Synchronisation of Biological Clock Signals

Capturing Coupled Repressilators from a Control Systems Perspective

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Modelling Oscillatory
Information Processing Group

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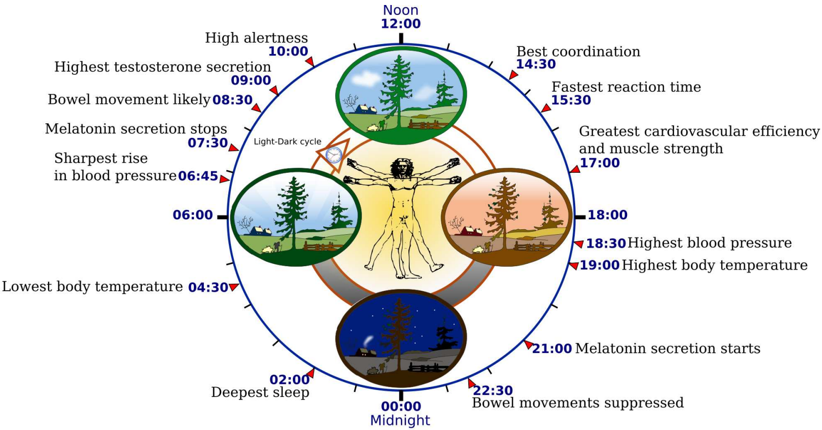
Bio-Inspired Systems and Signal
Processing (BIOSIGNALS2011)

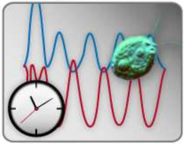
Human Daily Rhythm: Trigger and Control System



www.wikipedia.org

Biological Clocks

Significance



- Nanoscaled oscillatory reaction systems
- High precision and self-sustainability
- Robust and reliable control systems for manifold processes
- Adaptability to specific environmental conditions (e.g. cycles of light/darkness)
- Infradian (period $>$ 1 day), *circadian* ($\approx$ 1 day), and ultradian ($<$ 1 day) rhythms
- Several independent evolutionary origins
- Prototypes for fine-grained clock synchronisation
- Medicine, agriculture, bionics, material sciences, biology

$\Longrightarrow$ Keeping environmental time within living organisms

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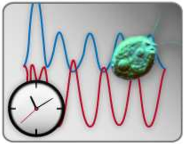

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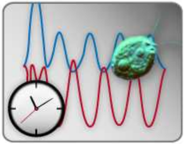

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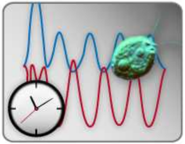

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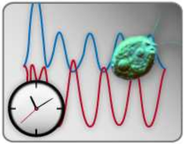

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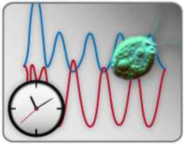

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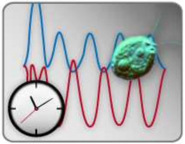

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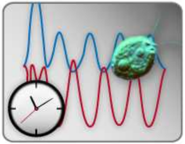

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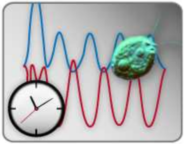

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Chronobiology

χρονοζ

time

βιοζ

life

λογοζ

science

ρυθμοζ

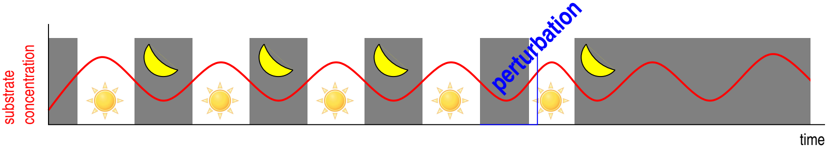
rhythm

science of biological rhythms and clock systems

FORSYS Partner Initiative

Circadian Clock

- Undamped biochemical oscillation
- Period *approx.* 24 hours persisting under constant environmental conditions
  (e.g. permanent darkness DD or permanent light LL)
- *Entrainment* – adaptation to external stimuli
  (e.g. light-dark cycles induced by sunlight)
- Temperature compensation within a physiological range
- Reaction systems with at least one feedback loop



