Biochemical Frequency Control by Synchronisation of Coupled Repressilators

An *In-silico* Study of Modules for Circadian Clock Systems

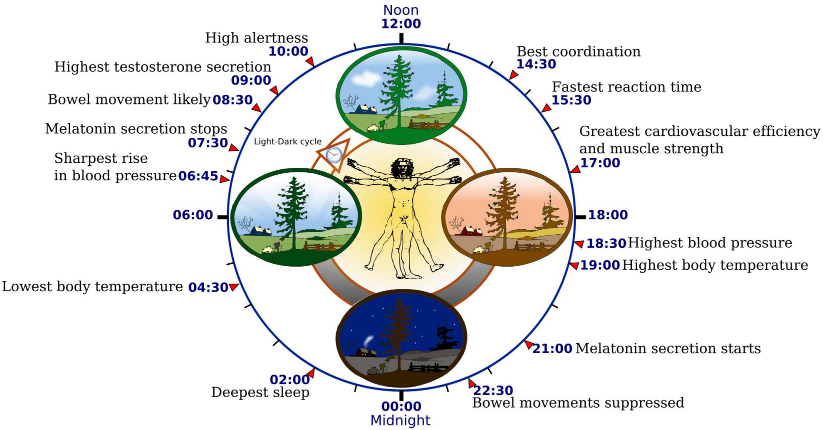
Thomas Hinze Mathias Schumann
Christian Bodenstein Ines Heiland Stefan Schuster

thomas.hinze@uni-jena.de

Friedrich Schiller University Jena
Department Bioinformatics at
School of Biology/Pharmacy

Modelling Oscillatory
Information Processing Group

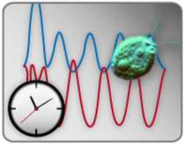
Human Daily Rhythm: Trigger and Control System



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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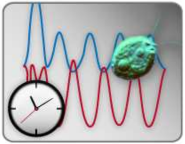

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FORSYS Partner Initiative

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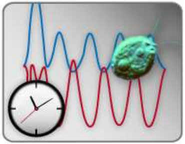

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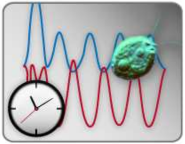

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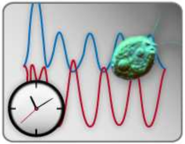

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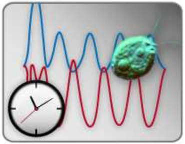

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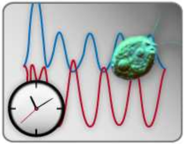

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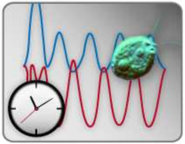

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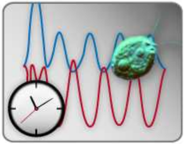

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Chronobiology

χρονοζ
time

βιοζ
life

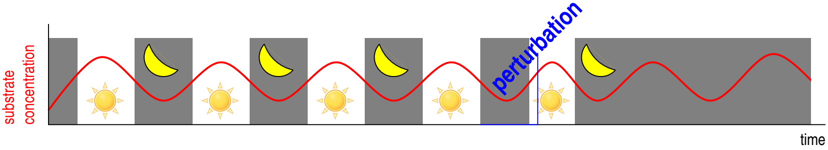
λογοζ
science

ρυθμοζ
rhythm

science of biological rhythms and clock systems

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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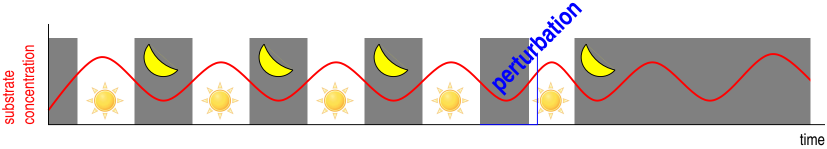
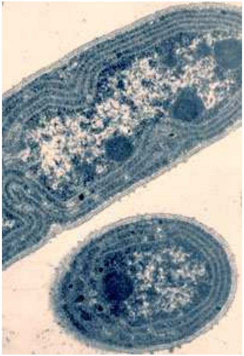


