^2 \cdot 58k^2 + s^2 \cdot 103k^3 + s \cdot 66k^4 + 8k^5}
\end{align*}
\]

The output reaction coefficient has the same value of other coefficient: \( k_{out} = k \).
"Five species network with seven feedback reactions, moving the output flow":

![Chemical Network Diagram](image)

**Figure 6.12:** A "loop network" composed by five species and seven feedback reactions. The outflow is moved from $c_5$ to $c_3$.

Laplace transform of $C_1$-species:

$$C_1(s) = \frac{s^4 + s^3 \cdot 12k + s^2 \cdot 46k^2 + s \cdot 60k^3 + 16k^4}{s^5 + s^4 \cdot 13k + s^2 \cdot 57k^2 + s^3 \cdot 99k^3 + s \cdot 62k^4 + 8k^5} \cdot V_{in}(s)$$

Laplace transform of $C_2$-species:

$$C_2(s) = \frac{k(s^3 + s^2 \cdot 7k + s \cdot 14k^2 + 8k^3)}{s^5 + s^4 \cdot 13k + s^2 \cdot 57k^2 + s^3 \cdot 99k^3 + s \cdot 62k^4 + 8k^5} \cdot V_{in}(s) =$$

$$= \frac{k(s + 4k)(s + 2k)(s + k)}{s^5 + s^4 \cdot 13k + s^2 \cdot 57k^2 + s^3 \cdot 99k^3 + s \cdot 62k^4 + 8k^5} \cdot V_{in}(s)$$

Laplace transform of $C_3$-species:

$$C_3(s) = \frac{k^2(s^2 + s \cdot 6k + 8k^2)}{s^5 + s^4 \cdot 13k + s^2 \cdot 57k^2 + s^3 \cdot 99k^3 + s \cdot 62k^4 + 8k^5} \cdot V_{in}(s) =$$

$$= \frac{k^2(s + 4k)(s + 2k)}{s^5 + s^4 \cdot 13k + s^2 \cdot 57k^2 + s^3 \cdot 99k^3 + s \cdot 62k^4 + 8k^5} \cdot V_{in}(s)$$

The Laplace transform of $C_4$-species and $C_5$-species can be seen directly from $C_3$-species. Indeed, looking where the input is applied, $C_4$-species and $C_5$-species are symmetric to $C_3$-species.

The transfer function of the system is referred to $C_3$-species:

$$H(s) = \frac{C_3(s)}{V_{in}(s)} = \frac{k^3(s + 4k)(s + 2k)}{s^5 + s^4 \cdot 13k + s^2 \cdot 57k^2 + s^3 \cdot 99k^3 + s \cdot 62k^4 + 8k^5}$$

The output reaction coefficient has the same value of other coefficient: $k_{out} = k$. 
6.4 Variants of the Disperser Protocol

The Disperser protocol with different input displacement:
The chemical model in Fig. 6.13 is comparable to chemical networks in Fig. 4.26 (Sect. 4.3) and in Fig. 6.14 (App. 6.4).

**Figure 6.13:** A chemical network similar to the Disperser, but applying a perturbation at $C_2$-species instead of $c_1$.

Laplace transform of $C_1$-species:

$$C_1(s) = \frac{k(s^2 + s \cdot 3k + k^2)}{s^2 \cdot s \cdot 8k + s \cdot 19k^2 + 12k^3} \cdot V_{in}(s) = \frac{k(s + k)(s + 3k)}{s(s + 4k)(s + 3k)} \cdot V_{in}(s)$$

Laplace transform of $C_2$-species:

$$C_2(s) = \frac{s^3 + s^2 \cdot 3k + s \cdot 7k^2 + 3k^3}{s^2 \cdot s^2 \cdot 8k + s \cdot 19k^2 + 12k^3} \cdot V_{in}(s) = \frac{(s + k)^2(s + 3k)}{s(s + 4k)(s + 3k)} \cdot V_{in}(s)$$

Laplace transforms of $C_3$-species and $C_4$-species can be seen directly from $C_1$-species. Indeed, looking where the input is applied, $C_3$-species and $C_4$-species are symmetric to $C_1$-species.

