Simulation of rhythmic processes: rhythms as a result of network properties

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RHYTHMS AS A RESULT OF NETWORK PROPERTIES

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1. INTRODUCTION

The recent development of powerful personal computers is changing the habits of scientific research even in biological sciences. Indeed, these machines are not only used as efficient systems to control, sample and treat data of experiments, but also as new tools allowing a broader community of researchers to better understand living organisms. This latter aspect is illustrated by modelisation and simulation which constitute a new non-intuitive method to validate (or invalidate) hypothesis in non-linear systems, where the classical intuitive methods are error prone (see also [18]).

To obtain a simulation with a computer it is necessary to construct a model of the system being studied in the form of mathematical equations in order to input coherent instructions to the machine. Future developments will probably attempt to simplify this step. However, a classical method to create a mathematical model of a dynamical system is through a set of differential equations.

At cellular and biochemical levels we can model a system by metabolic or genetic networks [18]. An example of a minimal metabolic network is represented by two enzymes, where the product of the first one is the substrate of the second one. The network is the whole system with the two enzymes and their matter or/and information (regulation) exchanges. The advantage of using the metabolic network concept is that we can define simple rules to construct models
with elementary "building blocks" (enzymes, transport, etc.) connected with matter or regulation equations that are derived by elementary functions obtained from experimental enzyme kinetic analysis.

Biological rhythms are largely present in living organisms. They are of very significant biological importance and nowadays are the topics of intense research [5]. In this paper, we will describe how to construct a model in the case of rhythmic phenomena by applying the rules of a metabolic network. We will use a negative feedback loop model to simulate virtual experiments (virtual mutagenesis and virtual biochemistry) of rhythmic processes.

2. HOW TO CONSTRUCT A METABOLIC NETWORK MODEL

A metabolic network is constituted by a given number of processes which transform matter (reactants) and regulatory information processes using reactants (but not transforming them) in order to activate or inhibit processes (systemic feedback). The goal of the model is to describe the dynamical properties (evolution of reactants in time) of the system. This is done in term of all reactants involving their rate equations associated with all transforming or information processes. In order to achieve this kind of model, we will present some simple and useful rules. As this approach is not new, the interested reader is referred to the more elaborate theory presented by Reich and Sel'kov [23].

2.1. Dynamical equations of matter

All reactants \(X_i\) in a metabolic network fluctuate in time according to:

\[
\frac{dX_i}{dt} = \sum_{\text{influx}} - \sum_{\text{outflux}} = \sum_j v_{iin} - \sum_k v_{iout}
\]

(1)

As an example, consider the following metabolic network where one reactant \(X\) is synthesised by three and destroyed by two different processes, respectively:
Each flux that synthesises $X$ is added and each flux that destroys $X$ is subtracted. We thus obtain the simple dynamical equation of matter as a linear function of the fluxes (rate equations):

$$\frac{dX}{dt} = v_1 + v_2 + v_3 - v_4 - v_5$$ \hspace{1cm} (2)

In metabolism, fluxes are described by rate equations which are function of the concentrations of the reactants. Some basic and useful expressions of fluxes (we will consider here only monosubstrate reactions) are given below:

- **Constant flux**
  $$v_i = k_i$$ \hspace{1cm} (3)

- **First order rate equation**
  $$v_i = k_i X_i$$ \hspace{1cm} (4)

- **First order with delay**
  $$v_i = k_i X_i(t - \text{delay})$$ \hspace{1cm} (5)

- **Michaelis-Menten**
  $$v_i = \frac{V_{m_i} X_i}{K_{m_i} + X_i}$$ \hspace{1cm} (6)

- **Hill equation**
  $$v_i = \frac{V_{m_i} X_i^n}{K_{m_i}^n + X_i^n}$$ \hspace{1cm} (7)

### 2. Equations of regulation

Regulatory functions which depend on the concentrations of some reactants are participating in the overall network by multiplication of target fluxes:

$$v_i^{rs} = v_i \prod_j f_j$$ \hspace{1cm} (8)

- $v_i^{rs}$: regulated flux
- $v_i$: unregulated flux
- $f_j$: $j$th regulatory function
- $\prod$: multiplication

