Stochastic simulations
Application to circadian clocks

Didier Gonze
Circadian rhythms allow living organisms to live in phase with the alternance of day and night...
Circadian rhythms in *Drosophila*

### Locomotor activity

A. normal

B. arrhythmic mutant

C. short-period mutant

D. long-period mutant

Expression of *per* gene

Molecular mechanism of circadian clocks

Core mechanism: negative feedback loop

<table>
<thead>
<tr>
<th>Organism</th>
<th>Clock Gene(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drosophila</td>
<td>per (period), tim (timeless)</td>
</tr>
<tr>
<td>Mammals</td>
<td>mper1-3 (period homologs)</td>
</tr>
<tr>
<td>Neurospora</td>
<td>frq (frequency)</td>
</tr>
</tbody>
</table>
Deterministic models for circadian rhythms
Goldbeter's 5-variable model

Goldbeter's 5-variable model

\[
\frac{dM_P}{dt} = v_s \frac{K_l^n}{K_l^n + P_N^n} - v_m \frac{M_P}{K_m + M_P}
\]

\[
\frac{dP_0}{dt} = k_s M_P - v_1 \frac{P_0}{K_1 + P_0} + v_2 \frac{P_1}{K_2 + P_1}
\]

\[
\frac{dP_1}{dt} = v_1 \frac{P_0}{K_1 + P_0} - v_2 \frac{P_1}{K_2 + P_1} - v_3 \frac{P_1}{K_3 + P_1} + v_4 \frac{P_2}{K_4 + P_2}
\]

\[
\frac{dP_2}{dt} = v_3 \frac{P_1}{K_3 + P_1} - v_4 \frac{P_2}{K_4 + P_2} - v_d \frac{P_2}{K_d + P_2} - k_1 P_2 + k_2 P_N
\]

\[
\frac{dP_N}{dt} = k_1 P_2 - k_2 P_N
\]

Goldbeter's 5-variable model

Dynamics of \( \text{per mRNA} (M_P) \): synthesis

\[
\frac{dM_P}{dt} = v_s \frac{K_l^n}{K_l^n + P_N^n} - v_m \frac{M_P}{K_m + M_P}
\]

Inhibition: Hill function

\[
\text{Inhibitor, } P_n
\]

Cooperativity
Goldbeter's 5-variable model

Dynamics of per mRNA ($M_P$): degradation

$$\frac{d M_P}{dt} = v_s \frac{K_I^n}{K_I^n + P_N^n} - v_m \frac{M_P}{K_m + M_P}$$

Degradation: Michaelis-Menten

Degradation rate vs. substrate, $M_P$

$E << M$

$k_1, k_{-1} \gg k_2$

$E_{tot} = E + ME$

$K_M = \frac{k_{-1} + k_2}{k_1}$

$v_m = k_2 E_{tot}$
Goldbeter's 5-variable model

Dynamics of PER protein \((P_0, P_1, P_2, P_N)\)

\[
\frac{dP_0}{dt} = k_s M_P - v_1 \frac{P_0}{K_1 + P_0} + v_2 \frac{P_1}{K_2 + P_1}
\]

PER synthesis: proportional to mRNA

\[
\frac{dP_1}{dt} = v_1 \frac{P_0}{K_1 + P_0} - v_2 \frac{P_1}{K_2 + P_1} - v_3 \frac{P_1}{K_3 + P_1} + v_4 \frac{P_2}{K_4 + P_2}
\]

\[
\frac{dP_2}{dt} = v_3 \frac{P_1}{K_3 + P_1} - v_4 \frac{P_2}{K_4 + P_2} - v_d \frac{P_2}{K_d + P_2} - k_1 P_2 + k_2 P_N
\]

\[
\frac{dP_N}{dt} = k_1 P_2 - k_2 P_N
\]
Goldbeter's 5-variable model

Dynamics of PER protein \((P_0, P_1, P_2, P_N)\)

\[
\frac{dP_0}{dt} = k_s M_P - v_1 \frac{P_0}{K_1 + P_0} + v_2 \frac{P_1}{K_2 + P_1}
\]