⟹ Biological counterpart of frequency control system

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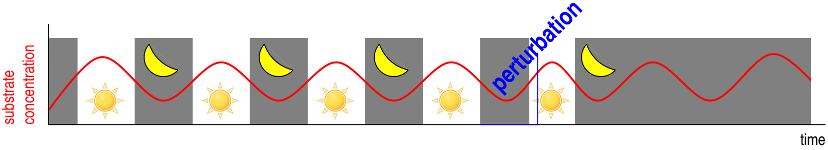
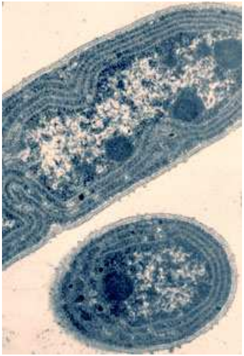


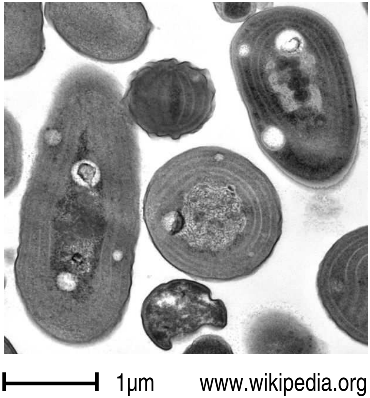
⟹ Biological counterpart of frequency control system

Cyanobacterium *Synechococcus elongatus*

"Simplest and earliest cells known to exhibit circadian phenomena"



www.genome.jgi−psf.org



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- Prokaryotic autotrophic picoplankton in tropical seas
- Assumed to be on earth for more than 3.5 billion years
- Clock: Phosphorylation cycle without gene expression

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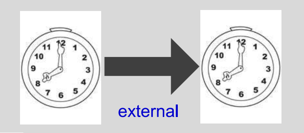
Entrainment vs. Synchronisation

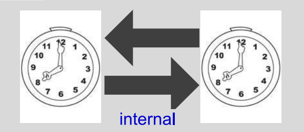
Entrainment

- Oscillating signal (frequency, phase, and amplitude) dynamically adapts to (varying) external stimulus. External stimulus itself not influenced.

Synchronisation

- *External:* Entrainment to external stimulus (e.g. light-dark cycle induced by sunlight) + adaptation to signal shape of external stimulus
- *Internal:* oscillating signals mutually adapt, converge to a common signal



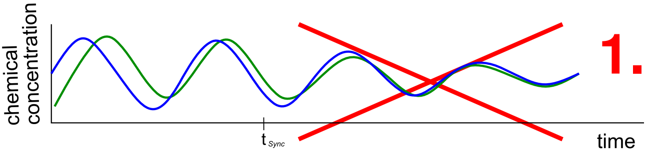


$\Longrightarrow$ Entrainment can be seen as special case of synchronisation

Properties of Synchronous Oscillations (I)

Undamped oscillations

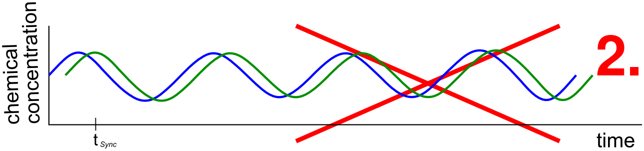
- Modelled oscillation results from solution of ordinary differential equations (ODEs) describing dynamical behaviour of the biochemical clok system
- Eigenvalues of Jacobian matrix (real parts < 0) mostly indicate undamped oscillations
- Limit cycles (represented by orbital courses) as method of choice for numerical data



Properties of Synchronous Oscillations (II)