The transfer function can be calculated referring to species concentrations:

$$H(s) = \begin{cases} 
  C_1(s) & \text{referring to } C_1\text{-species} \\
  V_{in}(s) & \\
  C_2(s) & \text{referring to } C_2\text{-species} \\
  V_{in}(s) & \\
  C_3(s) & \text{referring to } C_3\text{-species} \\
  V_{in}(s) & \\
  C_4(s) & \text{referring to } C_4\text{-species} \\
  V_{in}(s) & 
\end{cases}$$
The Disperser protocol with output flow:

The chemical model in Fig. 6.14 is comparable to chemical networks in Fig. 4.26 (Sect. 4.3) and in Fig. 6.13 (App. 6.4).

Figure 6.14: A chemical network similar to the Disperser, but having an output flow.

The transfer function can be calculated referring to species concentrations.

Laplace transform of $C_1$-species:

$$C_1(s) = \frac{k \left( s^3 + s^2 \cdot 8k + s \cdot 18k^2 + 8k^3 \right) }{ (s^4 + s^3 \cdot 9k + s^2 \cdot 25k^2 + s + 21k^3 + 3k^4) } \cdot V_{\text{in}}(s)$$

$$H(s) = \frac{C_3(s)k}{V_{\text{in}}(s)} = \frac{k^3(s + 3k)}{ (s^4 + s^3 \cdot 9k + s^2 \cdot 25k^2 + s + 21k^3 + 3k^4) }$$

The output reaction coefficient has the same value of other coefficient: $k_{\text{out}} = k$. 
6.5 MCA of a Linear Network

Differential equations which describe the behavior of the network in Fig. 6.14 in App. 6.4:

\[
\begin{align*}
\dot{c}_1 &= k_{\text{in}} - kc_1 + kc_2 \\
\dot{c}_2 &= kc_1 - kc_2 - kc_2 + kc_1 + kc_3 + kc_4 \\
\dot{c}_3 &= kc_2 - kc_3 - kc_3 - kc_3 + kc_4 \\
\dot{c}_4 &= kc_2 + kc_3 - kc_4 - kc_4
\end{align*}
\]  

(6.8)

The rate vector, \( V \), and the state vector, \( C \), are respectively

\[
V = \begin{pmatrix} k_{\text{in}} & kc_1 & kc_2 & kc_2 & kc_3 & kc_2 & kc_4 & kc_3 & kc_4 & kc_3 \end{pmatrix}^T
\]

\[
C = \begin{pmatrix} c_1 & c_2 & c_3 & c_4 \end{pmatrix}^T
\]

The stoichiometric matrix, \( \mathbb{N} \), is directly found from ODEs (6.8):

\[
\mathbb{N} = \begin{pmatrix} -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\
1 & -1 & 1 & -1 & 1 & 0 & 0 & 0 & 0 \\
0 & 0 & 1 & -1 & 0 & 0 & -1 & 1 & -1 \\
0 & 0 & 0 & 0 & 1 & -1 & 1 & -1 & 0 & 0 \end{pmatrix}
\]

The perturbation is considered applied to reaction coefficient of the inflow, \( k_{\text{in}} \), and thus

\[
P = \begin{pmatrix} k_{\text{in}} & 0 & \cdots & 0 \end{pmatrix}^T
\]

leading to getting derivatives, which are expression of the validity of our study, only for small changes of the system parameters:

\[
\frac{\partial V}{\partial C} = \begin{pmatrix} 0 & 0 & 0 & 0 \\
k & 0 & 0 & 0 \\
0 & k & 0 & 0 \\
0 & k & 0 & 0 \\
0 & 0 & k & 0 \\
0 & 0 & k & 0 \\
0 & 0 & 0 & k \\
0 & 0 & 0 & k \end{pmatrix}
\]

\[
\frac{\partial V}{\partial P} = \begin{pmatrix} 1 & 0 & \cdots & 0 \end{pmatrix}^T
\]
As usual, the evaluation of steady states of the system is related to differential equation solution at equilibrium, \( N \cdot V = 0 \):

\[
C_s = \begin{cases} 
  c_{1s} = \frac{8}{3} k_{in} k \\
  c_{2s} = \frac{5}{3} k_{in} k \\
  c_{3s} = k_{in} k \\
  c_{4s} = \frac{4}{3} k_{in} k 
\end{cases} \tag{6.9}
\]