For example, consider a network with two reactants ($X$ and $Y$), 4 fluxes of matter ($V_1$ to $V_4$), and 2 regulations ($f_1$ and $f_2$) on the synthesis of $X$ as follows:
We can write the dynamical equations of the whole network with two equations:

\[
\frac{dX}{dt} = v_1 + v_2 f_1 f_2 - v_3
\]  

(9)

\[
\frac{dY}{dt} = v_3 - v_4
\]  

(10)

In metabolic networks, regulatory functions which can either inhibit or activate a given process, are scaled between 0 and 1. Some basic and useful functions are:

Inhibition:

\[
f_j = \begin{cases} 
1 & \text{if } R_j \leq K_i > 0 \\
0 & \text{if } R_j \geq K_i 
\end{cases}
\]  

(11)

- **Simple regulation**

\[
f_j = \frac{K_i}{K_i + R_j}
\]  

(12)

- **Cooperative regulation**

\[
f_j = \frac{K_i^n}{K_i^n + R_j^n}
\]  

(13)

Activation:

\[
f_j = \begin{cases} 
0 & \text{if } R_j \leq K_s > 0 \\
1 & \text{if } R_j \geq K_s 
\end{cases}
\]  

(14)

- **Simple regulation**

\[
f_j = \frac{R_j}{K_s + R_j}
\]  

(15)
Cooperative regulation  \[ f_j = \frac{R_j^n}{K^n + R_j^n} \]  

Where \( R_j \) is the concentration of a regulatory reactant.

### 3. THE NEGATIVE FEEDBACK LOOP NETWORK MODEL

Starting from the Lotka-Volterra [17, 27] equations, established as early as 1910, various models have been presented that exhibit oscillatory behaviour in different "biological" situations [28, 21, 20, 15, 14, 9, 10, 6, 2]. However, all these models, though they are of great interest, are not always relevant in the case of metabolic networks. Indeed, the models need to be based on relevant realistic metabolic equations such as enzyme kinetics, regulation, transport, etc. Moreover, in order to avoid the introduction of artefactual non-linear properties in the equations, a minimal approach needs to be followed. The minimal approach has been successfully applied for metabolic networks in modelling and simulating the intracellular calcium oscillations [4, 7] or the mitotic oscillator [8], for example.

Among these models we have selected one of them to simulate rhythmic processes: the negative feedback loop network (hereafter abbreviated NFL). This choice should by no way be interpreted as being in favour of a unique model of biological clocks of that kind, but rather as one of the multiple possibilities to generate biological rhythms in some specific situations.

NFL models for biological rhythms have been studied since 1963 ([9, 10, 11, 14, 16, 26], for an historical presentation see [22]). However, until recently, involvement of NFL in biological clocks was not really supported by experimental evidences, so that interest in this kind of model has not been sustained. Moreover, positive feedback loops have been successfully used in the case of oscillating behaviour. This result lead some authors [6, 23] to consider, also in accordance with theoretical arguments [25, 11], that the NFL could not be the basis of low frequency biological rhythms (e.g. rhythms with a period of about one day = circadian rhythms). It is clear that the main purpose of NFL is to stabilise fluxes and concentrations of substances in a metabolic network and not to oscillate. However, we present here some arguments that allow us to further study and use NFL as a possible good basis for describing rhythmic behaviour and even for some circadian rhythms.

First, NFL is of a rather widespread occurrence in living organisms. Second, from an evolutionary point of view, it may well be that non oscillating NFL became oscillating under cyclic environmental pressure giving advantages to organisms possessing such properties. Stabilisation and convergence towards the correct period could be obtained through classical mutation and selection. This
convergence to periodicity from a non oscillating NFL is possible since potentially most NFL can oscillate. Finally, there exists now very strong experimental evidences that NFL are part of biological rhythms as, for example, the circadian CREM (cAMP-Response Element Modulator) system in mammals [19], the period protein circadian rhythm in Drosophila [12, 13] and the circadian frequency gene expression in Neurospora [1, 3].