Asymptotic or total adaption

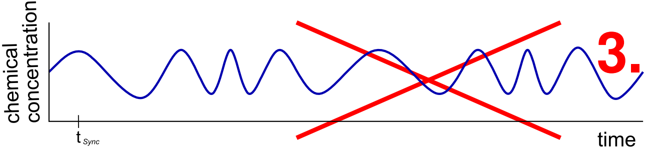
- Harmonisation of oscillating substrate concentration
    - after finite time t_{sync} within
    - arbitrarily selectable ε-neighbourhood



Properties of Synchronous Oscillations (III)

Monofrequential oscillation after t_{sync}

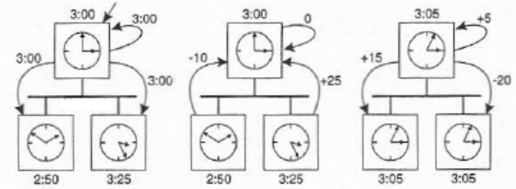
- Fast Fourier Transformation / Fourier analysis (discrete data processing and comparison of peaks)
- Laplace transform and subsequent algebraic processing (preferably for sinusoidal signals)
- Numerical exploration (e.g. sampling)



Internal Clock Synchronsiation: Technical Protocols

Each node in a bidirectionally coupled computer network

- Comprises a specific clock (potential deviations to others)
- Can communicate with all other nodes by sending/receiving local time stamps
- Requests time stamps from others (mutually exchange)
- Successively adjusts its local clock (Lamport, Christian, Berkeley algorithms)

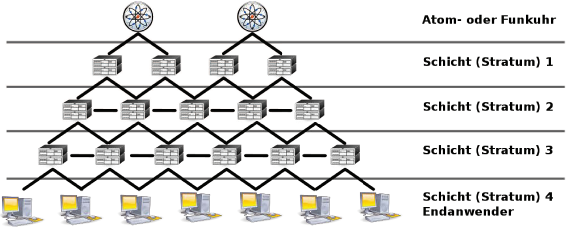


Berkeley algorithm. A. S. Tanenbaum and M. van Steen, *Distributed Systems Principles and Paradigms*, 2001

External Clock Synchronsiation: Technical Protocols

Each node in unidirectionally coupled computer network

- Comprises a specific clock (potential deviations to others)
- Localised within hierarchial network structure
- Retrieves time stamps exclusively from upper layers (unidirectional signal transduction)
- Successively adjusts its local clock by propagating time stamps from clock(s) in root position



Network Time Protocol (NTP). de.wikipedia.org/wiki/Network_Time_Protocol

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Repressilator Prototype

In-vitro Oscillating Gene Regulatory Network

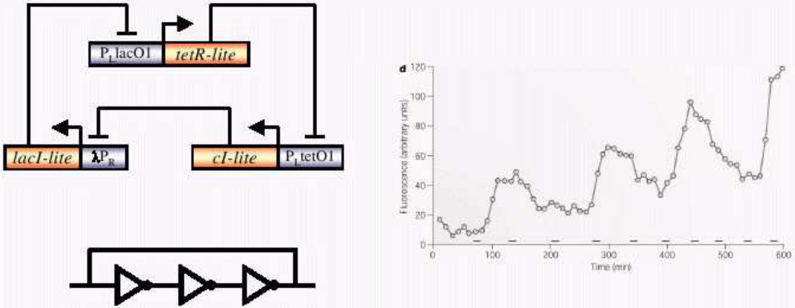
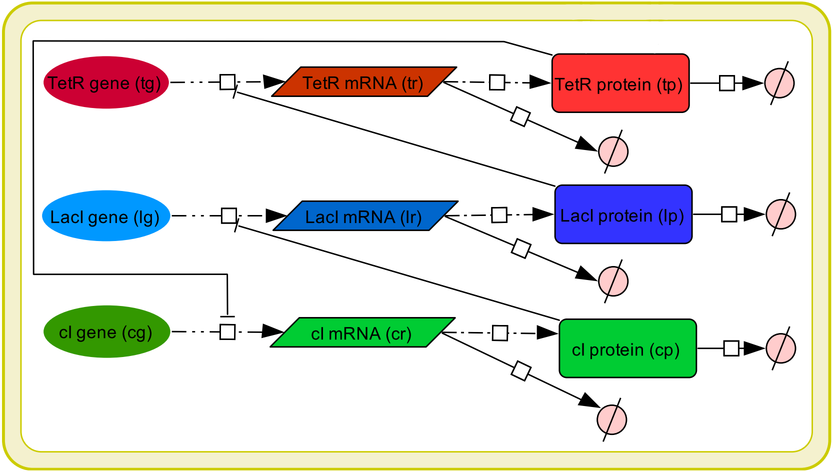