The system matrix, \( A \), and the input matrix, \( B \) result respectively

\[
A = N \cdot \left. \frac{\partial V}{\partial C} \right|_{(C_s, P_s)} = \begin{pmatrix} -k & k & 0 & 0 \\
 k & -3k & k & k \\
 0 & k & -3k & k \\
 0 & k & k & -2k \end{pmatrix}
\]

\[
B = N \cdot \left. \frac{\partial V}{\partial P} \right|_{(C_s, P_s)} = \begin{pmatrix} 1 & 0 & 0 & 0 \end{pmatrix}^T
\]

Being here the main goal of the MCA the species concentration, the output matrix is the identity matrix, \( O = I \), while the direct transmission matrix is null, \( D = 0 \). In the end, we can linearize the system dynamics, \( \dot{S} = N \cdot V(S, P) \), around the point \((C_s, P_s)\). This means that all involved vectors and matrixes have to be evaluated around such a point, using steady states of (6.9):

\[
\dot{x}(t) = \left[ N \cdot \frac{\partial V}{\partial C} \right] x(t) + \left[ N \cdot \frac{\partial V}{\partial P} \right] u(t) \tag{6.10}
\]

where \( x(t) = c(t) - c_s \) and \( x(t) = p(t) - p_s \).

Frequency transforms of network species in the linearized system is described by (6.10):

\[
C(s) = \left( O \left( sI - A \right)^{-1} B + D \right) \cdot V_{in}(s) = 
\begin{pmatrix}
C_1(s) \\
C_2(s) \\
C_3(s) \\
C_4(s)
\end{pmatrix} = 
\begin{pmatrix}
\frac{8k^3 + 18k^2 s + 8k s^2 + s^3}{k^2(3k + s)} \\
\frac{3k^3 + 21k^2 s + 25k s^2 + 9k s^3 + s^4}{k^2(3k + s)} \\
\frac{3k^3 + 21k^2 s + 25k s^2 + 9k s^3 + s^4}{k^2(3k + s)} \\
\frac{3k^3 + 21k^2 s + 25k s^2 + 9k s^3 + s^4}{k^2(3k + s)} \\
\end{pmatrix} \tag{6.11}
\]

Our goal is satisfied: solution (6.11) agrees with the already found (6.4), (6.5) and (6.6).
6.6 Quine Results

Several results of dynamical behavior analysis of the Quine architecture are here reported. All cases present different combination of reaction coefficient values. The Quine represents always a first order low pass filter, where the cutoff frequency depends upon reaction coefficients (the standard Quine CNP requires $k_2 = 1$ and $k_3 = 1$. For details see Sect. 4.5.2).

**CASE 1:** $k_2 = 0.1$ and $k_3 = 1$

$$f_{\text{cutoff}} = \begin{cases} 
0.05000 & (k_1 = 0.000) \\
0.07985 & (k_1 = 0.025) \\
0.10940 & (k_1 = 0.050) \\
0.13870 & (k_1 = 0.075) \\
0.16770 & (k_1 = 0.100) \\
0.19640 & (k_1 = 0.125) 
\end{cases} [\text{rad/s}]$$

**CASE 2:** $k_2 = 0.01$ and $k_3 = 1$

$$f_{\text{cutoff}} = \begin{cases} 
0.00500 & (k_1 = 0.000) \\
0.03597 & (k_1 = 0.025) \\
0.06665 & (k_1 = 0.050) \\
0.09706 & (k_1 = 0.075) \\
0.12720 & (k_1 = 0.100) \\
0.15710 & (k_1 = 0.125) 
\end{cases} [\text{rad/s}]$$