PER phosphorylation/dephosphorylation: Michaelis-Menten

\[
\frac{dP_1}{dt} = v_1 \frac{P_0}{K_1 + P_0} - v_2 \frac{P_1}{K_2 + P_1} - v_3 \frac{P_1}{K_3 + P_1} + v_4 \frac{P_2}{K_4 + P_2}
\]

\[
\frac{dP_2}{dt} = v_3 \frac{P_1}{K_3 + P_1} - v_4 \frac{P_2}{K_4 + P_2} - v_d \frac{P_2}{K_d + P_2} - k_1 P_2 + k_2 P_N
\]

\[
\frac{dP_N}{dt} = k_1 P_2 - k_2 P_N
\]
Goldbeter's 5-variable model

**Dynamics of PER protein \((P_0, P_1, P_2, P_N)\)**

\[
\frac{dP_0}{dt} = k_s M_P - v_1 \frac{P_0}{K_1 + P_0} + v_2 \frac{P_1}{K_2 + P_1}
\]

\[
\frac{dP_1}{dt} = v_1 \frac{P_0}{K_1 + P_0} - v_2 \frac{P_1}{K_2 + P_1} - v_3 \frac{P_1}{K_3 + P_1} + v_4 \frac{P_2}{K_4 + P_2}
\]

\[
\frac{dP_2}{dt} = v_3 \frac{P_1}{K_3 + P_1} - v_4 \frac{P_2}{K_4 + P_2} - \frac{P_2}{K_d + P_2} - k_1 P_2 + k_2 P_N
\]

\[
\frac{dP_N}{dt} = k_1 P_2 - k_2 P_N
\]

**PER degradation:**
Michaelis-Menten
Goldbeter's 5-variable model

Dynamics of PER protein \((P_0, P_1, P_2, P_N)\)

\[
\frac{dP_0}{dt} = k_s M_P - v_1 \frac{P_0}{K_1 + P_0} + v_2 \frac{P_1}{K_2 + P_1}
\]

\[
\frac{dP_1}{dt} = v_1 \frac{P_0}{K_1 + P_0} - v_2 \frac{P_1}{K_2 + P_1} - v_3 \frac{P_1}{K_3 + P_1} + v_4 \frac{P_2}{K_4 + P_2}
\]

\[
\frac{dP_2}{dt} = v_3 \frac{P_1}{K_3 + P_1} - v_4 \frac{P_2}{K_4 + P_2} - v_d \frac{P_2}{K_d + P_2} - k_1 P_2 - k_2 P_N
\]

PER nuclear transport: linear

\[
\frac{dP_N}{dt} = k_1 P_2 - k_2 P_N
\]
Goldbeter's 5-variable model

Limit-cycle oscillations

- Mutants (long-period, short-period, arrhythmic)
- Entrainment by light-dark cycles
- Phase shift induced by light pulses
- Suppression of oscillations by a light pulse
- Temperature compensation
- ...

![Graph showing limit-cycle oscillations](image-url)

*Graph showing the relationship between per mRNA, M (nM) and cytosolic PER protein, P_d (nM)*
Molecular mechanism of circadian clocks

Interlocked positive and negative feedback loops

<table>
<thead>
<tr>
<th></th>
<th>Clock gene</th>
<th>Activator</th>
<th>Effect of light</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drosophila</td>
<td>per, tim</td>
<td>clk, cyc</td>
<td>TIM degradation</td>
</tr>
<tr>
<td>Mammals</td>
<td>mper1-3, cry1,2</td>
<td>clock, bmal1</td>
<td>per transcription</td>
</tr>
<tr>
<td>Neurospora</td>
<td>frq</td>
<td>wc-1, wc-2</td>
<td>frq transcription</td>
</tr>
</tbody>
</table>

Molecular mechanism of circadian clocks

Example: circadian clock in mammals

Model for the mammalian circadian clock

16-variable model including *per, cry, bmal1, rev-erbα*

Stochastic models for circadian rhythms
Circadian clocks limited by noise?

