On proving the absence of oscillations in models of genetic circuits*

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Abstract. Using computer algebra methods to prove that gene regulatory networks cannot oscillate appears to be easier than expected. We illustrate this claim on a family of models related to historical examples.

1 Introduction

The authors belong to a pluridisciplinary working group whose goal is to model the gene regulatory network controlling the circadian clock of a unicellular green alga [1]. See [2] for a survey on circadian rhythms and [3, Chapter 9] or [4, 5] for more general texts about oscillations in biology. In doing so, they have gained some experience in designing models of oscillating gene regulatory networks.

One of the main problems faced by our working group can be formulated as follows: given a system of parametric ordinary differential equations built using mass action law kinetics, does there exist ranges of values for the model parameters and variables which are both meaningful from a biological point of view and where oscillating trajectories, i.e. limit cycles, can be found ?

This issue is theoretically very difficult. It is related to the unsolved Hilbert sixteenth problem. Indeed, systems of parametric ordinary differential equations which oscillate may do so only for very restricted ranges of parameters values. The difficulty is strengthened by the number of parameters arising in biochemical models, which can quickly become very large.

A related but easier problem consists of searching for the existence of parameter and variable values which are both meaningful from a biological point of view and give rise to a Hopf (more precisely Poincaré–Andronov–Hopf) bifurcation. See [6, Chapter 11], [5, Section 3.5] or [7, Section I.16]. In the neighborhood of a Hopf bifurcation indeed, a stable steady point of the model under study gives birth to a small stable limit cycle under some general hypotheses. Note that searching for Hopf bifurcations is not as general as searching for limit cycles:

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first, some Hopf bifurcations (e.g. the subcritical ones) do not strictly imply the existence of stable limit cycles; second, there may exist limit cycles not related to Hopf bifurcations; third, a model may involve a Hopf bifurcation for parameters and variables values which are *close* to but *outside* of the biologically meaningful parameter domain values and generate limit cycles inside this domain.

There exist software packages such as AUTO or XPPAUT [8, 9] which locate Hopf bifurcations by means of numerical calculations. They allow one to evidence the existence of Hopf bifurcations but not to prove their absence, and thus cannot be used to discard a model. Theoretically, the existence or the absence of Hopf bifurcations can be decided algebraically. See e.g. [10–13]. In particular, it can be decided by means of computer algebra methods which rely on Sturm sequences computations and algebraic elimination. Practitioners usually seem to avoid these methods because of their huge complexity in the worst case. In particular, we tried[3] the QEPCAD [14] package, which is based on quantifier elimination methods. However, we could not solve the problems addressed in this paper with it. We did not try the REDLOG package [15] and the software described in [10] for they rely on QEPCAD for the quantifier elimination process. An attempt to solve the addressed problem using the RAGLib library [16] is in progress, with the help of its author.

By comparison, the computer algebra methods described in this paper are very light. They take advantage of the special structure of the equations and of the biological constraints. This indicates that if used carefully, computer algebra methods may apply on more complex examples than one might expect.

In order to illustrate the core ideas of this paper in a simple setting, we do not study realistic models of circadian clocks but focus on a simple family of models depending on an integer parameter n and featuring a negative feedback loop, one of the core ingredients for generating oscillations [3]. These abstract models are closely related to models studied by Goodwin and Griffith in the 60's [17–19]. In particular, Griffith considered a model of a gene regulated by a polymer formed of n copies of its own protein. We study the same problem, but in a slightly more general case, where gene activation is not assumed to be fast. We conclude with the absence of Hopf bifurcation in our family of models for $n \leq 8$ and their existence for $n \geq 9$. Although we do focus here on biology, it should be stressed that a cooperativity of order 9 is not as unrealistic as it may seem. In particular, gene regulation by an octamer has been reported [20]. Moreover, an effective cooperativity of order 9 may also be obtained as a consequence of reducing a higher-dimensional, more realistic, model to a three-variable one. Finally, our conclusions are consistent with those of Griffith [3, Pages 244–246] and of other works devoted to more sophisticated variants of the Goodwin model [21–23], and thus we believe that the interest of the present paper goes beyond illustrating computer algebra methods. The application of these methods to more realistic biological models is in progress and is left for a future paper.

[3] QEPCAD was downloaded from `www.cs.usna.edu/~qepcad` and installed on a computer endowed with a Pentium 4 and 512 MB of RAM. Tests were performed by increasing the default number of cells up to its maximal limit: 200 millions of cells.

Our paper is organized as follows. Section 2 describes the family of models we study. Some basic facts about Hopf bifurcations are recalled in section 3. In section 4, we use computer algebra methods to prove the absence of Hopf bifurcation in our model for $n \leq 8$ and the occurrence of Hopf bifurcations for $n \geq 9$. The methods involved are Gröbner basis theory [24, 25] and Descartes' rule of signs [26]. Computations are performed using the MAPLE 9 computer algebra software. Our proofs were constructed after carrying out intensive numerical simulations which strongly suggested the results.