Figure 9. The repressilator circuit consists of three proteins and their three corresponding promoters, arranged such that each protein P_x represses the expression of a different protein P_y which does not repress P_x. These proteins include a synthetic tag, signified by the suffix “lite”, that targets the proteins for fast decay in the cell. The gene network configuration corresponds to a ring oscillator logic circuit.

Eulowitz et al., Nature 403:335-338, 2000

Repressilator Model: Network Topology



Based on M.B. Elowitz, S. Leibler. A synthetic oscillatory network of transcriptional regulators. Nature **403**:335-338, 2000

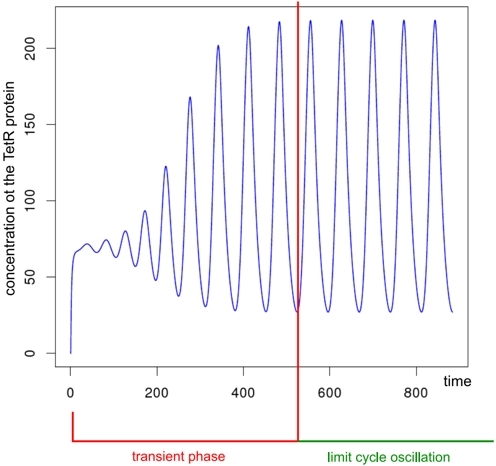
ODEs Formalising Repressilator’s Dynamic Behaviour

$$
\begin{aligned}
\frac{\mathrm{d}\ \mathit{LacI\_Protein}}{\mathrm{d}\ t} &= \mathrm{k\_tl} \cdot \mathit{LacI\_mRNA} - \mathrm{k\_p} \cdot \mathit{LacI\_Protein}\\
\frac{\mathrm{d}\ \mathit{TetR\_Protein}}{\mathrm{d}\ t} &= \mathrm{k\_tl} \cdot \mathit{TetR\_mRNA} - \mathrm{k\_p} \cdot \mathit{TetR\_Protein}\\
\frac{\mathrm{d}\ \mathit{cI\_Protein}}{\mathrm{d}\ t} &= \mathrm{k\_tl} \cdot \mathit{cI\_mRNA} - \mathrm{k\_p} \cdot \mathit{cI\_Protein}\\
\frac{\mathrm{d}\ \mathit{LacI\_mRNA}}{\mathrm{d}\ t} &= \mathrm{a0\_tr} + \frac{\mathrm{a\_tr} \cdot \mathrm{KM}^{\mathrm{n}}}{\mathrm{KM}^{\mathrm{n}} + \mathit{cI\_Protein}} - \mathrm{k\_tl} \cdot \mathit{LacI\_mRNA} - \mathrm{k\_r} \cdot \mathit{LacI\_mRNA}\\
\frac{\mathrm{d}\ \mathit{TetR\_mRNA}}{\mathrm{d}\ t} &= \mathrm{a0\_tr} + \frac{\mathrm{a\_tr} \cdot \mathrm{KM}^{\mathrm{n}}}{\mathrm{KM}^{\mathrm{n}} + \mathit{LacI\_Protein}} - \mathrm{k\_tl} \cdot \mathit{TetR\_mRNA} - \mathrm{k\_r} \cdot \mathit{TetR\_mRNA}\\
\frac{\mathrm{d}\ \mathit{cI\_mRNA}}{\mathrm{d}\ t} &= \mathrm{a0\_tr} + \frac{\mathrm{a\_tr} \cdot \mathrm{KM}^{\mathrm{n}}}{\mathrm{KM}^{\mathrm{n}} + \mathit{TetR\_Protein}} - \mathrm{k\_tl} \cdot \mathit{cI\_mRNA} - \mathrm{k\_r} \cdot \mathit{cI\_mRNA}
\end{aligned}
$$