Circadian clocks limited by noise
Goldbeter's 5-variable model

\[
\frac{dM_P}{dt} = v_s \frac{K_P^m}{K_P^n + P_N^n} - v_m \frac{M_P}{K_m + M_P}
\]

\[
\frac{dP_0}{dt} = k_s M_P - v_1 \frac{P_0}{K_1 + P_0} + v_2 \frac{P_1}{K_2 + P_1}
\]

\[
\frac{dP_1}{dt} = \frac{v_1 P_0}{K_1 + P_0} - v_2 \frac{P_1}{K_2 + P_1} - \frac{v_3 P_1}{K_3 + P_1} + \frac{v_4 P_2}{K_4 + P_2}
\]

\[
\frac{dP_2}{dt} = \frac{v_3 P_1}{K_3 + P_1} - v_4 \frac{P_2}{K_4 + P_2} - v_d \frac{P_2}{K_d + P_2} - k_1 P_2 + k_2 P_N
\]

\[
\frac{dP_N}{dt} = k_1 P_2 - k_2 P_N
\]

Fluctuations are due to the limited number of molecules (molecular noise). They can be assessed thanks to stochastic simulations.

Such an approach requires a description in terms of the number of molecules (instead of concentrations).

Here, we will focus on several robustness factors:

- Number of molecules
- Degree of cooperativity
- Periodic forcing (LD cycle)
- Proximity of a bifurcation point
- Coupling between cells
Successive binding of 4 $P_N$ molecules to the gene G

\[ G + P_N \rightleftharpoons GP_N \]
\[ GP_N + P_N \rightleftharpoons GP_{N2} \]
\[ GP_{N2} + P_N \rightleftharpoons GP_{N3} \]
\[ GP_{N3} + P_N \rightleftharpoons GP_{N4} \]

\[ [G, GP_{N1}, GP_{N2}, GP_{N3}] \rightarrow M + [G, GP_{N1}, GP_{N2}, GP_{N3}] \]

\[ M + E_m \rightleftharpoons C_m \rightarrow E_m \]

\[ M \rightarrow M + P_0 \]

\[ P_0 + E_1 \rightleftharpoons C_1 \rightarrow P_1 + E_1 \]
\[ P_1 + E_2 \rightleftharpoons C_2 \rightarrow P_0 + E_2 \]
\[ P_1 + E_3 \rightleftharpoons C_3 \rightarrow P_2 + E_3 \]
\[ P_2 + E_4 \rightleftharpoons C_4 \rightarrow P_1 + E_4 \]

\[ P_2 + E_d \rightleftharpoons C_d \rightarrow E_d \]

\[ P_2 \rightleftharpoons P_n \]
A reaction rate $w_i$ is associated to each reaction step. These probabilities are related to the kinetics constants.

Initial number of molecules of each species are specified.

The time interval is computed stochastically according the reaction rates.

At each time interval, the reaction that occurs is chosen randomly according to the probabilities $w_i$ and both the number of molecules and the reaction rates are updated.