2 Our family of models

Figure 1 displays a gene regulated by a polymer obtained by combining n times a protein. The model variables are the state G of the gene, the mRNA concentration M and the concentration P of the protein translated from the mRNA. Greek letters represent parameters. The initial model involves $n+2$ differential

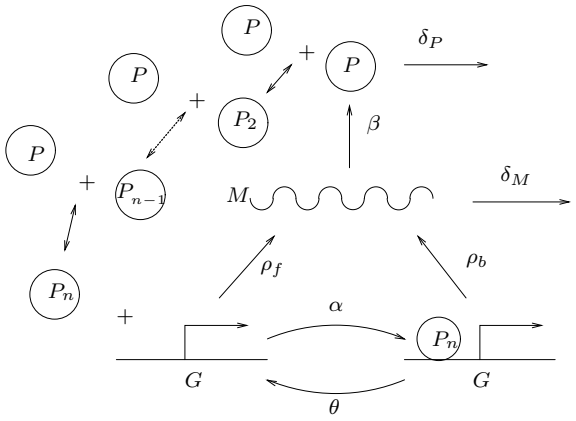


Fig. 1. A gene regulated by a polymer of its protein

equations depending on $2\,n+5$ parameters. By means of a suitable quasi-steady state approximation, described in section 2.1, one obtains the following reduced model, involving only three equations:

$$\begin{aligned} \dot{G} &= \theta\,(\gamma_0 - G - G\,P^n), \\ \dot{P} &= n\,\alpha\,(\gamma_0 - G - G\,P^n) + \delta\,(M - P), \\ \dot{M} &= \lambda\,G + \gamma_0\,\mu - M. \end{aligned} \tag{1}$$

All variables and parameters are positive apart λ, which is allowed to be negative. The protein P reacts with itself, forming a polymer. Gene activity is regulated by the polymer as it binds to the gene promoter. Depending on the sign of λ, the polymer is an activator or a repressor: if $\lambda < 0$ then mRNA transcription

is enhanced when polymer is bound to promoter ; if $\lambda > 0$ then mRNA transcription is reduced. The G variable takes values in the range $[0, \gamma_0]$ and can be viewed as an averaged gene activity. The values $G = 0$ and $G = \gamma_0$ correspond repectively to a polymer being bound to the gene promoter or not.

2.1 Model reduction

The chemical system involves $n + 5$ reactions, described below. Denote P_i the polymer obtained by combining i proteins P with the convention $P_1 = P$.

$$G + P_n \underset{\theta}{\overset{\alpha}{\rightleftharpoons}} G : P_n, \quad G \xrightarrow{\rho_f} G + M, \quad G : P_n \xrightarrow{\rho_b} G : P_n + M,$$
$$M \xrightarrow{\beta} M + P, \quad M \xrightarrow{\delta_M} \emptyset, \quad P \xrightarrow{\delta_P} \emptyset, \qquad P_i + P \underset{k_i^-}{\overset{k_i^+}{\rightleftharpoons}} P_{i+1} \quad (1 \le i \le n-1).$$

The dynamics of these reactions is governed by the following equations, where $A_i = (1/\varepsilon)(k_{i+1}^- P_{i+1} - k_{i+1}^+ P_i P)$:

$$\begin{aligned}
\dot{G} &= \theta\,(\gamma_0 - G) - \alpha\, G\, P_n, \\
\dot{M} &= \rho_f\, G + \rho_b\,(\gamma_0 - G) - \delta_M\, M, \\
\dot{P} &= \beta\, M - \delta_P\, P + 2\, A_1 + A_2 + \cdots + A_{n-1}, \\
\dot{P}_i &= -A_{i-1} + A_i \qquad (2 \le i \le n-1), \\
\dot{P}_n &= -A_{n-1} + \theta\,(\gamma_0 - G) - \alpha\, G\, P_n
\end{aligned}$$

The $1/\varepsilon$ factor is introduced to express the fact that the various steps of protein polymerization are assumed to be fast compared to other reactions (transcription, translation, degradation, binding of polymer to the gene). Eliminating the A_i by means of quasi steady state assumptions ($\dot{P}_2, \ldots, \dot{P}_n$ are assumed to be small), one reformulates the third differential equation as:

$$\dot{P} = \beta\, M - \delta_P\, P + n\,(\theta\,(\gamma_0 - G) - \alpha\, G\, P_n).$$

The P_n variable can be reexpressed as

$$P_n = \frac{k_1^+ \cdots k_{n-1}^+}{k_1^- \cdots k_{n-1}^-}\, P^n + \text{a term multiplied by } \varepsilon.$$

Neglecting the term multiplied by ε and introducing a new parameter $\bar{\alpha}$, one is thereby led to the following system of three differential equations:

$$\begin{aligned}
\dot{G} &= \theta\,(\gamma_0 - G) - \alpha\,\bar{\alpha}\, G\, P^n, \\
\dot{P} &= n\,\theta\,(\gamma_0 - G) - n\,\alpha\,\bar{\alpha}\, G\, P^n + \beta\, M - \delta_P\, P, \\
\dot{M} &= \rho_f\, G + \rho_b\,(\gamma_0 - G) - \delta_M\, M.
\end{aligned}$$

The model can now be simplified by rescaling all parameters and indeterminates G, M and P. Since the reduction involves many (easy) intermediate computations, we only sketch it here. First replace $\alpha\,\bar{\alpha}$ by α. Then replace P by