⟹ Biological counterpart of frequency control system

Cyanobacterium *Synechococcus elongatus*

“Simplest and earliest cells known to exhibit circadian phenomena”



www.genome.jgi–psf.org

www.wikipedia.org

- 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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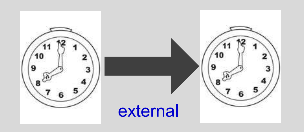
Entrainment vs. Synchronisation

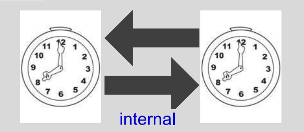
Entrainment

- 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



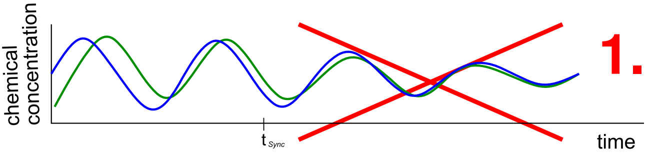


$\Longrightarrow$ Entrainment can be seen as special case of synchronisation

Properties of Synchronous Oscillations (I)

Undamped oscillations

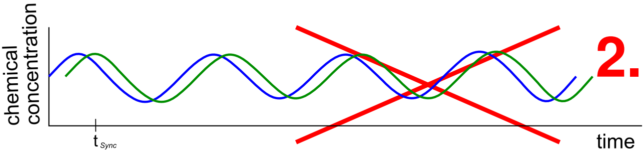
- Modelled oscillation results from solution of ordinary differential equations (ODEs) describing dynamical behaviour of the biochemical clok system
- Eigenvalues of Jacobian matrix (real parts < 0) mostly indicate undamped oscillations
- Limit cycles (represented by orbital courses) as method of choice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



Properties of Synchronous Oscillations (II)

Asymptotic or total adaption

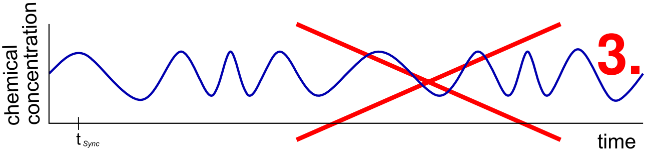
- Harmonisation of oscillating substrate concentration
  - after finite time t_{sync} within
  - arbitrarily selectable ε-neighbourhood



Properties of Synchronous Oscillations (III)

Monofrequential oscillation after t_{sync}

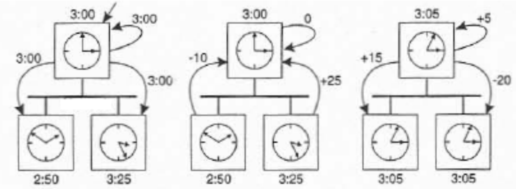
- Fast Fourier Transformation / Fourier analysis (discrete data processing and comparison of peaks)
- Laplace transform and subsequent algebraic processing (preferably for sinusoidal signals)
- Numerical exploration (e.g. sampling)



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)

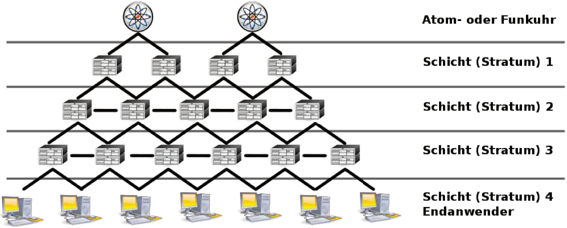


Berkeley algorithm. A. S. Tanenbaum and M. van Steen, *Distributed Systems Principles and Paradigms*, 2001