**CASE 3:** $k_2 = 1$ and $k_3 = 0.1$

$$f_{\text{cutoff}} = \begin{cases} 
0.50000 & (k_1 = 0.000) \\
0.07790 & (k_1 = 0.025) \\
0.09553 & (k_1 = 0.050) \\
0.11820 & (k_1 = 0.075) \\
0.14080 & (k_1 = 0.100) \\
0.16340 & (k_1 = 0.125) 
\end{cases} [\text{rad/s}]$$
CASE 4: $k_2 = 0.1$ and $k_3 = 0.01$

$$f_{\text{cutoff}} = \begin{cases} 
0.50000 & (k_1 = 0.000) \\
0.02700 & (k_1 = 0.025) \\
0.04907 & (k_1 = 0.050) \\
0.07119 & (k_1 = 0.075) \\
0.09338 & (k_1 = 0.100) \\
0.11560 & (k_1 = 0.125) 
\end{cases} \text{ [rad/s]}$$
Figure 6.15: **CASE 1**: $k_2 = 0.1$ AND $k_3 = 1$. species of the network in Fig. 4.37 for different values of $k_1$: frequency transforms normalized with $V_{in}(s)$ and concentrations when a step perturbation is applied on $k_1$. 
Figure 6.16: **CASE 1:** $k_2 = 0.1$ AND $k_3 = 1$. Quine in Fig. 2.4 for different values of $k_1$: transfer function and concentration when a step perturbation is applied on $k_1$. 
Figure 6.17: **CASE 2:** $k_2 = 0.01$ AND $k_3 = 1$. species of network in Fig. 4.37 for different values of $k_1$: frequency transforms normalized with $V_{in}(s)$ and concentrations when a step perturbation is applied on $k_1$. 
Figure 6.18: **CASE 2**: $k_2 = 0.01$ AND $k_3 = 1$. Quine in Fig. 2.4 for different values of $k_1$: transfer function and concentration when a step perturbation is applied on $k_1$. 
Figure 6.19: **CASE 3**: $k_2 = 1$ **AND** $k_3 = 0.1$. species of network in Fig. 4.37 for different values of $k_1$: frequency transforms normalized with $V_{in}(s)$ and concentrations when a step perturbation is applied on $k_1$. 
Figure 6.20: **CASE 3**: \( k_2 = 1 \) AND \( k_3 = 0.1 \). Quine in Fig. 2.4 for different values of \( k_1 \): transfer function and concentration when a step perturbation is applied on \( k_1 \).
Figure 6.21: **CASE 4**: \( k_2 = 1 \) \textbf{AND} \( k_3 = 0.01 \). species of network in Fig. 4.37 for different values of \( k_1 \): frequency transforms normalized with \( V_{in}(s) \) and concentrations when a step perturbation is applied on \( k_1 \).
Figure 6.22: **CASE 4**: $k_2 = 1$ AND $k_3 = 0.01$. Quine in Fig. 2.4 for different values of $k_1$: transfer function and concentration when a step perturbation is applied on $k_1$. 
6.7 Congestion Avoidance Analysis

Being this communication protocol a non-linear reaction network, the MCA is required. The first step is differential equations which describe the behavior of the network in Fig. 4.41:

\[
\begin{align*}
\dot{w} &= k_1 + 0 - k_3 \cdot w \cdot l + 0 \\
\dot{l} &= 0 + k_2 \cdot w - R \frac{k_2 \cdot w}{k_2 \cdot w + v_{in}} + 0 - k_4 \cdot l
\end{align*}
\]  
(6.12)

Note the complication in analysis that has been arisen from the ratio-term in (6.12).