---


### Stochastic description of the model

<table>
<thead>
<tr>
<th>Reaction number</th>
<th>Reaction step</th>
<th>Probability of reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>( G + P_3 \xrightarrow{k_1} GP_3 )</td>
<td>( w_1 = a_1 \times G \times P_3 / \Omega )</td>
</tr>
<tr>
<td>2</td>
<td>( GP_3 \xrightarrow{k_2} G + P_3 )</td>
<td>( w_2 = d_1 \times GP_3 )</td>
</tr>
<tr>
<td>3</td>
<td>( GP_N + P_2 \xrightarrow{k_3} GP_{N2} )</td>
<td>( w_3 = a_1 \times GP_2 \times P_2 / \Omega )</td>
</tr>
<tr>
<td>4</td>
<td>( GP_{N2} \xrightarrow{k_4} GP_N + P_3 )</td>
<td>( w_4 = d_1 \times GP_{N2} )</td>
</tr>
<tr>
<td>5</td>
<td>( GP_{N2} + P_3 \xrightarrow{k_5} GP_{N3} )</td>
<td>( w_5 = a_1 \times GP_{N2} \times P_3 / \Omega )</td>
</tr>
<tr>
<td>6</td>
<td>( GP_{N3} \xrightarrow{k_6} GP_{N2} + P_3 )</td>
<td>( w_6 = d_1 \times GP_{N3} )</td>
</tr>
<tr>
<td>7</td>
<td>( GP_{N3} + P_3 \xrightarrow{k_7} GP_{N4} )</td>
<td>( w_7 = a_1 \times GP_{N3} \times P_3 / \Omega )</td>
</tr>
<tr>
<td>8</td>
<td>( GP_{N4} \xrightarrow{k_8} GP_{N3} + P_3 )</td>
<td>( w_8 = d_1 \times GP_{N4} )</td>
</tr>
<tr>
<td>9</td>
<td>( {G, GP_N, GP_{N2}, GP_{N3}} \xrightarrow{v_4} M_P )</td>
<td>( w_9 = v_4 \times {G + GP_N + GP_{N2} + GP_{N3}} )</td>
</tr>
<tr>
<td>10</td>
<td>( M_P + E_m \xrightarrow{k_{10}} C_m )</td>
<td>( w_{10} = k_{10} \times M_P \times E_m / \Omega )</td>
</tr>
<tr>
<td>11</td>
<td>( C_m \xrightarrow{k_{11}} M_P + E_m )</td>
<td>( w_{11} = k_{11} \times C_m )</td>
</tr>
<tr>
<td>12</td>
<td>( C_m \xrightarrow{k_{12}} E_m )</td>
<td>( w_{12} = k_{12} \times C_m )</td>
</tr>
<tr>
<td>13</td>
<td>( M_P \xrightarrow{k_{13}} M_P + p_0 )</td>
<td>( w_{13} = k_{13} \times M_P )</td>
</tr>
<tr>
<td>14</td>
<td>( p_0 + E_3 \xrightarrow{k_{14}} C_1 )</td>
<td>( w_{14} = k_{14} \times P_3 \times E_3 / \Omega )</td>
</tr>
<tr>
<td>15</td>
<td>( C_1 \xrightarrow{k_{15}} P_3 + E_1 )</td>
<td>( w_{15} = k_{15} \times C_1 )</td>
</tr>
<tr>
<td>16</td>
<td>( C_1 \xrightarrow{k_{16}} P_0 + E_1 )</td>
<td>( w_{16} = k_{16} \times C_1 )</td>
</tr>
<tr>
<td>17</td>
<td>( P_1 + E_2 \xrightarrow{k_{17}} C_2 )</td>
<td>( w_{17} = k_{17} \times P_1 \times E_2 / \Omega )</td>
</tr>
<tr>
<td>18</td>
<td>( C_2 \xrightarrow{k_{18}} P_1 + E_2 )</td>
<td>( w_{18} = k_{18} \times C_2 )</td>
</tr>
<tr>
<td>19</td>
<td>( C_2 \xrightarrow{k_{19}} P_0 + E_2 )</td>
<td>( w_{19} = k_{19} \times C_2 )</td>
</tr>
<tr>
<td>20</td>
<td>( P_1 + E_3 \xrightarrow{k_{20}} C_3 )</td>
<td>( w_{20} = k_{20} \times P_1 \times E_3 / \Omega )</td>
</tr>
<tr>
<td>21</td>
<td>( C_3 \xrightarrow{k_{21}} P_3 + E_3 )</td>
<td>( w_{21} = k_{21} \times C_3 )</td>
</tr>
<tr>
<td>22</td>
<td>( C_3 \xrightarrow{k_{22}} P_0 + E_3 )</td>
<td>( w_{22} = k_{22} \times C_3 )</td>
</tr>
<tr>
<td>23</td>
<td>( P_3 + E_4 \xrightarrow{k_{23}} C_4 )</td>
<td>( w_{23} = k_{23} \times P_3 \times E_4 / \Omega )</td>
</tr>
<tr>
<td>24</td>
<td>( C_4 \xrightarrow{k_{24}} P_3 + E_4 )</td>
<td>( w_{24} = k_{24} \times C_4 )</td>
</tr>
<tr>
<td>25</td>
<td>( C_4 \xrightarrow{k_{25}} P_0 + E_4 )</td>
<td>( w_{25} = k_{25} \times C_4 )</td>
</tr>
<tr>
<td>26</td>
<td>( P_3 + E_4 \xrightarrow{k_{26}} C_4 )</td>
<td>( w_{26} = k_{26} \times P_3 \times E_4 / \Omega )</td>
</tr>
<tr>
<td>27</td>
<td>( C_4 \xrightarrow{k_{27}} P_0 + E_4 )</td>
<td>( w_{27} = k_{27} \times C_4 )</td>
</tr>
<tr>
<td>28</td>
<td>( C_4 \xrightarrow{k_{28}} E_4 )</td>
<td>( w_{28} = k_{28} \times C_4 )</td>
</tr>
<tr>
<td>29</td>
<td>( P_3 \xrightarrow{k_{29}} P_N )</td>
<td>( w_{29} = k_{29} \times P_3 )</td>
</tr>
<tr>
<td>30</td>
<td>( P_N \xrightarrow{k_{30}} P_3 )</td>
<td>( w_{30} = k_{30} \times P_N )</td>
</tr>
</tbody>
</table>
Stochastic oscillations and limit cycle