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

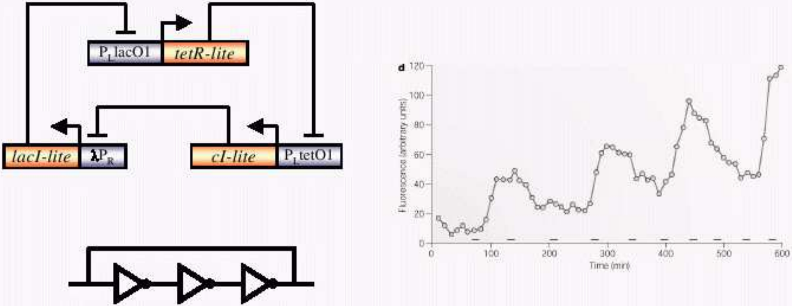
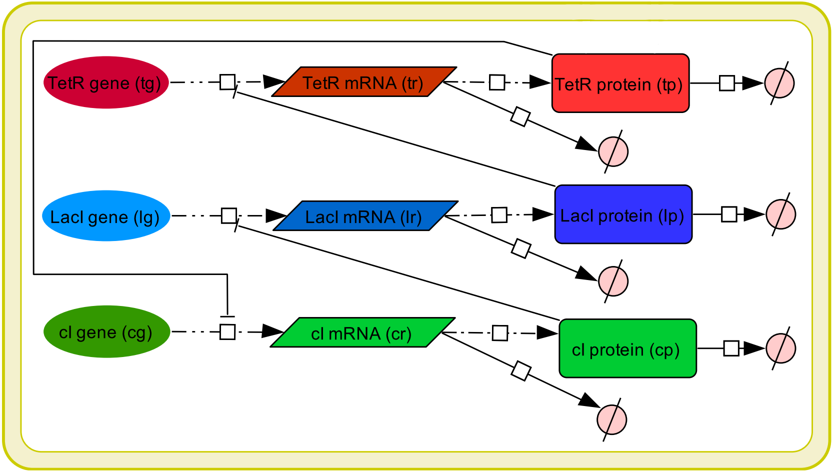


Figure 9. The repressilator circuit consists of three proteins and their three corresponding promoters, arranged such that each protein P_x represses the expression of a different protein P_y which does not repress P_x. These proteins include a synthetic tag, signified by the suffix “lite”, that targets 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

$$\frac{\mathrm{d}\, \mathit{LacI\_Protein}}{\mathrm{d}\, t} = \mathrm{k\_tl} \cdot \mathit{LacI\_mRNA} - \mathrm{k\_p} \cdot \mathit{LacI\_Protein}$$

$$\frac{\mathrm{d}\, \mathit{TetR\_Protein}}{\mathrm{d}\, t} = \mathrm{k\_tl} \cdot \mathit{TetR\_mRNA} - \mathrm{k\_p} \cdot \mathit{TetR\_Protein}$$

$$\frac{\mathrm{d}\, \mathit{cI\_Protein}}{\mathrm{d}\, t} = \mathrm{k\_tl} \cdot \mathit{cI\_mRNA} - \mathrm{k\_p} \cdot \mathit{cI\_Protein}$$

$$\frac{\mathrm{d}\, \mathit{LacI\_mRNA}}{\mathrm{d}\, t} = \mathrm{a0\_tr} + \frac{\mathrm{a\_tr} \cdot \mathrm{KM}^{\mathrm{n}}}{\mathrm{KM}^{\mathrm{n}} + \mathit{cI\_Protein}} - \mathrm{k\_tl} \cdot \mathit{LacI\_mRNA} - \mathrm{k\_r} \cdot \mathit{LacI\_mRNA}$$