$(\theta/\alpha)^{1/n}\,P$. Expand the equation in $\dot{M}$, replace $\rho_f - \rho_b$ by λ and $\rho_b\,\gamma_0$ by μ. This implies that λ may be *positive or negative*. Then apply rescale time by replacing t by $\delta_M\,t$. This last transformation simplifies the term $-\delta_M M$ into $-M$. At this stage, one gets the following system:

$$\dot{G} = \frac{\theta}{\delta_M}\,(\gamma_0 - G - G\,P^n), \quad \dot{M} = \frac{\lambda}{\delta_M}\,G + \frac{\mu}{\delta_M} - M,$$
$$\dot{P} = n\,\left(\frac{\alpha}{\theta}\right)^{\frac{1}{n}}\,\frac{\theta}{\delta_M}\,(\gamma_0 - G - G\,P^n) + \left(\frac{\alpha}{\theta}\right)^{\frac{1}{n}}\,\frac{\beta}{\delta_M}\,M - \frac{\delta_P}{\delta_M}\,P.$$

Then discard all the δ_M by replacing β/δ_M, δ_P/δ_M, λ/δ_M and μ/δ_M by β, δ, λ and μ. Then replace α^n/θ^{n-1} by α. Then replace θ/δ_M and α/δ_M by θ and α. Using the fact that M occurs only in linear terms, renormalize last M so that $\beta = \delta_P$ and update λ and μ. One finally gets our reduced model (1).

Comments. Gene activity is regulated by P^n. The reduced model is designed so that the steady state depends only on parameters λ, μ and γ_0 while θ, α and δ control time scales. Note that Griffith model is recovered by letting θ and α tend towards $+\infty$, keeping the ratio θ/α constant. When translation is equal to degradation i.e. $\delta = 0$, $n\,G - (\theta/\alpha)\,P$ is constant which expresses the fact that DNA binding and unbinding do not modify the total quantity of proteins.

3 Hopf bifurcations

3.1 Hurwitz determinants

Let $\dot{x} = F(x)$ be a differential system in m dependent variables. The steady points of the differential system are the zeros of the system (that we assume to be polynomial or rational) $F(x) = 0$. To each steady point, one may associate a linear system $\dot{x} = J\,x$ where J is the $m \times m$ jacobian matrix of the differential system, evaluated over the steady point. The stability of the steady state is determined by the eigenvalues of J. It is stable if and only if all eigenvalues have negative real parts. Thus to each steady point, one may associate the characteristic polynomial $C(\sigma) = \sigma^m + a_1\,\sigma^{m-1} + \cdots + a_m$ $(a_0 = 1)$ of J. Thanks to the Routh-Hurwitz criterion, the stability of the steady points can be studied by analyzing the sign of the Hurwitz determinants $c_{k,0}$. These ones can be directly computed from the coefficients of the characteristic polynomial, as shown below. Following [7, Section I.13], compute the Sturm sequence:

$$p_0(\omega) = \Re\left(\frac{C(i\,\omega)}{i^m}\right), \quad p_1(\omega) = -\Im\left(\frac{C(i\,\omega)}{i^m}\right) \tag{2}$$
$$p_{k+2}(\omega) = -\operatorname{rem}(p_k,\, p_{k+1},\, \omega) \qquad (k \geq 0).$$

Denote $p_k(\omega) = c_{k,0}\,\omega^{m-k} + c_{k,1}\,\omega^{m-k-2} + c_{k,2}\,\omega^{m-k-4} + \cdots$ Observe that the computation of p_k must be performed carefully (e.g. using subresultant sequences) to ensure that $c_{k,0}$ actually is a Hurwitz determinant. See [10]. Indeed,

$$c_{0,0} = 1, \quad c_{1,0} = a_1, \quad c_{2,0} = a_1\,a_2 - a_3, \quad \ldots, \quad c_{m,0} = a_m\,c_{m-1,0}.$$

The two following propositions are well known. The first one is nearly a corollary to the Routh Theorem [7, Theorem 13.4].

Proposition 1. *With the same notations, if all the Hurwitz determinants $c_{k,0}$ are positive, apart perhaps $c_{m,0}$, then J has no pure imaginary eigenvalue.*

Proof. If all the Hurwitz determinants $c_{k,0}$ are positive ($0 \leq k < m$) then they are *a fortiori* nonzero. Assume J has pure imaginary eigenvalues $\pm i\,\bar{\omega}$ (they are necessarily conjugate). These values $\pm\bar{\omega}$ are then common zeros of p_0 and p_1. The gcd of p_0 and p_1 has thus degree greater than or equal to 2. This gcd is the last nonzero polynomial in the sequence $p_0, \ldots, p_{m-1}$. Thus one polynomial p_k with $0 \leq k < m$ must vanish identically. Therefore the corresponding Hurwitz determinant $c_{k,0}$ must vanish also.

Proposition 2. *With the same notations, if all the Hurwitz determinants $c_{k,0}$ are positive ($0 \leq k \leq m-2$) and $c_{m-1,0} = 0$ and $c_{m-2,1} < 0$ then all the eigenvalues of J have negative real parts except a purely imaginary conjugate pair.*

Proof. The polynomial p_{m-1} has the special form $p_{m-1} = c_{m-1,0}\,\omega$. We have $c_{m-1,0} = 0$. Then p_0 and p_1 have a degree two gcd, p_{m-2}, which has the special form $p_{m-2} = c_{m-2,0}\,\omega^2 + c_{m-2,1}$. We have $c_{m-2,1} < 0$ and $c_{m-2,0} > 0$ thus, the common roots $\pm\bar{\omega}$ of p_0 and p_1 are real. Therefore J has one pair of purely imaginary conjugate eigenvalues $\pm i\,\bar{\omega}$. Now, compute the Sturm sequence (2) over the polynomial $\bar{C}(\sigma) = C(\sigma)/(\sigma^2+\bar{\omega}^2)$. This Sturm sequence $\bar{p}_0, \bar{p}_1, \ldots, \bar{p}_{\bar{m}}$ can actually be derived from that of C:

$$\bar{p}_0(\omega) = \frac{p_0}{\sigma^2+\bar{\omega}^2}, \quad \bar{p}_1(\omega) = \frac{p_1}{\sigma^2+\bar{\omega}^2}, \quad \ldots, \quad \bar{p}_{\bar{m}}(\omega) = c_{m-2,0}.$$

All the corresponding Hurwitz determinants are positive. According to the Routh Theorem [7, Theorem 13.4], all the roots of $\bar{C}$ have negative real parts. This concludes the proof of the proposition.