Reaction rates and parameter setting: $\mathrm{k\_tl} = 6.93$, $\mathrm{k\_p} = 0.069$, $\mathrm{k\_r} = 0.347$, $\mathrm{a0\_tr} = 0.03$, $\mathrm{a\_tr} = 29.97$, $\mathrm{KM} = 40$, $\mathrm{n} = 3$ resulted from parameter fitting based on available experimental data (Garcia-Ojalvo et al.).

System implies sustained limit-cycle oscillations after transient phase.

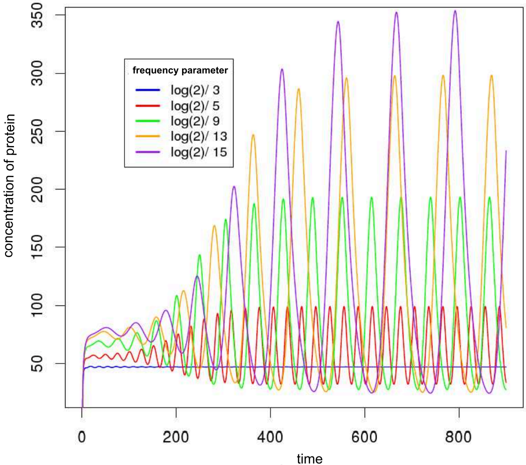
Dynamical Behaviour of the Repressilator (TetR)



Initialisation at limit cycle avoids transient phase

$\Longrightarrow$ Eliminates its influence on synchronisation time

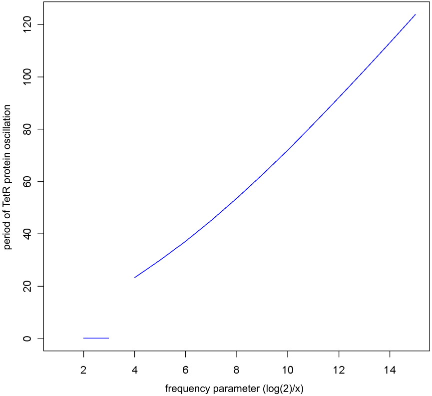
Period Control by Velocity of Protein Degradation



Variable degradation rates $\mathrm{k\_p} = \ln(2)/x$ (frequence parameter $x = 3, \ldots, 15$) of proteins sufficient for clock advance or delay.

Frequence control: prerequisite for synchronisability.

Repressilator’s Transfer Function



Correlation between velocity of protein degradation and period.

Identification of minimal period delimiting sustained oscillations.

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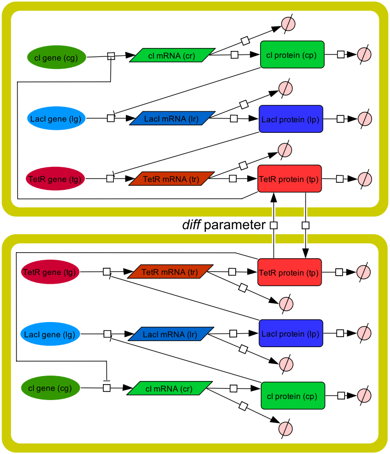
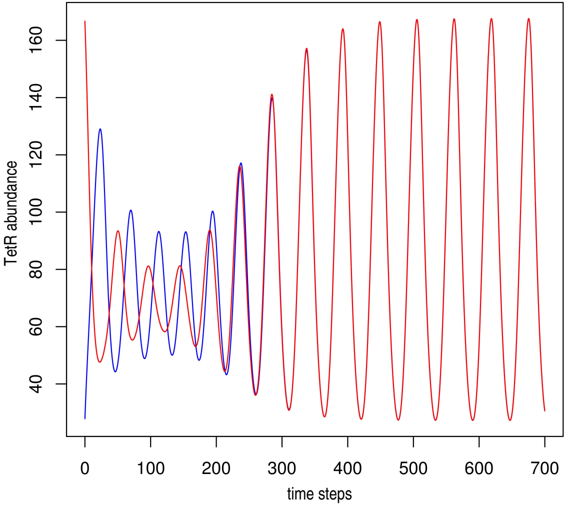
Coupled Repressilators for Internal Synchronisation