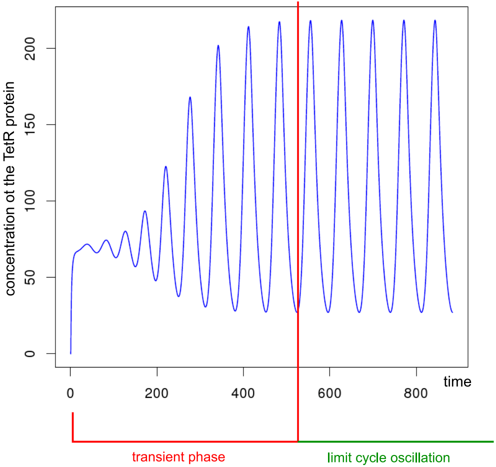
$$\frac{\mathrm{d}\, \mathit{TetR\_mRNA}}{\mathrm{d}\, t} = \mathrm{a0\_tr} + \frac{\mathrm{a\_tr} \cdot \mathrm{KM}^{\mathrm{n}}}{\mathrm{KM}^{\mathrm{n}} + \mathit{LacI\_Protein}} - \mathrm{k\_tl} \cdot \mathit{TetR\_mRNA} - \mathrm{k\_r} \cdot \mathit{TetR\_mRNA}$$

$$\frac{\mathrm{d}\, \mathit{cI\_mRNA}}{\mathrm{d}\, t} = \mathrm{a0\_tr} + \frac{\mathrm{a\_tr} \cdot \mathrm{KM}^{\mathrm{n}}}{\mathrm{KM}^{\mathrm{n}} + \mathit{TetR\_Protein}} - \mathrm{k\_tl} \cdot \mathit{cI\_mRNA} - \mathrm{k\_r} \cdot \mathit{cI\_mRNA}$$

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.

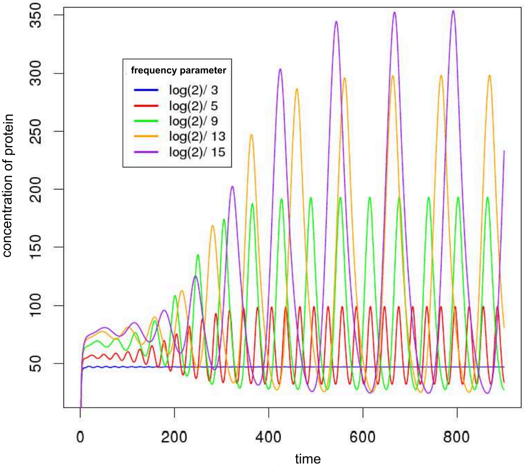
Dynamical Behaviour of the Repressilator (TetR)



Initialisation at limit cycle avoids transient phase

⟹ Eliminates its influence on synchronisation time

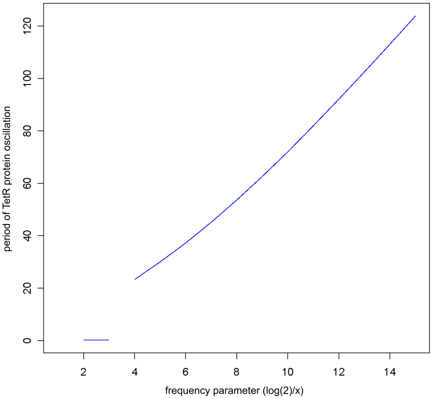
Period Control by Velocity of Protein Degradation