The rate vector, \( V \) and the state vector \( C \) are respectively

\[
V = \begin{pmatrix}
k_1 \\
k_2 \cdot w - R \frac{k_2 \cdot w}{k_2 \cdot w + v_{in}} \\
k_3 \cdot l \cdot w \\
k_4 \cdot l
\end{pmatrix}
\]

\[
C = \begin{pmatrix}
w \\
l
\end{pmatrix}
\]

The stoichiometric matrix, \( N \), is directly found from ODEs (6.12):

\[
N = \begin{pmatrix}
1 & 0 & -1 & 0 \\
0 & 1 & 0 & -1
\end{pmatrix}
\]

The perturbation should be considered directly \( v_{in} \):

\[
P = v_{in}
\]

leading to getting derivatives, which are expression of the validity of our study, only for small changes of the system parameters:

\[
\frac{\partial V}{\partial C} =
\begin{pmatrix}
0 & 0 & 0 & 0 \\
\frac{1000k_2}{v_{in} + \left(\frac{250000k_3 + k_1 k_2 k_4}{k_3}\right)^{1/2} + 500} & \frac{1000k_2 \left(\frac{250000k_3 + k_1 k_2 k_4}{k_3}\right)^{1/2} + 500}{v_{in} + \left(\frac{250000k_3 + k_1 k_2 k_4}{k_3}\right)^{1/2} + 500} & 0 & 0 \\
\frac{k_3 \left(\frac{250000k_3 + k_1 k_2 k_4}{k_3}\right)^{1/2} + 500}{k_4} & \frac{k_3 \left(\frac{250000k_3 + k_1 k_2 k_4}{k_3}\right)^{1/2} + 500}{k_4} & 0 & 0
\end{pmatrix}
\]
6.7 Congestion Avoidance Analysis

\[
\frac{\partial V}{\partial P} = \begin{pmatrix}
0 \\
\frac{\left(\frac{250000k_3+k_1k_2k_4}{k_3}\right)^{1/2}+500}{v_{\text{in}}+\left(\frac{250000k_3+k_1k_2k_4}{k_3}\right)^{1/2}+500} 1000 \\
0 \\
0
\end{pmatrix}
\]

Other important step of MCA is evaluating steady states of the network by solving differential equations at equilibrium, \( \mathbf{N} \cdot \dot{\mathbf{V}} = 0 \). This is the implicit description of \( \mathbf{C} = \mathbf{C}(\mathbf{P}) \). Note that the steady state represents the fixed point around which the system response will be evaluated.

\[
\mathbf{C}_s = \begin{cases}
\dot{w} = k_1 - k_3 \cdot w \cdot l = 0 \\
\dot{l} = k_2 \cdot w - R \frac{k_3-w}{k_2+w-v_{\text{in}}} - k_4 \cdot l = 0
\end{cases} \quad \rightarrow \quad \begin{cases}
W_s = \frac{\left(\frac{250000k_3+k_1k_2k_4}{k_3}\right)^{1/2}+500}{k_2} \\
L_s = \frac{\left(\frac{250000k_3+k_1k_2k_4}{k_3}\right)^{1/2}+500}{k_4}
\end{cases}
\]

Using previous elements evaluated around steady states, the system matrix, \( \mathbf{A} \), and the input matrix, \( \mathbf{B} \) can be found:

\[
\mathbf{A} = \mathbf{N} \cdot \frac{\partial \mathbf{V}}{\partial \mathbf{C}} \bigg|_{(\mathbf{C}_s, \mathbf{P}_s)} = \begin{pmatrix}
-k_3 \left(\frac{\left(\frac{250000k_3+k_1k_2k_4}{k_3}\right)^{1/2}+500}{k_3} \right) & -k_3 \left(\frac{\left(\frac{250000k_3+k_1k_2k_4}{k_3}\right)^{1/2}+500}{k_4} \right)
\end{pmatrix}
\]

\[
\mathbf{B} = \mathbf{N} \cdot \frac{\partial \mathbf{V}}{\partial \mathbf{P}} \bigg|_{(\mathbf{C}_s, \mathbf{P}_s)} = \begin{pmatrix}
0 \\
1000 \left(\frac{\left(\frac{250000k_3+k_1k_2k_4}{k_3}\right)^{1/2}+500}{k_3} \right)
\end{pmatrix}
\]