3.1 The negative feedback loop model

Already published studies have shown that there exists some minimum requirements for the feedback negative loop model in order that it can exhibit an oscillatory behaviour [26]. We will thus elaborate the model with five reactants (A to E) involving six processes (e.g. enzymes: \( V_0 \), \( V_A \) to \( V_E \) and a negative cooperative feedback of the 4th order (\( f_E \)). The scheme of the model is:

It is necessary to define the nature of the process which synthesises A \((V_0)\). We can assume that it is a constant flux, modulated by the regulatory feedback inhibitory function \( f_E \). Processes (fluxes) \( V_A \) to \( V_E \) could be either of the first order or of the Michaelis-Menten type. With these considerations and using the simple rules presented above, it is straightforward to obtain the set of differential equations of the dynamical system as:

First order flux:

\[
\frac{dA}{dt} = V_0 \frac{K_4^{A}}{K_4^{E} + E^4} - k_A A \quad (17)
\]

\[
\frac{dB}{dt} = k_A A - k_B B \quad (18)
\]

\[
\frac{dC}{dt} = k_B B - k_C C \quad (19)
\]

\[
\frac{dD}{dt} = k_C C - k_D D \quad (20)
\]
\[
\frac{dE}{dt} = k_D D - k_E E
\]

(21)

Michaelis-Menten:

\[
\frac{dA}{dt} = V_0 \frac{K_{M_E}^A}{K_{M_E}^A + A} - \frac{V_{m_A} A}{K_m A + A}
\]

(22)

\[
\frac{dB}{dt} = \frac{V_{m_A} A}{K_m A + A} - \frac{V_{m_B} B}{K_m B + B}
\]

(23)

\[
\frac{dC}{dt} = \frac{V_{m_B} B}{K_m B + B} - \frac{V_{m_C} C}{K_m C + C}
\]

(24)

\[
\frac{dD}{dt} = \frac{V_{m_C} C}{K_m C + C} - \frac{V_{m_D} D}{K_m D + D}
\]

(25)

\[
\frac{dE}{dt} = \frac{V_{m_D} D}{K_m D + D} - \frac{V_{m_E} E}{K_m E + E}
\]

(26)

The last step before computer simulation is to fix the constant values, respectively \(V_0\), \(K_{M_E}\), \(k_a\) to \(k_e\), \(V_{m_A}\) to \(V_{m_E}\), and \(K_m A\) to \(K_m E\). This is a critical point since no value can be obtained experimentally. In this case one should perform some mathematical stability analysis which would give some indications about the different behavioural states of the dynamical system (equilibrium, oscillations, chaos,...) depending on the values of these constants. In our case, it is known that a necessary condition is that the \(k_j\)s should not be of a too large difference in magnitude. In the Michaelis-Menten model, \(V_{m_i}/K_{m_i}\) ratios should also not be too different [26]. In order to simplify these estimations, it is possible to start with all these constants equal to 1. The results of the simulation for both models are shown in Figure 1.

Once the simulation is working, it is possible to use the basic dynamical system to test its behaviour in different situations according to experimental-like protocols used in different disciplines (physiology, biochemistry, genetics, evolution, etc.) and to compare the results of these simulations with the experimental data. In order to give some more details on this approach, we will present two simplified virtual experiments on the negative feedback loop model: virtual mutagenesis and biochemistry.
Figure 1. Simulation of the negative feedback loop network model with five reactants. For clarity only A, C and E reactant dynamics are shown. A: first order reactions rate model with $v_0=5, k_a=k_b=k_c=k_d=k_e=1, K_i=0.5$ and $n=4)$. B: Michaelis-Menten reactions rate model with $v_0=1, V_m=1, K_m=1, K_i=1$ and $n=4)$. 
4. VIRTUAL MUTAGENESIS OF THE NEGATIVE FEEDBACK LOOP MODEL