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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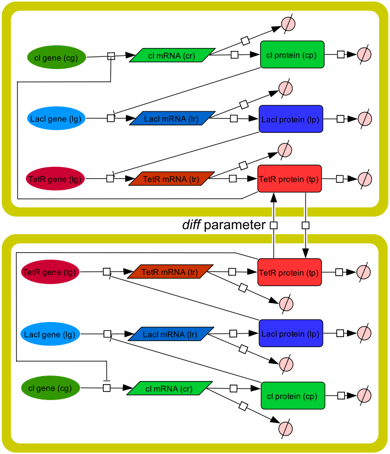
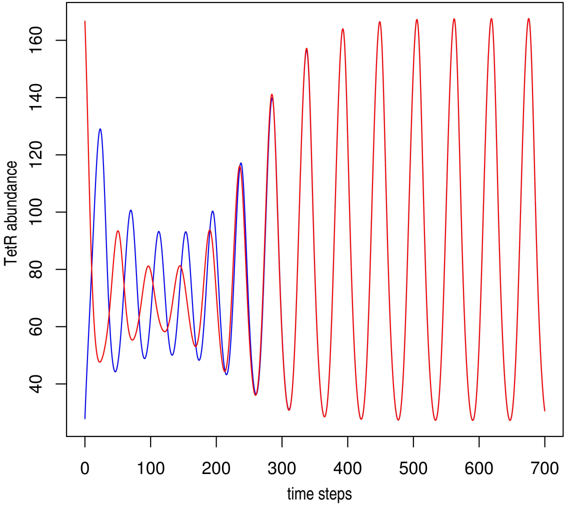
Coupled Repressilators for Internal Synchronisation



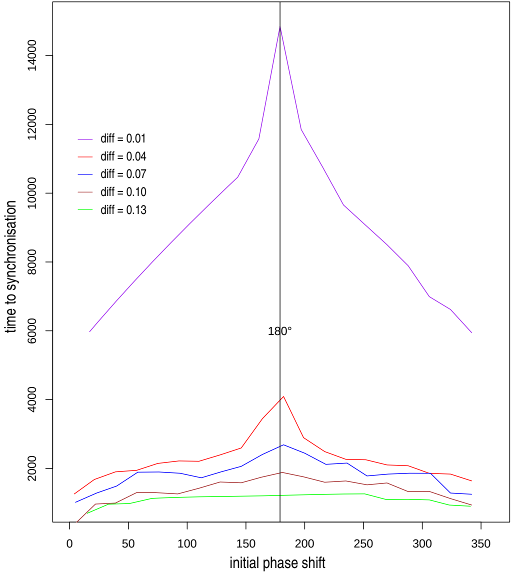
Bidirectional diffusion of TetR proteins between either repressilators enable internal synchronisation. Diffusion parameter *diff* as additional rate constant (linear kinetics)

Typical Synchronisation Run



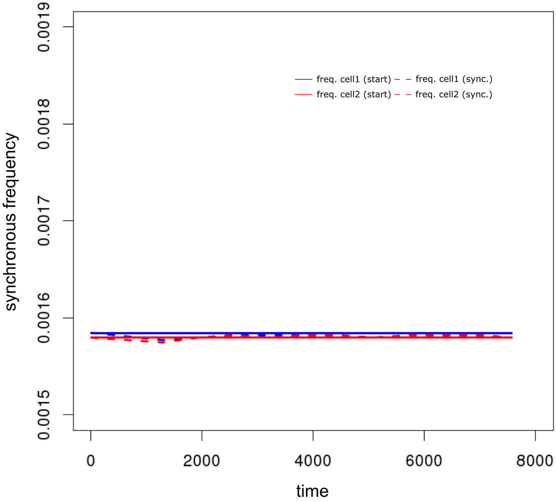
Typical synchronisation run of two TetR-coupled repressilators, coupling strength $\mathit{diff} = 0.04$, initial phase shift 182°.