For $m = 3$ we have $c_{m-2,1} = -a_3$. For $m = 4$ we have $c_{m-2,1} = -a_1\,a_4$.

3.2 Hopf bifurcations

The differential systems encountered in biological modelling involve parameters. Let $\dot{x} = F(x, \theta)$ be a differential system in m variables and p parameters θ. If some real values are assigned to the parameters then one gets a system such as the one described in section 3.1. If these real values continuously vary then the steady points and their associated eigenvalues continuously vary also.

Definition 1. *With notations as above, a Hopf bifurcation arises for a steady point when all the eigenvalues associated to the steady point have negative real parts except one complex conjugate pair, which crosses the imaginary axis because of a variation in the system parameters.*

3.3 In computer algebra

In computer algebra, an important point is to avoid to compute the steady points, i.e. not to solve the system $F(x,\,\theta) = 0$. The Hurwitz determinants can be computed generically. They depend on the system parameters. Their sign is studied modulo the ideal I generated by the polynomial system $F(x,\,\theta) = 0$. The absence of Hopf bifurcation is established, thanks to proposition 1 and definition 1, by proving that the Hurwitz determinants $c_{0,0}, \ldots, c_{m-1,0}$ are positive for all x and θ, considering that x and θ satisfy $F(x,\,\theta) = 0$ plus, usually, some extra (positivity) conditions such as $x,\,\theta > 0$.

The Hurwitz determinants $c_{k,0}$ get reformulated by computing their normal forms $\bar{c}_{k,0}$ w.r.t. any Gröbner basis of the ideal I. Reference books for the Gröbner basis theory are [24, 25]. Indeed, the difference $c_{k,0} - \bar{c}_{k,0}$ belongs to I. Over any steady point of the differential system, it is thus zero, thus the two polynomials $c_{k,0}$ and $\bar{c}_{k,0}$ have the same value hence the same sign.

In practice moreover, Gröbner bases can be computed in dimension zero. Computing in dimension zero corresponds to some generic computation, which may be false for particular values of the system variables and parameters. However, in biological models, parameters (and thus variables) have no accurate values and zero dimensional computing makes sense.

4 Application to our models

To permit the reader to reproduce our computations, we provide the sequence of MAPLE 9 commands which prove that no Hopf bifurcation may arise in our models for positive values of the system variables and parameters (apart λ).

The *LinearAlgebra* package, the *Groebner* package and the *Jacobian* function of the *VectorCalculus* package are loaded. The list of the model variables is assigned to *vars*.

```
with (LinearAlgebra):
with (VectorCalculus,Jacobian):
with (Groebner):
vars := [G, P, M]:
```

The list of the right–hand sides of the model equations is assigned to the *equilibria* variable. The zeroes of this polynomial system provide the steady points of the model.

```
equilibria := [
    theta*(gamma0 - G - G*P^n),
    n*alpha*(gamma0 - G - G*P^n) + delta*(M-P),
    lambda*G + gamma0*mu - M]:
```

In general, one cannot compute a Gröbner basis of the ideal I generated by such a system if a symbolic n is left as an exponent. But in our case, generic Gröbner bases exist, at least w.r.t. some admissible orderings. Let us fix the pure lexicographical ordering given by $\lambda > M > \gamma_0$. The other model variables

and parameters are considered as algebraically independent elements of the base field of the equations. The Gröbner basis is thus computed in dimension zero i.e. in the polynomial ring $\mathbb{K}[\lambda, M, \gamma_0]$ where $\mathbb{K}$ denotes the field obtained by adjoining all the remaining variables and parameters to the field of the rational numbers. Here are the Gröbner basis elements. The leading terms appear on the left–hand side of the equations:

$$\gamma_0 = G + G\,P^n, \quad M = P, \quad \lambda = \frac{P - \mu\,G - \mu\,G\,P^n}{G}.$$

Observe that for any particular value of n, the Gröbner basis can be computed by the following sequence of MAPLE commands (these commands do not permit to obtain the generic form directly):

```
ordre := plex (lambda, M, gamma0):
basis := gbasis (equilibria, ordre):
seq (leadterm (basis [i], ordre) =
    solve (basis [i], leadterm (basis [i], ordre)),
    i = 1 .. nops (basis));
```