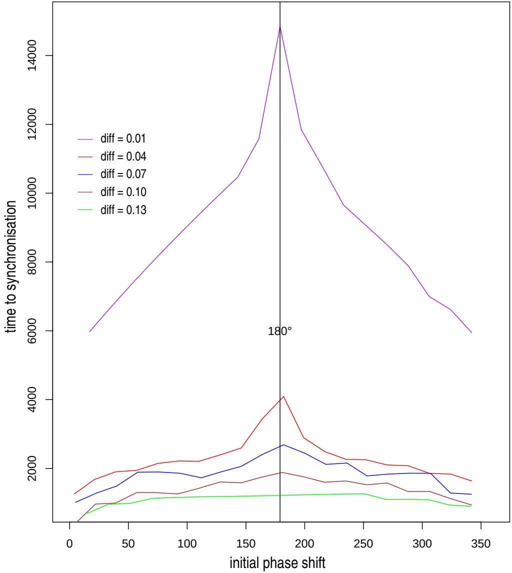
Bidirectional diffusion of TetR proteins between either repressilators enable internal synchronisation. Diffusion parameter *diff* as additional rate constant (linear kinetics)

Typical Synchronisation Run



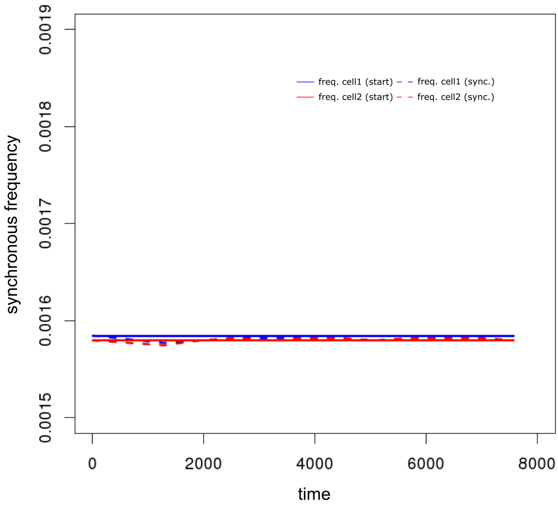
Typical synchronisation run of two TetR-coupled repressilators, coupling strength *diff* $= 0.04$, initial phase shift 182°.

Time to Synchronisation for Various Initial Phase Shifts



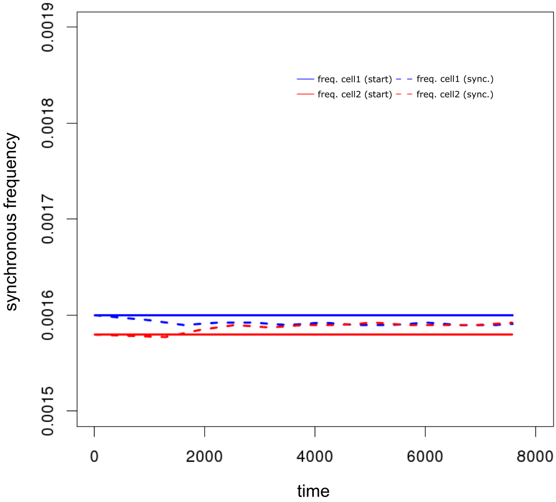
Time to synchronisation subject to various initial phase shifts. Parameter $\mathit{diff} = 0.01, \ldots, 0.13$ denotes coupling strength from weak to strong coupling. Initial antiphase rhythmicity (phase shift 180°) between both repressilators causes the highest effort to synchronise both oscillatory signals by mutual forcing.