**Deterministic**

**Stochastic**

Effect of the number of molecules, $\Omega$  

$\Omega=1000$  

$\Omega=100$  

$\Omega=10$
Effect of the degree of cooperativity, $n$

$n = 4$

$n = 1$

Quantification of the effect of noise

Auto-correlation function

Effect of the number of molecules, $\Omega$

Effect of the degree of cooperativity, $n$
Effect of a periodic forcing (LD cycle)

Light-dark cycle
LD 12:12

light induces
PER protein
degradation, $v_d$
Cooperative protein-DNA binding

We define $\gamma$:

$$a_i \rightarrow a_i / \gamma \quad (i = 1, \ldots, 4)$$

$$d_i \rightarrow d_i / \gamma \quad (i = 1, \ldots, 4)$$
Influence of the protein-DNA binding rate

\( \gamma = 100 \)

\( \gamma = 1000 \)

Developed deterministic model

\[ \frac{dG}{dt} = -a_1GP + d_1[GP] , \]

\[ \frac{d[GP]}{dt} = a_1GP - d_1[GP] - a_2[GP]P_N + d_2[GP_N] , \]

\[ \frac{d[GP_N]}{dt} = a_2[GP_N]P_N - d_2[GP_N] - a_3[GP_N]P_N + d_3[GP_N] , \]

\[ \frac{d[GP_{N1}]}{dt} = a_3[GP_{N1}]P_N - d_3[GP_{N1}] - a_4[GP_{N1}]P_N + d_4[GP_{N1}] , \]

\[ \frac{d[GP_{N2}]}{dt} = a_4[GP_{N2}]P_N - d_4[GP_{N2}] - d_3[GP_{N2}] , \]

\[ \frac{dM}{dt} = \nu_1(G + [GP_N] + [GP_{N2}] + [GP_{N3}]) - k_{11}ME_m + k_{12}C_m , \]

\[ \frac{dE_m}{dt} = -k_{11}ME_m + k_{m2}C_m + k_{m3}C_m , \]

\[ \frac{dC_m}{dt} = k_{m1}ME_m - k_{m2}C_m - k_{m3}C_m , \]

\[ \frac{dP_0}{dt} = k_1M - k_{11}P_0E_1 + k_{12}C_1 + k_{32}C_2 , \]

\[ \frac{dE_1}{dt} = -k_{11}P_0E_1 + k_{12}C_1 + k_{13}C_1 , \]

\[ \frac{dC_1}{dt} = k_{11}P_0E_1 - k_{12}C_1 + k_{13}C_1 , \]