The computed Gröbner basis has two striking properties: its leading terms are plain variables ; apart for λ which is allowed to be negative, the right–hand sides of the three other equations of the Gröbner basis are necessarily positive. The first property implies that the quotient ring is a free algebra: a polynomial ring. In particular, the product of two normal forms is itself a normal form. The second property implies that there are no constraints on the values that can be assigned to the model variables and parameters occuring in the right–hand sides of the equations since positivity is the only requirement for the values of γ_0 and M. Therefore, to evaluate the Routh–Hurwitz criterion over the model steady point, it is sufficient to replace each element by its normal form in the Jacobian matrix J of the model.

```
J := Jacobian (equilibria, vars):
```

$$J := \begin{pmatrix} -\theta\,(1+P^n) & -n\,\theta\,G\,P^{n-1} & 0 \\ -n\,\alpha\,(1+P^n) & -n^2\,\alpha\,G\,P^{n-1} - \delta & \delta \\ \lambda & 0 & -1 \end{pmatrix}$$

The generic normal form of J is:

$$J = \begin{pmatrix} -\theta\,(1+P^n) & -n\,\theta\,G\,P^{n-1} & 0 \\ -n\,\alpha\,(1+P^n) & -n^2\,\alpha\,G\,P^{n-1} - \delta & \delta \\ \dfrac{P - \mu\,G - \mu\,G\,P^n}{G} & 0 & -1 \end{pmatrix}$$

From now on, J is assumed to be under normal form. This implies in particular that all the expressions computed from J are free of the parameter λ. These expressions thus only involve positive variables. For any particular value of n, the normal form of the jacobian can be computed by the following command:

```
J := map (normalf, J, basis, ordre):
```

The characteristic polynomial of J writes: $\sigma^3 + a_1\,\sigma^2 + a_2\,\sigma + a_3$. Its coefficients are stored in indexed variables:

```
pol := CharacteristicPolynomial (J, sigma):
for i from 1 to nops (vars) do
    a[i] := coeff (pol, sigma, nops (vars) - i)
od:
```

The Hurwitz determinants $c_{k,0}$ can now be computed from the coefficients a_k of the characteristic polynomial.

```
c[0,0] := 1:
c[1,0] := a[1]:
c[2,0] := a[1]*a[2]-a[3]:
c[3,0] := a[3]*(a[1]*a[2]-a[3]):
```

In order to apply propositions 1 and 2, one needs to study the positivity of the Hurwitz determinants $c_{k,0}$ for $0 \leq k \leq m-1 = 2$. The coefficient $c_{0,0}$ is obviously positive. So is the coefficient $c_{1,0}$ (below), for it is generically equal to a sum of monomials involving positive variables and coefficients:

$$1 + n^2\,\alpha\,G\,P^{n-1} + \delta + \theta + \theta\,P^n$$

The coefficient $c_{2,0}$ is more complicated. However it has a very special form. It is equal to a sum of monomials with positive coefficients minus the single following monomial: $n\,\theta\,\delta\,P^{n+2}$. Readers who would like to reproduce the computations may want to use our *negterms* function, described in section 4.4:

```
negterms(c[2,0]);
```

$$[[-n\,\theta\,\delta\,P^{n+2}], []]$$

4.1 Cases $n = 1$ and $n = 2$

Those two cases are easy. Indeed, for these values of n, the negative term in $c_{2,0}$ is cancelled by another coefficient (namely $2\,\theta\,\delta\,P^{n+2}$). This implies that $c_{2,0}$ is always positive. Thanks to proposition 1 no Hopf bifurcation may occur.

```
negterms(subs(n=1, c[2,0]));
```

$$[[], []]$$

```
negterms(subs(n=2, c[2,0]));
```

$$[[], []]$$

4.2 Cases $3 \leq n \leq 8$

In these cases, $c_{2,0}$ is also always positive. The proof here is less straightforward than above. It relies on Descartes' rule of signs. See [26].

Proposition 3. *(Descartes' rule of signs)*
Let $p = a_0\,x^d + \cdots + a_{d-1}\,x + a_d$ be a polynomial in one indeterminate x and real coefficients. Denote $r(p)$ the number of positive real roots of p, counted with multiplicities, and $v(p)$ the number of sign changes in the sequence $a_0, \ldots, a_d$ (the zero coefficients must be removed). Then $v(p) \geq r(p)$. In particular, if $v(p) = 0$ then $r(p) = 0$; if $v(p) = 1$ then $r(p) = 1$.

To simplify the positivity proof of $c_{2,0}$, one first cancels all the monomials not depending on θ, δ and P (i.e. the variables and parameters occuring in the negative term). One then clears the denominator P^2 out. One is thus led to prove the positivity of the *cond* polynomial computed below.

```
X := indets (c[2,0]) minus indets (negterms (c[2,0]));
```

$$X := \{\alpha,\, G,\, \mu\}$$

```
cond := numer (subs (seq (X[i]=0, i=1..nops(X)), c[2,0]))/P^2:
```

The polynomial *cond* is actually a polynomial in P^n. By a change of variables, one is led to study the positivity of the following degree 2 polynomial in P:

```
cond := collect (subs (P^n=P, cond), P):
```

$$(\theta^2\,\delta+\theta^2)\,P^2+(2\,\theta^2\,\delta+\theta+2\,\theta\,\delta+\delta^2\,\theta+2\,\theta^2-\theta\,\delta\,n)\,P+(2\,\theta\,\delta+\delta^2\,\theta+\delta^2+\theta^2\,\delta+\theta+\delta+\theta^2)$$