Time to Synchronisation for Various Initial Frequencies



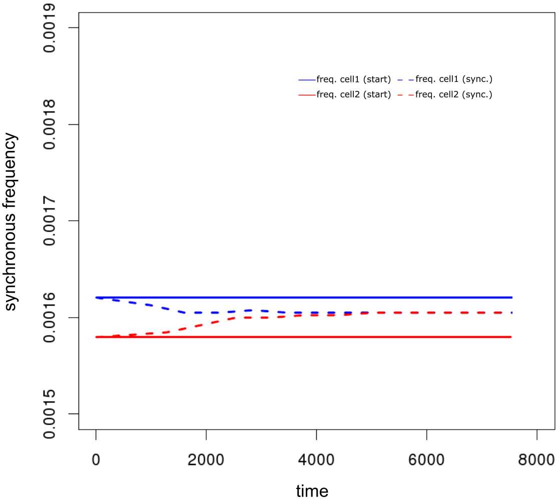
Weak diffusion, *diff* =0.01, frequency parameter *x* ratio: 9.475 / 9.5

Time to Synchronisation for Various Initial Frequencies



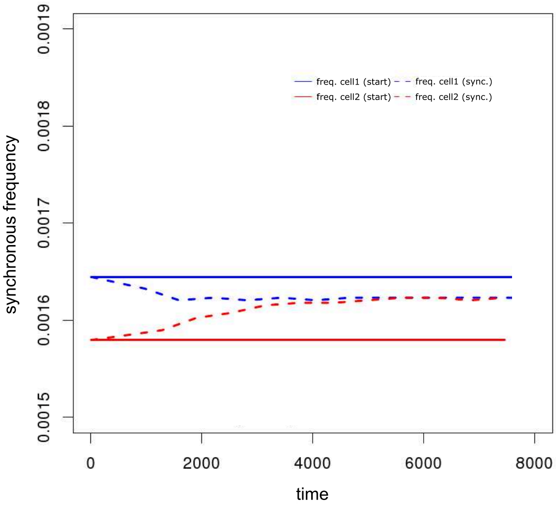
Weak diffusion, *diff* =0.01, frequency parameter x ratio: 9.4 / 9.5

Time to Synchronisation for Various Initial Frequencies



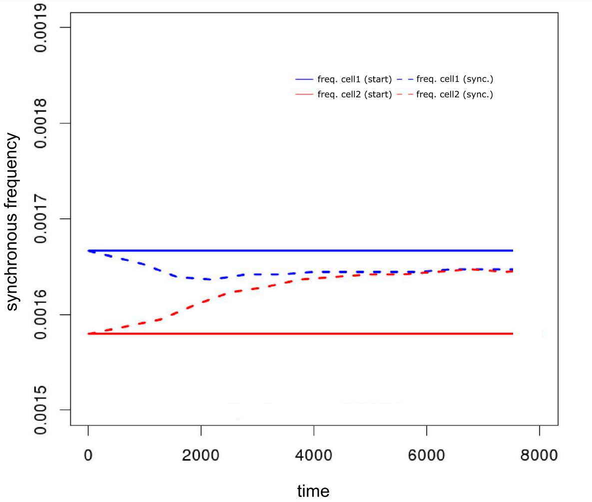
Weak diffusion, *diff* =0.01, frequency parameter *x* ratio: 9.3 / 9.5

Time to Synchronisation for Various Initial Frequencies



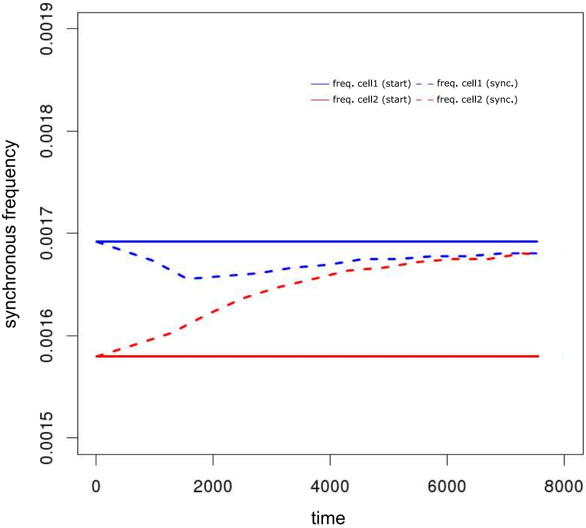
Weak diffusion, *diff* =0.01, frequency parameter *x* ratio: 9.2 / 9.5

