Capturing Circadian Clocks from the Perspective of Phase-Locked Loops

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Network Reconstruction: Complementary Strategies

Top-down

- From functional components to interacting network modules
- Successive refinement
- Identification, exploration and exchange of module candidates

Bottom-up

- From a monolithic behavioural specification to functional components
- Successive modularisation
- Identification of subnetworks acting as interfaced modules

⇒ We introduce a top-down strategy inspired by control systems.
Network Reconstruction: Complementary Strategies

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Circadian Clocks: General Schematic Representation

Frequency Control Systems with Phase-Locked Loop

Adapted from J.L. Stensby. Phase-locked loops. CRC Press, 1997
Considering Elementary Oscillators

- One or several coupled elementary oscillator(s)
- Global feedback path (loop filter for damping and delay)
- Local feedback(s)
- Error signal
- Tuning signal
- Affects frequency
- Output signal
- Signal comparator (phase difference or frequency deviation)
- External stimuli (reference)

Collaboration with C. Bodenstein and B. Schau, FSU Jena

Capturing Circadian Clocks from the Perspective of Phase-Locked Loops

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Elementary Oscillators under Study

- Sinusoidal function / Fourier series (dummy oscillator)
- Goodwin oscillator (original form)
- Goodwin oscillator with Michaelis-Menten degradation
- First attempts towards *Chlamydomonas* core oscillator
- Brusselator (autocatalysis, exclusively positive feedback loops)
- Sirius oscillator (resonator, clock signal generator)
- Repressilator (gene regulatory network, well-studied)
- Suprachiasmatic nucleus (single neuron oscillator, well-studied)

⇒ How to vary frequency? Obtaining response curves
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⇒ How to vary frequency? Obtaining response curves
Example: Repressilator

Protein Half-Life Parameter Controls Frequency

protein_hl = 3, . . . , 15 influences protein-degradation rates (LacI, cl, TetR)

M. Schumann, T. Hinze, S. Schuster. Synchronisation of clocks: Comparing mechanisms in biological and technical distributed systems, submitted
Repressilator: Response Curve – I/O Mapping
(period length subject to protein_hl)

M. Schumann, T. Hinze, S. Schuster. Synchronisation of clocks: Comparing mechanisms in biological and technical distributed systems, submitted

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Considering Low-Pass Filters

Motivation

Elementary Oscillators

Low-Pass Filters

Signal Comparator

Discussion

Elementary Oscillators

Low-Pass Filters

Signal Comparator

Discussion

Considering Low-Pass Filters

coupled one or several elementary oscillator(s) local feedback(s)

damping and delay) (loop filter for frequency signal tuning)

(error signal)

output signal

external stimuli (reference)

one or several coupled elementary oscillator(s)

local feedback(s)

signal comparator (phase difference or frequency deviation)

Collaboration with C. Bodenstein and B. Schau, FSU Jena

Capturing Circadian Clocks from the Perspective of Phase-Locked Loops
Effect of Low-Pass Filters to Oscillatory Signals

Frequency response – I/O mapping

- Low frequency oscillations pass through
- High frequency oscillations eliminated
- Signal smoothing, damping, and delay (desensibilise global feedback)
Signal Transduction Cascade Acts as Low-Pass Filter


Capturing Circadian Clocks from the Perspective of Phase-Locked Loops
Frequency Response Depends on Cascade Topology

Low-Pass Filter by Moving Average Elements

Excursus: the DAX

- Common principle for smoothening oscillatory signals
- Length of average window determines frequency response
- Needs a buffer and produces a delay
Low-Pass Filter by Moving Average Elements

heavily varying inflow

molecular buffer

prototype of a diffusion system with delay

slightly varying outflow
Considering Signal Comparators

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Tasks

- Compare elementary oscillator’s output to reference signal(s) (i.e. external stimuli)
- Obtain a weighted measure for dynamical signal deviance
- Execute *arithmetic operations* on signal values
Functional Units

Obtain phase difference and/or frequency deviance

Low-pass filter
- Signal transduction cascade or moving average element for both comparator inputs

FFT (Fast Fourier Transformation)
- Obtain fundamental oscillation of the form 
  \[ a_0 + a_1 \cdot \sin(\omega t + \phi) \] for both signals

Sampling and Accumulation
- Superpositioning of sampling data
- Nonlinear regression
- Approximation by trigonometric function

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Third Root Network (achieved by SBMLLevolver)

initial conc. of input species $\rightarrow$ steady state conc. of output species

Addition

Let $k_1 = k_2 = k_3 > 0$.

Steady state:

$$y = \lim_{t \to \infty} \left( 1 - e^{-k_1 t} \right) \cdot \left( x_1 + x_2 \right) = x_1 + x_2$$

Non-Negative Subtraction

\[
\begin{align*}
\frac{dx_1}{dt} &= 0 \\
\frac{dx_2}{dt} &= 0 \\
\frac{dy}{dt} &= -k_2 yz - k_1 y + k_1 x_1 \\
\frac{dz}{dt} &= k_1 x_2 - k_2 yz \\
\end{align*}
\]

Let \( k_1 > 0 \) and \( k_2 > 0 \).

Steady state:

\[
y = \begin{cases} 
  x_1 - x_2 & \text{iff } x_1 > x_2 \\
  0 & \text{otherwise}
\end{cases}
\]
Multiplication

\[
\frac{dx_1}{dt} = 0 \quad \frac{dx_2}{dt} = 0 \quad \frac{dy}{dt} = k_1 x_1 x_2 - k_2 y
\]

Let \( k_1 = k_2 > 0 \).

Steady state:
\[
y = \lim_{t \to \infty} \left( 1 - e^{-k_1 t} \right) \cdot x_1 \cdot x_2 = x_1 \cdot x_2
\]

Let $k_1 = k_2 > 0$. Steady state:

$$y = \begin{cases} 
\lim_{t \to \infty} \left(1 - e^{-k_1 t}\right) \cdot \frac{x_2}{x_1} & \text{iff } x_1 > 0 \\
\lim_{t \to \infty} \int k_2 x_2 dt & \text{otherwise} \\
\frac{x_2}{x_1} & \text{iff } x_1 > 0 \\
\infty & \text{iff } x_1 = 0 \text{ and } x_2 > 0 \\
0 & \text{iff } x_1 = 0 \text{ and } x_2 = 0
\end{cases}$$
Considering Phase-Locked Loops

Motivation

- Elementary Oscillators
- Low-Pass Filters
- Signal Comparator

Discussion

- Considering Phase-Locked Loops
  - Coupled one or several elementary oscillator(s)
  - Local feedback(s)
  - Global feedback path (loop filter for damping and delay)
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Combine Modular Components

Motivation
Elementary Oscillators
Low-Pass Filters
Signal Comparator
Discussion

Combine Modular Components

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Take-Home Message

- Circadian clocks can be seen as biological frequency control systems
- Adopting the concept of phase-locked loops seems promising
- Proposing network candidates for each module gives high flexibility in top-down network inference
- Hypothesis testing flanked by experiments (variation of external stimuli over time with respect to oscillator output)
Special Thanks go to ... 

... my coworkers

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Department Bioinformatics, FSU Jena

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... you for your attention. Questions?