Time to Synchronisation for Various Initial Phase Shifts



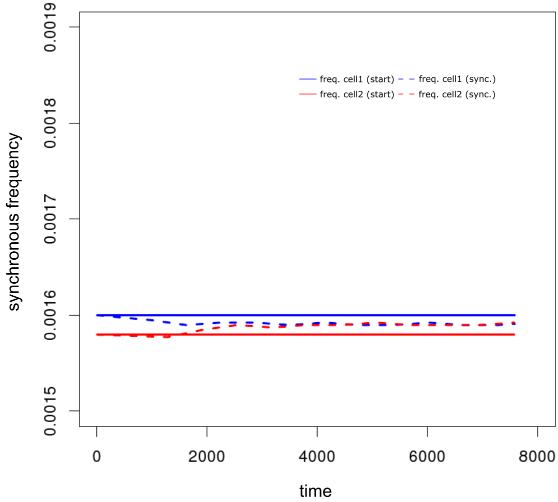
Time to synchronisation subject to various initial phase shifts. Parameter *diff* $= 0.01, \ldots, 0.13$ denotes coupling strength from weak to strong coupling. Initial antiphase rhythmicity (phase shift 180°) between both repressilators causes the highest effort to synchronise both oscillatory signals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

Time to Synchronisation for Various Initial Frequencies



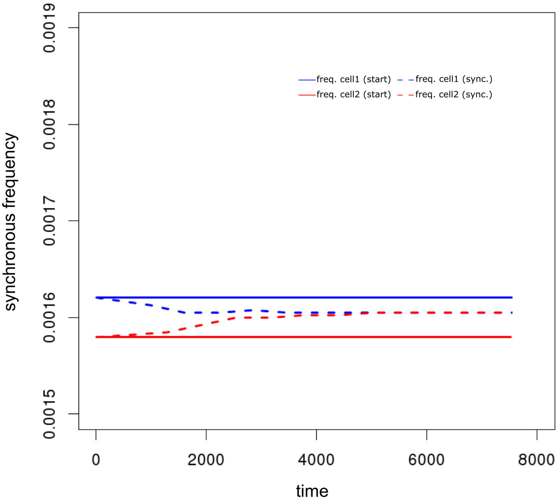
Weak diffusion, *diff* =0.01, frequency parameter *x* ratio: 9.475 / 9.5

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

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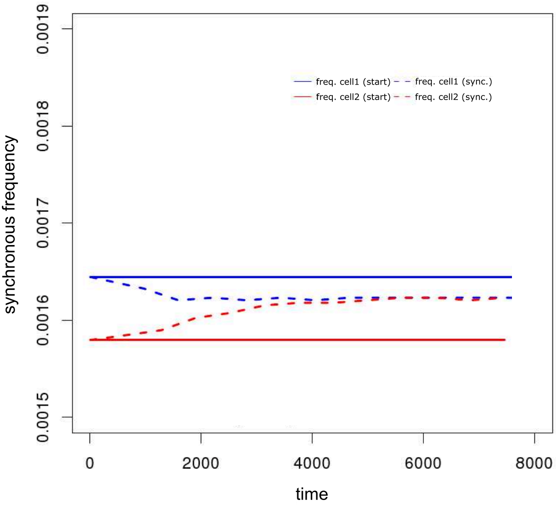
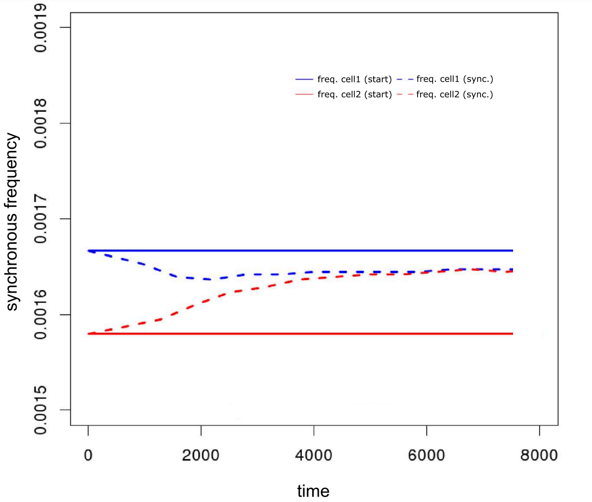