Time to Synchronisation for Various Initial Frequencies



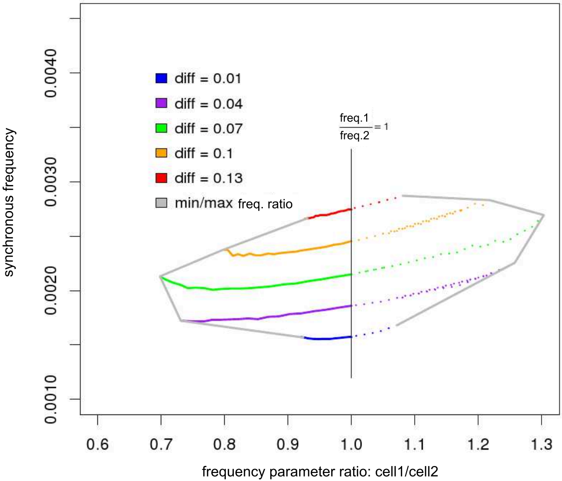
Weak diffusion, *diff* =0.01, frequency parameter *x* ratio: 9.1 / 9.5

Time to Synchronisation for Various Initial Frequencies



Weak diffusion, *diff* =0.01, frequency parameter *x* ratio: 9.0 / 9.5

Frequency Synchronisation Window



Ratios of initial frequencies subject to synchronous frequency considering variety of coupling strengths *diff* $= 0.01, \ldots, 0.13$: variant of an Arnold tongue

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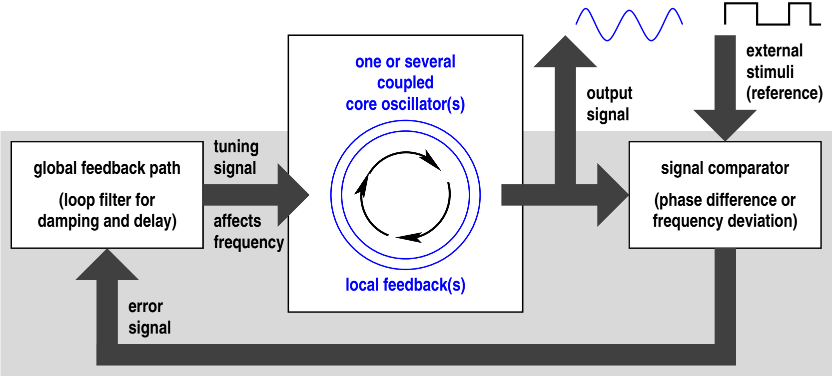
Frequency Control System with Phase-Locked Loop



one or several coupled core oscillator(s)

local feedback(s)

output signal

external stimuli (reference)

global feedback path

(loop filter for damping and delay)

tuning signal

affects frequency

signal comparator

(phase difference or frequency deviation)

error signal

Coupled repressilators as core oscillator of frequency control system able to manage external synchronisation to external stimuli (reference oscillation)

Conclusions and Take Home Message

- Repressilator as promising biochemical *in-vitro* model system to explore synchronisation of circadian oscillations
- Inherent oscillation similar but not equal to sinusoidal course (hence not “symmetric”)
- Repressilator coupling by diffusion of TetR protein enables internal synchronisation.
- Arbitrary initial phase shifts (also antiphasic behaviour) become harmonised while adaptation to different initial frequencies spans a synchronisation window.
- Coupled repressilators can be considered as part of a frequency control system based on phase-locked loop (PLL) utilising external synchronisation.

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... you for your attention. Questions?