Mutagenesis is the modification of genetic information (DNA) which ultimately leads to changes in the wild-type functioning of the cell. The mutations can affect the activities of enzymes mostly by decreasing their actual catalytic rate or sometimes by increasing it. These mutations happen at random and with a random magnitude. To simulate this aspect with the NFL network model, we assume that the mutation also affects the kinetic constants at random with a random magnitude. The range or magnitude of the mutation is greatly in favor of a negative modification which is the most likely to occur. The parameters used for the NFL with first order flux (Equations 17-21) are specified below:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>&quot;locus&quot;</th>
<th>Wild type value</th>
<th>Range of change</th>
<th>Probability of change</th>
</tr>
</thead>
<tbody>
<tr>
<td>(v_0)</td>
<td>1</td>
<td>5</td>
<td>-90 to +50%</td>
<td>2/11</td>
</tr>
<tr>
<td>(n)</td>
<td>2</td>
<td>4</td>
<td>-25, -50, -75%</td>
<td>2/11</td>
</tr>
<tr>
<td>(K_{IE})</td>
<td>3</td>
<td>0.5</td>
<td>-90 to +100%</td>
<td>2/11</td>
</tr>
<tr>
<td>(k_a)</td>
<td>4</td>
<td>1</td>
<td>-90 to 50%</td>
<td>1/11</td>
</tr>
<tr>
<td>(k_b)</td>
<td>5</td>
<td>1</td>
<td>-90 to 50%</td>
<td>1/11</td>
</tr>
<tr>
<td>(k_c)</td>
<td>6</td>
<td>1</td>
<td>-90 to 50%</td>
<td>1/11</td>
</tr>
<tr>
<td>(k_d)</td>
<td>7</td>
<td>1</td>
<td>-90 to 50%</td>
<td>1/11</td>
</tr>
<tr>
<td>(k_e)</td>
<td>8</td>
<td>1</td>
<td>-90 to 50%</td>
<td>1/11</td>
</tr>
</tbody>
</table>

The probability of change is assumed to be slightly different in some cases to account for more complex reactions. For example, \(K_{IE}\) is a function of more than one elementary kinetic (k) constant.

After running the simulation with this "virtual mutagenesis" treatment, a great number of virtual phenotypes are obtained. Some typical and interesting examples are shown in Figure 2. It can be seen that either longer (e.g. tictac2(4,0.5)^L) or shorter (e.g. tictac181(1,0.4)^S) periods can be obtained. Aperiodic mutants are also present (e.g. tictac75(8,0.2)^o or tictac9(7,0.2)^o). The amplitude can also be changed with respect to the wild type. It can be higher or lower. An interesting situation is encountered with the mutant tictac33(4,0.3)^L, which possess a slowly damping rhythm: the simulation shows that, whereas the substrate A is oscillating, an experimental measure of E would certainly lead (due to errors of measurements and noise) to an apparent arrhythmicity of E. In fact, this is not the case. The conclusions from this virtual experiments are of great biological interest. First, different mutations can create phenotypes that can be similar to each other. Second, the oscillation can be affected by mutations at different loci, because there is a lot of reactions involved as in the case of the
Figure 2. Virtual mutagenesis. Simulation of dynamical behavior for the negative feedback loop network model (first order rate equations). The graphs represent the evolution of reactants $A$ and $E$ in arbitrary unit of concentration as a function of arbitrary units of time. The two upper graphs represent the dynamical behavior of the wild type model (see text for details). Some examples of interesting "mutants" are also shown with longer ($L$), shorter ($S$) or an aperiodic ($O$) evolution of reactants.
period protein in Drosophila, where an other mutation also affects the period of this protein (see tim mutation [24]).

The same simulations can be done with the Michaelis-Menten model (Equations 22-26), where the number of targets for virtual mutagenesis is greater and more complex, due to the terms $V_{mi}$ and $K_{mi}$. Similar results are obtained which show an even greater variability of phenotypes. Some examples are presented in Figure 3 with longer, shorter and aperiodic mutants. With the appropriate change of scale of $K_m$ and $V_m$ values, it is possible to convert any arbitrary unit (AU) of time to any value wanted. Thus, assuming that the period of the wild type is of 24 h, one obtains the following corresponding periods for the virtual mutants:

<table>
<thead>
<tr>
<th>Type</th>
<th>&quot;Locus&quot;</th>
<th>Mutation</th>
<th>Period (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>-</td>
<td>-</td>
<td>24</td>
</tr>
<tr>
<td>chronos1L</td>
<td>$V_{md}$</td>
<td>negative from 1 to 0.7</td>
<td>28.5</td>
</tr>
<tr>
<td>chronos2S</td>
<td>$V_{me}$</td>
<td>negative from 1 to 0.4</td>
<td>19.6</td>
</tr>
<tr>
<td>chronos3O</td>
<td>n</td>
<td>negative from 4 to 1</td>
<td>aperiodic</td>
</tr>
</tbody>
</table>