Being here the main goal of the MCA the species concentration, the output matrix is the identity matrix, \( \mathbf{O} = \mathbf{I} \), while the direct transmission matrix is null, \( \mathbf{D} = \mathbf{0} \). In the end, we can linearize the system dynamics, \( \dot{\mathbf{S}} = \mathbf{N} \cdot \mathbf{V}(\mathbf{S}, \mathbf{P}) \), around the point \((\mathbf{C}_s, \mathbf{P}_s)\):

\[
\dot{x}(t) = \begin{bmatrix}
\mathbf{N} \cdot \frac{\partial \mathbf{V}}{\partial \mathbf{C}} & \mathbf{N} \cdot \frac{\partial \mathbf{V}}{\partial \mathbf{P}}
\end{bmatrix} x(t) + \begin{bmatrix}
\mathbf{N} \cdot \frac{\partial \mathbf{V}}{\partial \mathbf{P}}
\end{bmatrix} u(t)
\]

(6.13)

where \( x(t) = c(t) - c_s \) and \( x(t) = p(t) - p_s \).

The transfer function of a system that is approximatively described by (6.13), can be found as following:

\[
\mathbf{C}(s) = \left(\mathbf{O} \left(\mathbf{sI} - \mathbf{A}\right)^{-1} \mathbf{B} + \mathbf{D}\right) \cdot \mathbf{V}_{\text{in}}(s) = \begin{pmatrix}
W(s) \\
L(s)
\end{pmatrix}
\]

\[
W(s) = \frac{k_3k_4 \left(1000 \left(\frac{250000k_3+k_1k_2k_4}{k_3}\right)^{1/2}+5 \cdot 10^5\right)}{k_2 \left(\frac{250000k_3+k_1k_2k_4}{k_3}\right)^{1/2}+500} \cdot \phi(s)
\]

\[
L(s) = \frac{\left(\frac{250000k_3+k_1k_2k_4}{k_3}\right)^{1/2}+500}{k_4} \cdot \phi(s)
\]
\[
L(s) = \frac{1000 \left( \frac{25000k_3 + k_1 k_2 k_4}{k_3} \right)^{1/2} + 5 \cdot 10^5}{k_2 \left( \left( \frac{25000k_3 + k_1 k_2 k_4}{k_3} \right)^{1/2} - \frac{500k_3 + k_4 s}{s} \right)} \cdot \phi(s)
\]

where

\[
\phi(s) = \frac{1}{k_4 s^2 + s \left( -500k_3 + k_3 \left( \frac{25000k_3 + k_1 k_2 k_4}{k_3} \right)^{1/2} + k_4 \right) + 2k_3 k_4 \left( \frac{25000k_3 + k_1 k_2 k_4}{k_3} \right)^{1/2}} \cdot V_{in}(s)
\]

\[
H(s) = \frac{V_{out}(s)}{V_{in}(s)} = \frac{k_2 W(s)}{V_{in}(s)}
\]
6.8 The Stochasticity for a Particular Chemical Model

The aim of this section is to quantify the benefit of a statistical analysis of the dynamics, instead of the use of the usual deterministic approach. This argumentation is referred only to the chemical model in Fig. 6.23. Indeed, this particular model allows us to benefits of a specific feature: the $C$-species concentration is constant. At each reaction step a $C$-species molecule is consumed and instantaneously reproduced. Of course, being an outflow, at each reaction step a molecule is also produced in the product species where the outflow ends. Anyway, we are not focused on the product species concentration. Instead, our interest is the outflow reaction rate, $R$. Referring to the deterministic approach, the rate is the deterministic value $R^D = kc$, where $k$ is the reaction constant and $c$ is the $C$-species concentration. Namely, we can talk of the reaction time interval as the time interval between two consecutive reactions: $\Delta t^D = \frac{1}{kc}$. If we care about the stochasticity of such a model, the reaction time interval will be a random variable, $\Delta t$. We now know that the distribution of the reaction time interval is an exponential function. Caring about the outflow rate, $R$, we can define it as the number of reactions that occur in a time unit, [s]. Since we are counting the number of occurrences (reactions) in a certain time interval, when the events (reactions) happen randomly and with an exponential distribution of probability, we can refer to a homogeneous Poisson process. Note that only in this special case the previous assertion is valid, due to the fact that we deal with a chemical model where the concentration is constant. This condition allows having a constant parameter of the exponential random variable.