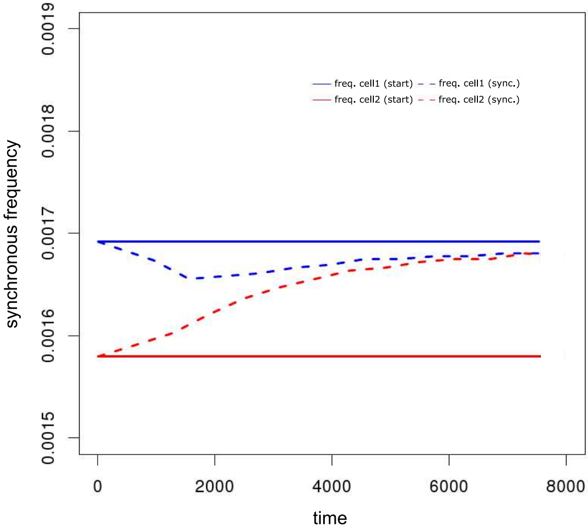
Weak diffusion, *diff* =0.01, frequency parameter x ratio: 9.2 / 9.5

Time to Synchronisation for Various Initial Frequencies



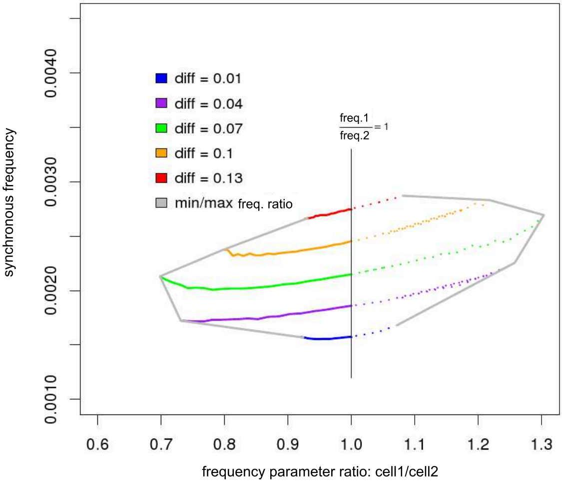
Weak diffusion, *diff* =0.01, frequency parameter x ratio: 9.1 / 9.5

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 $diff = 0.01, \dots, 0.13$: variant of an Arnold tongue

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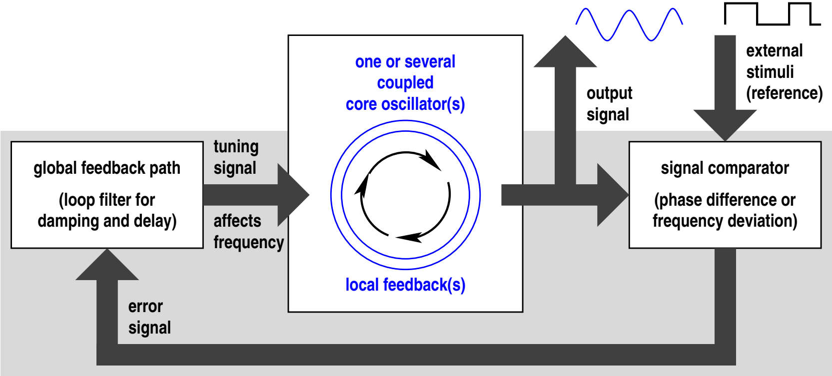
Frequency Control System with Phase-Locked Loop



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

Conclusions and Take Home Message

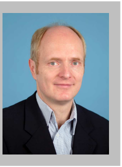
- Repressilator as promising biochemical *in-vitro* model system to explore synchronisation of circadian oscillations
- Inherent oscillation similar but not equal to sinusoidal course (hence not “symmetric”)
- Repressilator coupling by diffusion of TetR protein enables internal synchronisation.
- Arbitrary 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.

Special Thanks go to . . .

... my coworkers

Mathias Schumann
Department Bioinformatics, FSU Jena

Stefan Schuster
Department Bioinformatics, FSU Jena



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... you for your attention. Questions?

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