It is interesting to observe in this particular example that negative mutations (decrease in the maximum rate velocity $V_{max}$ of the processes, e.g. enzymes) can yield either an increase or a decrease of the period depending on where the mutation occurs. This result is not intuitively obvious, but could be anticipated, if we recall that the system analysed is dealing with non-linear dynamics.

5. VIRTUAL BIOCHEMISTRY OF THE NEGATIVE FEEDBACK LOOP MODEL

Among a lot of different possibilities, one can consider a biochemistry-like virtual approach of the NFL model network by using some simple common standard procedures adopted in this field. As an example, we can consider the Michaelis-Menten model as a network catalysed by different enzymes that could be isolated in the computer then characterised with classical enzyme kinetic methods. The results of such measurements can be displayed on a virtual spectrophotometer. For a practical example, we can examine what can be obtained by isolating two particular processes of the NFL network, namely $P_0$ and $P_E$: 
Figure 3. Virtual mutagenesis. Simulation of dynamical behavior for the negative feedback loop network model (Michaelis-Menten rate equations). The graphs represent the evolution of reactants A and E in arbitrary unit of concentration as a function of arbitrary units of time. The upper graph represents the dynamical behavior of the wild type model (see text for details). Some examples of interesting "mutants" are also shown with longer (L), shorter (S) or an aperiodic (O) evolution of reactants.
Isolating the process $P_E$ is equivalent to remove it from the rest of the network. For the simulation in a virtual spectrophotometer, this can be done by supplying different concentrations of $E$ and following the disappearance of this substrate. The equation for the simulation is thus:

$$\frac{dE}{dt} = \frac{V_m E}{K_m + E}$$  \hspace{0.5cm} (27)$$

Similarly, the process $P_0$ is removed from the network and the appearance of the reactant $A$ monitored in the virtual spectrophotometer by:

$$\frac{dA}{dt} = v_0 \frac{K^4}{K^4 + E^4}$$  \hspace{0.5cm} (28)$$

The results of the virtual experiments with different mutants are shown in Figure 4. It is important to observe that the virtual biochemistry approach clearly shows that isolated processes do not show any oscillation. In this regard one may wonder where the oscillation with its characteristic period value is generated. The answer in this context is very simple: the rhythmic behaviour is a property of the network itself, the oscillatory property being generated by specific relationships (and half-life time of the reactants) of the whole integrated network. If this is true, the search for one specific gene of the rhythm could well be an elusive goal. In this regard it has to be noted that the rhythmic period protein expression in *Drosophila* is not only affected by mutations in the *per* gene but also in an other gene, the *tim* one [24].

5. CONCLUSION

Simple metabolic networks, such as the one presented here, namely the negative feedback loop, exhibit complex behaviour. It has also been shown that other relatively simple metabolic networks can exhibit even more complex patterns such as chaos, intermittence, bursting etc. [6]. All these complexities are intrinsic properties of non-linear dynamical systems. It is a matter of fact that non-linear patterns are difficult to interpret and understand, i.e. it is difficult to
Figure 4. Virtual results of the isolated $P_E$ and $P_0$ processes measured in a virtual spectrophotometer. Different specified concentrations of reactant $E$ are added in order to study the concentration dependence of each process with different mutants.
distinguish if the complex behaviour is the result of non-linear properties deriving from a simple or relatively simple series of molecular events, or if the underlying molecular events are very complex. In this perspective, the use of minimal models, which contain the minimum relevant information that could simulate correctly enough the experimental results, is a useful step in the elucidation of the molecular mechanisms involved. Indeed, they can pinpoint to the core of the underlying investigated phenomenon. It is our opinion that simulation will become an indispensable tool in the domain of non-linear phenomena in biology, since unravelling the apparent dynamical complexity of these networks is something that the human brain can not intuitively resolve.

REFERENCES