Let us write *cond* as $A\,P^2 + B\,P + C$. The coefficients A and C are positive for they only involve monomials with positive coefficients. Therefore, the conditions $B^2 - 4\,A\,C \geq 0$ (to ensure the existence of real roots) and $B < 0$ (to ensure the existence of positive roots by Descartes' rule of signs) are necessary and sufficient to have $cond = 0$ for $P > 0$. Condition $B^2 - 4\,A\,C \geq 0$ leads to $\theta \leq \theta_0$ where

```
theta0 := solve (discrim (cond, P) / theta^2, theta):
```

$$\theta_0 := \frac{\delta^4 - 2\,\delta^3\,n + \delta^2\,n^2 - 4\,\delta^2\,n + 1 - 2\,\delta^2 - 2\,\delta\,n}{4\,\delta\,n\,(1+\delta)}.$$

The condition $B < 0$ leads to $\theta < \theta_1$, where

```
theta1 := solve (coeff (cond, P, 1) / theta, theta);
```

$$\theta_1 := \frac{-1 - 2\delta - \delta^2 + \delta\,n}{2\,(1+\delta)}.$$

One is thus led to prove that the two conditions

$$0 < \theta \leq \theta_0, \quad 0 < \theta < \theta_1 \tag{3}$$

cannot be satisfied at the same time. One may convince oneself by plotting curves (see Figure 2). Let us continue the analysis more algebraically. For $\delta = 0$ we have $\theta_1 < 0$. Therefore, $\theta_1(\delta) > 0$ only if δ lies in the interval bounded by the two real roots of θ_1 i.e.

$$\frac{n - \sqrt{n^2 - 4\,n}}{2} - 1 < \delta < \frac{n + \sqrt{n^2 - 4\,n}}{2} - 1.$$

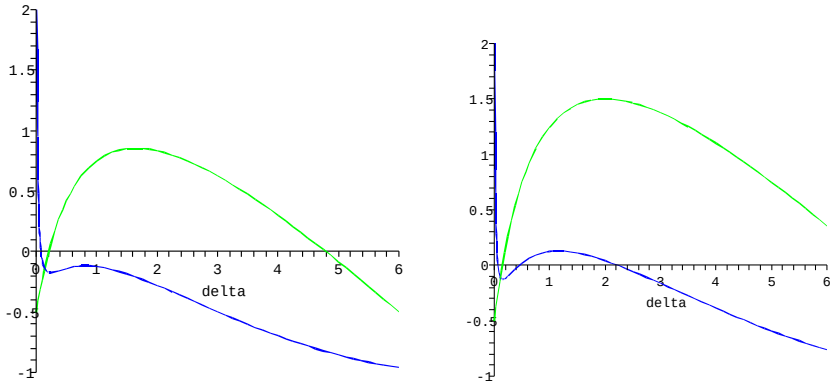


Fig. 2. The curves $\theta_0(\delta)$ and $\theta_1(\delta)$ are not simultaneously positive for $n = 7$ (left) while they are simultaneously positive for $n = 9$ (right). The curve for $\theta_1(\delta)$ starts with a negative value.

which only holds for $n > 4$. Hence no Hopf bifurcation may occur for $n \leq 4$, thanks to proposition 1.

To solve the remaining cases $5 \leq n \leq 8$, it is sufficient to compute a table of variations. For each value of n, one may isolate the positive real roots of $\theta_0(\delta)$ and $\theta_1(\delta)$ in arbitrary small intervals, ensuring that all the intervals are disjoint. Evaluating these two expressions for values of δ outside these intervals, one easily proves that conditions (3) cannot hold simultaneously. Hence no Hopf bifurcation may occur for $5 \leq n \leq 8$. Here is such a table for $n = 7$:

δ		0.07			0.21		4.79		12.58	
θ_0	+	0	−	−	−	−	−	0	+	
θ_1	−	−	−	0	+	0	−	−	−	

The *realroot* function of MAPLE may be used to isolate the roots, the following commands show. This function implements the algorithm described in [27]. Its is based on Descartes' rule of signs.

```
readlib (realroot):
realroot (subs (n=7, numer (theta0)), 1e-3);
```

$$\left[\left[\frac{81}{1024}, \frac{41}{512}\right], \left[\frac{12879}{1024}, \frac{805}{64}\right]\right]$$

```
realroot (subs (n=7, numer (theta1)), 1e-3);
```

$$\left[\left[\frac{213}{1024}, \frac{107}{512}\right], \left[\frac{2453}{512}, \frac{4907}{1024}\right]\right]$$

4.3 Case $n = 9$

Hopf bifurcations may arise for $n = 9$. One can be found using proposition 2. Using a table of variations or looking at Figure 2, we see that $\delta = 1$ and $\theta = 1/10$ satisfy conditions (3). Replace α, μ and G, which were set to zero in *cond*, by small positive values (say 1/1000). The Hurwitz determinant $c_{2,0}$ is then a polynomial in P with real roots. The following commands compute one of them.

```
vals := n=9,delta=1,theta=1/10,alpha=1/1000,mu=1/1000,G=1/1000:
c20 := normal (subs (vals, c[2,0])):
valP := realroot (c20, 1e-6) [1];
```

$$valP = \left[\frac{41381}{32768}, \frac{1324193}{1048576}\right]$$

The above values cancel the Hurwitz determinant $c_{2,0}$. The following command checks that $c_{1,1} = -a_3 < 0$. Observe that the computation could be performed more carefully by means of interval arithmetics.

```
evalf(subs(vals,P=valP[1],-a[3])), evalf(subs(vals,P=valP[2],-a[3]));
```

$$-8.268768356,\ -8.268823875$$

According to proposition 2, a Hopf bifurcation should occur. Numerical simulations show that this Hopf bifurcation gives birth to oscillations.