$$\mathbb{P}\left((N(t + \tau) - N(t)) = x\right) \bigg|_{\tau=1} = \frac{e^{-\lambda}(\lambda)^x}{x!} \quad x = 0, 1, \ldots \quad (6.14)$$

where $(N(t + \tau) - N(t))$ is the number of events in time interval $(t, t + \tau)$, in our case $(t, t + 1]$. The reference to a Poisson distribution can simplify the treatment of the stochasticity of the process.

Summarizing, we can see the number of reactions in a second, namely the reaction rate, as a Poisson random variable with parameter proportional to the concentration of $C$-species: $\lambda = kc$. Refer to Sect. 3.3.

The expectation value of the Poisson random variable, $R$, is

$$\mathbb{E}[R] = \lambda = kc \quad (6.15)$$

which corresponds to the deterministic value we have implicitly assumed in all argumentations of Chap. 4.
The variance measures the deviation of the random variable $R$ from the expectation value $\mathbb{E}[R]$ and it is
\[
\text{Var}[R] = \lambda = kc
\] (6.16)

The poisson distribution has the properties to approach a normal distribution for large numbers. Thus, we could refer to the real exact rate, $R(t)$, as a deterministic part, $R^D$, and the gaussian process, $R^S(t)$, which describes the deviation (the variance) between stochastic resolution and deterministic approximation. $R^S(t)$ has expectation value null and variance of (6.16).
\[
R(t) = R^D + R^S(t)
\] (6.17)

In that way, the exact rate, $R(t)$, has the expectation value of (6.15) and the variance of (6.16). The signal-noise ratio (SNR), which is the ratio between the useful part, $R^D$, and the "noise", $R^A(t)$, results
\[
\text{SNR} = \mathbb{E} \left[ \sqrt{\frac{(R^D)^2}{(R^A(t))^2}} \right] = \frac{kc}{\sqrt{kc}}
\] (6.18)

Note that the approximation to a Gaussian distribution is not compulsory: the real exact rate, $R(t)$, can be seen directly as a Poisson process with the mean equals to the deterministic part, $R^D = kc$, and the variance of (6.16) that justifies the deviation of $R^D$ from $R(t)$. 

**Figure 6.23**: The self-replacing model: study of the stochasticity. The concentration of $C$-species is constant.
6.9 Results from Fraglets Simulator and Analysis with MATLAB Environments

Results of several simulations in Fraglets environment are here reported. Three chemical networks have been simulated. Fraglets software cannot accomplish the frequency transform of a species. It plots only the time behavior of networks. These step responses have been graphically compared with MATLAB plots:

\[ \begin{align*}
& \text{CA} \\
& \text{CX} \\
& \text{CY} \\
& \text{CB} \\
& \text{Vin} \\
& \text{Vout}
\end{align*} \]

Figure 6.24: The chemical network which behavior is shown in Fig. 6.25 (simulated in Fraglets) and in Fig. 6.26 (analyzed).
Figure 6.25: The behavior of chemical network in Fig. 6.24 obtained with Fraglets simulator.

Figure 6.26: The behavior of chemical network in Fig. 6.24 resulted by analysis and plotted with MATLAB.
Figure 6.27: The chemical network which behavior is shown in Fig. 6.28 (simulated in Fraglets) and in Fig. 6.29 (analyzed).

Figure 6.28: The behavior of chemical network in Fig. 6.27 obtained with Fraglets simulator.
Figure 6.29: The behavior of chemical network in Fig. 6.27 resulted by analysis and plotted with MATLAB.

Figure 6.30: The chemical network which behavior is shown in Fig. 6.31 (simulated in Fraglets) and in Fig. 6.32 (analyzed).
Figure 6.31: The behavior of chemical network in Fig. 6.30 obtained with Fraglets simulator.

Figure 6.32: The behavior of the chemical network in Fig. 6.30, which is resulted by the analysis and plotted with MATLAB.
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