\[ \frac{dP_1}{dt} = -k_{22}P_1E_2 + k_{22}C_2 + k_{32}C_1 - k_{31}P_1E_3 + k_{32}C_3 + k_{43}C_4 , \]

\[ \frac{dE_2}{dt} = -k_{21}P_1E_2 + k_{22}C_2 + k_{33}C_2 , \]

\[ \frac{dC_2}{dt} = k_{21}P_1E_2 - k_{22}C_2 - k_{33}C_2 , \]

\[ \frac{dP_2}{dt} = k_{33}C_3 - k_{31}P_2E_4 + k_{32}C_4 - k_{41}P_2E_4 + k_{42}C_4 - k_{11}P_2 + k_{33}P_N , \]

\[ \frac{dE_3}{dt} = -k_{31}P_1E_3 + k_{32}C_3 + k_{33}C_3 , \]

\[ \frac{dC_3}{dt} = k_{31}P_1E_3 - k_{32}C_3 - k_{33}C_3 , \]

\[ \frac{dE_4}{dt} = -k_{31}P_2E_4 + k_{32}C_4 + k_{33}C_4 , \]

\[ \frac{dC_4}{dt} = k_{31}P_2E_4 - k_{32}C_4 - k_{33}C_4 , \]

\[ \frac{dC_4}{dt} = k_{31}P_2E_4 + k_{32}C_4 + k_{33}C_4 , \]

\[ \frac{dC_4}{dt} = k_{31}P_2E_4 - k_{32}C_4 - k_{33}C_4 , \]

\[ \frac{dP_N}{dt} = -a_1GP_N + d_1[GP_N] - a_2[GP_N]P_N + d_2[GP_N]P_N + d_3[GP_N]P_N + a_4[GP_N]P_N + d_4[GP_N] + k_{11}P_2 - k_{33}P_N \]

with \( G_{net} = G + GP_N + GP_{N2} + GP_{N3} + GP_{N4} = 1. \)
Deterministic model: bifurcation diagram

- $M_P^{\text{max}}$
- $M_P^{\text{SS}(u)}$
- $M_P^{\text{SS}(s)}$
- $M_P^{\text{min}}$

Parameters:
- Fast binding rate
- Slow binding rate
Developed deterministic model: excitability

\[ \gamma = 1000 \]

\[ \gamma = 100 \]

\[ \gamma = 1 \]
Mechanisms of noise-resistance in genetic oscillators


\[
\frac{dR}{dt} = \frac{\beta_R}{\delta_{MR}} \frac{\alpha_R \theta_R + \alpha'_R \gamma_R \bar{A}(R)}{\theta_R + \gamma_R \bar{A}(R)} - \gamma_C \bar{A}(R)R + \delta_A C - \delta_R R
\]

\[
\frac{dC}{dt} = \gamma_C \bar{A}(R)R - \delta_A C
\]
Stochastic resonance in circadian clock?

Internal noise stochastic resonance in a circadian clock system

Light-noise induced supra-threshold circadian oscillations and coherent resonance in *Drosophila*
Robust circadian oscillations are observed for a limited number of molecules, i.e. some tens mRNA molecules and hundreds proteins molecules.

Cooperativity increases the robustness of the oscillations.

The periodic forcing of the oscillations (LD cycle) increases the robustness by stabilizing the phase of the oscillations.

The proximity of a bifurcation point decreases the robustness of the oscillations. In particular, near an excitable steady state, highly irregular oscillations are observed.

Coupling between cells increases the robustness of the oscillations.
Acknowledgements

Albert Goldbeter
*Unité de Chronobiologie Théorique*
*Université Libre de Bruxelles, Belgium*
Jean-Christophe Leloup
José Halloy
Geneviève Dupont
Atilla Altinok
Claude Gérard

Hanspeter Herzel
*Institute for Theoretical Biology*
*Humboldt Universität zu Berlin, Germany*
Samuel Bernard
Christian Waltermann
Sabine Becker-Weimann
Florian Geier

Funding
Fonds National Belge de la Recherche Scientifique (FNRS).
Deutsche Forschungsgemeinschaft (SFB)
European Network BioSimulation.