4.4 Comments

A general analysis. It is possible to prove the absence of Hopf bifurcation for $n \leq 8$ without discussing all the cases. The idea, which is only sketched here, starts by noticing that the real roots of $\theta_0(\delta)$ belong to the range[4] $]1/n,\ n-2[$.

One first computes the resultant w.r.t. δ between $\theta_1(\delta)$ and its first derivative w.r.t. δ. It vanishes only for $n = 8$. Thus, apart for this case which may be handled separately, all the roots of $\theta_1(\delta)$ are simple.

One then proves that $\theta_1(\delta)$ admits one real root in the range $]0,\ 1/n[$. This can be done by performing a change of variables over $\theta_1(\delta)$, mapping the range $]0,\ 1/n[$ to the range $]0,\ +\infty[$ in order to apply Descartes' rule of signs. Here are the corresponding MAPLE commands:

```
z := collect (numer (theta0), delta):
z1 := collect (numer (subs (delta=1/(n*(sigma+1)), z)), sigma);
```

$$z_1 := n^4\,\sigma^4 + 2\,n^4\,\sigma^3 + (n^4 - 4\,n^3 - 2\,n^2)\,\sigma^2 - (8\,n^3 + 6\,n^2)\,\sigma - 4\,n^3 - 4\,n^2 + 1$$

For any $n \geq 1$, the number of changes of signs is equal to 1. By Descartes' rule of signs, $z_1(\sigma)$ admits exactly one positive real root whence[5] $\theta_1(\delta)$ admits exactly one positive root smaller than $1/n$. Therefore, $\theta_1(\delta) < 0$ after crossing this root.

[4] one denotes $]a,\ b[$ the open interval $[a,\ b]$.

[5] This is the very argument applied by the *realroot* function!

Performing similar changes of variables and using Descartes' rule of signs, one easily studies the existence of real roots of $\theta_1(\delta)$ in the ranges such $]1/n,\, 1[$ and $]1,\, n-2[$. The respective changes of variables are:

$$\delta = \frac{n+\sigma}{n\,(\sigma+1)} \quad \text{and} \quad \delta = 1 + \frac{n-3}{\sigma+1}\cdot$$

Applying Descartes' rule of signs over the resulting expressions, one concludes that $\theta_1(\delta)$ has no root located in the range defined by the two real roots of $\theta_0(\delta)$ for $n < 8$ and always two roots located in that range for $n \geq 9$. This is sufficient to prove that no Hopf bifurcation occurs for $n < 8$.

To prove that Hopf bifurcations occur for $n \geq 9$ by using proposition 2, one still needs to prove that $c_{1,1} = -a_3$ (below) may be negative for all these values. The variables μ and G may be assigned arbitrary small positive values. Therefore $c_{1,1}$ may be negative for each $n \geq 9$. This concludes the (sketched) proof that Hopf bifurcation arise for any $n \geq 9$.

$$\theta\,\delta\,(\mu\,G\,(n\,P^{n-1} + n\,P^{2\,n-1}) - (n+1)\,P^n - 1)$$

The γ_0 parameter. It is tempting to avoid the γ_0 parameter in the model and use the value 1 instead. However, over some of the models we tried, the shape of the Gröbner basis was nicer, naming this 1 and eliminating it.

The Gröbner basis. The choice of the ordering is important. It is at least necessary to eliminate the problematic λ parameter and, more generally, every variable or parameter which is allowed to be negative. Other Gröbner bases can be used to prove that $c_{2,0}$ is positive over the model steady points (at least for $n \leq 2$). A such example is obtained by replacing γ_0 by G in the ordering.

The *negterms* function. The MAPLE code of the *negterms* function is provided here. It gathers as input a rational fraction *expr*. It returns a pair of lists L_1 and L_2. The list L_1 (resp. L_2) is the list of the monomials of the numerator (resp. denominator) of *expr* which have negative coefficients.

```
negterms := proc (expr)
    local f, p, koeffs, terms, result;
    f := proc (x,y) if x < 0 then x*y else NULL fi end:
    result := NULL;
    for p in [expand (numer (expr)), expand (denom (expr))] do
        if indets (p) <> {} then
            koeffs := coeffs (p, indets (p), 'terms');
            result := result, zip (f, [koeffs], [terms])
        else
            result := result, []
        fi;
    od;
    [result]
end:
```

5 Conclusion

We have studied a simple system depending on an integer n, which describes the regulation of a gene by a polymer of order n of its protein. We have shown that no Hopf bifurcation may occur for $n \leq 8$ and that Hopf bifurcations arise for $n \geq 9$, taking into account that biologically relevant values of most of the model variables and parameters must be positive. Strictly speaking, this is not sufficient to prove the absence of limit cycles for $n \leq 8$. However, our analysis is confirmed by extensive numerical simulations.

Our study led us to study the positivity of complicated rational fractions modulo the ideal I generated by the steady points equations. This problem is in general a difficult problem in computer algebra though it is theoretically solved [28]. Our study was however much simplified by the fact that we could compute a Gröbner basis of the ideal I having a very nice shape and by the fact that most of model variables and parameters are positive.

We believe that these simplifying properties occur more often that expected and that they imply that, at least in the domain of biological modeling, computer algebra methods are not necessarily restricted to academic problems.

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References

1. Morant, P.E., Vandermoere, C., Parent, B., Lemaire, F., Corellou, F., Schwartz, C., Bouget, F.Y., Lefranc, M.: Oscillateurs génétiques simples. Applications à l'horloge circadienne d'une algue unicellulaire. In: proceedings of the *Rencontre du non linéaire*, Paris (2007) `http://nonlineaire.univ-lille1.fr`.
2. McClung, C.R.: Plant Circadian Rhythms. The Plant Cell **18** (April 2006) 792–803
3. Fall, C.P., Marland, E.S., Wagner, J.M., Tyson, J.J.: Computational Cell Biology. Volume 20 of Interdisciplinary Applied Mathematics. Springer Verlag (2002)
4. Goldbeter, A.: Biochemical Oscillations and Cellular Rhythms: The Molecular Bases of Periodic and Chaotic Behaviour. Cambridge University Press (2004)
5. Françoise, J.P.: Oscillations en biologie. Volume 46 of Mathématiques et Applications. Springer Verlag (2005)
6. Hale, J.K., Koçak, H.: Dynamics and Bifurcations. Volume 3 of Texts in Applied Mathematics. Springer–Verlag, New York (1991)
7. Hairer, E., Norsett, S.P., Wanner, G.: Solving ordinary differential equations I. Nonstiff problems. 2 edn. Volume 8 of Springer Series in Computational Mathematics. Springer–Verlag, New York (1993)
8. Doedel, E.: AUTO software for continuation and bifurcation problems in ODEs. `http://indy.cs.concordia.ca/auto` (1996)
9. Ermentrout, B.: Simulating, Analyzing, and Animating Dynamical Systems: A Guide to XPPAUT for Researchers and Students. Volume 14 of Software, Environments, and Tools. SIAM (2002)

10. El Kahoui, M., Weber, A.: Deciding Hopf bifurcations by quantifier elimination in a software–component architecture. Journal of Symbolic Computation **30**(2) (2000) 161–179
11. Wang, D., Xia, B.: Stability Analysis of Biological Systems with Real Solution Classification. In: proceedings of ISSAC 2005, Beijing, China (2005) 354–361
12. Gatermann, K., Hosten, S.: Computational algebra for bifurcation theory. Journal of Symbolic Computation **40** (2005) 1180–1207
13. Gatermann, K., Eiswirth, M., Sensse, A.: Toric Ideals and graph theory to analyze Hopf bifurcations in mass action systems. Journal of Symbolic Computation **40** (2005) 1361–1382
14. Brown, C.W.: QEPCAD B: a program for computing with semi-algebraic sets using CADs. SIGSAM Bulletin **37**(4) (2003) 97–108
15. Dolzmann, A., Sturm, T.: Redlog: computer algebra meets computer logic. SIGSAM Bulletin **31**(2) (1997) 2–9
16. El Din, M.S.: RAGLib (Real Algebraic Library Maple package). `http://www-calfor.lip6.fr/~safey/RAGLib` (2003)
17. Goodwin, B.C.: Temporal Organization in Cells. Academic Press, London (1963)
18. Goodwin, B.C. In: Advances in Enzyme Regulation. Volume 3., Oxford, Pergamon Press (1965) 425
19. Griffith, J.S.: Mathematics of Cellular Control Processes. I. Negative Feedback to One Gene. Journal of Theoretical Biology **20** (1968) 202–208
20. Selleck, W., Howley, R., Fang, Q., Podolny, V., Fried, M.G., Buratowski, S., Tan, S.: A histone fold TAF octamer within the yeast TFIID transcriptional coactivator. Nature Structural Biology **8**(8) (2001) 695–700
21. Ruoff, P., Rensing, L.: The Temperature–Compensated Goodwin Model Simulates Many Circadian Clock Properties. Journal of Theoretical Biology **179** (1996) 275–285
22. Ruoff, P., Vinsjevik, M., Mohsenzadeh, S., Rensing, L.: The Goodwin Model: Simulating the Effet of Cycloheximide and Heat Shock on the Sporulation Rhythm of *Neurospora crassa*. Journal of Theoretical Biology **196** (1999) 483–494
23. Kurosawa, G., Mochizuki, A., Iwasa, Y.: Comparative Study of Circadian Clock Models, in Search of Processes Promoting Oscillation. Journal of Theoretical Biology **216** (2002) 193–208
24. Cox, D., Little, J., O'Shea, D.: Ideals, Varieties and Algorithms. An introduction to computational algebraic geometry and commutative algebra. Undergraduate Texts in Mathematics. Springer Verlag, New York (1992)
25. Becker, T., Weispfenning, V.: Gröbner Bases: a computational approach to commutative algebra. Volume 141 of Graduate Texts in Mathematics. Springer Verlag (1991)
26. Basu, S., Pollack, R., Roy, M.F.: Algorithms in Real Algebraic Geometry. Volume 10 of Algorithms and Computation in Mathematics. Springer Verlag (2003)
27. Collins, G.E., Akritas, A.G.: Polynomial real root isolation using Descartes'rule of signs. In: proceedings of ISSAC 1976, Yorktown Heights NY (1976) 272–275
28. Collins, G.E.: Quantifier Elimination for the Elementary Theory of Real Closed Fields by Cylindrical Algebraic Decomposition. Lecture Notes in Computer Science **33** (1975) 134–183