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.

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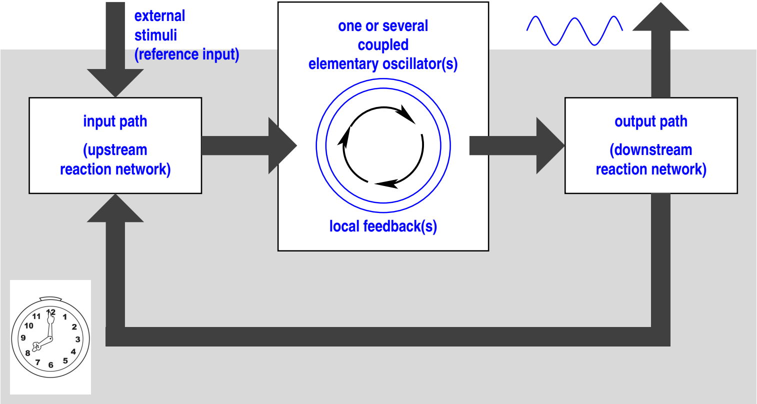
Circadian Clocks: General Schematic Representation



external stimuli (reference input)

one or several coupled elementary oscillator(s)

input path

(upstream reaction network)

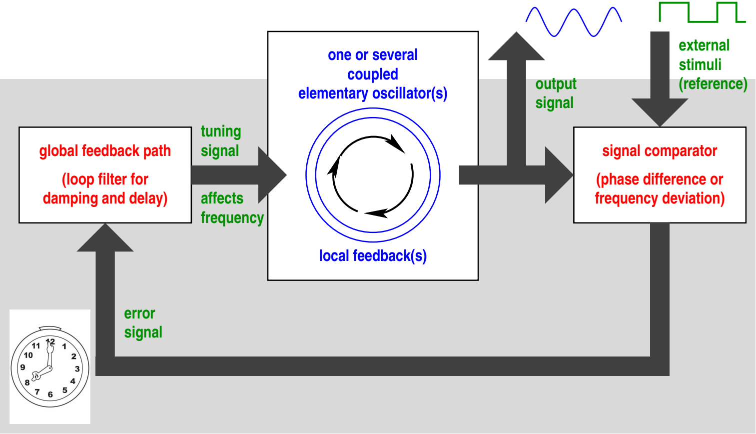
output path

(downstream reaction network)

local feedback(s)

Adapted from M.J. Gardner et al. How plants tell the time. Review in Biochem. J. **397**:15-24, 2006

Frequency Control Systems with Phase-Locked Loop



one or several coupled elementary oscillator(s)

local feedback(s)

output signal

external stimuli (reference)

signal comparator

(phase difference or frequency deviation)

error signal

global feedback path

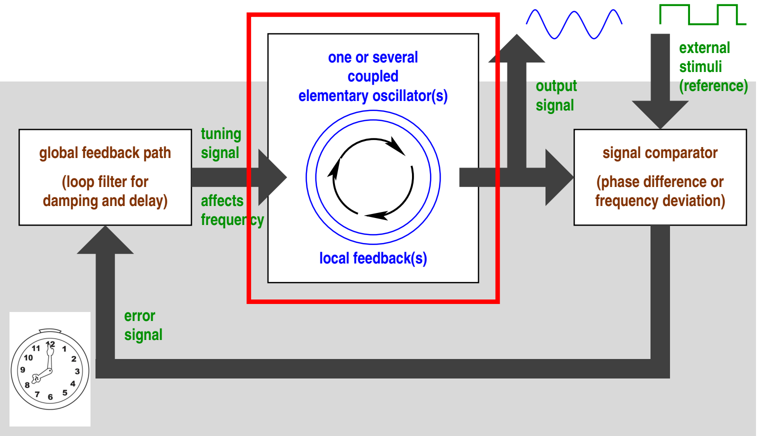
(loop filter for damping and delay)

tuning signal

affects frequency

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

Considering Elementary Oscillators



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

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)

$\Longrightarrow$ How to vary frequency? Obtaining response curves

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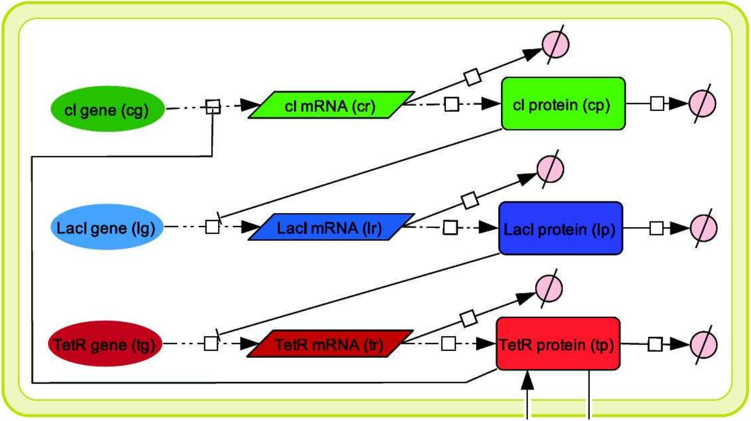
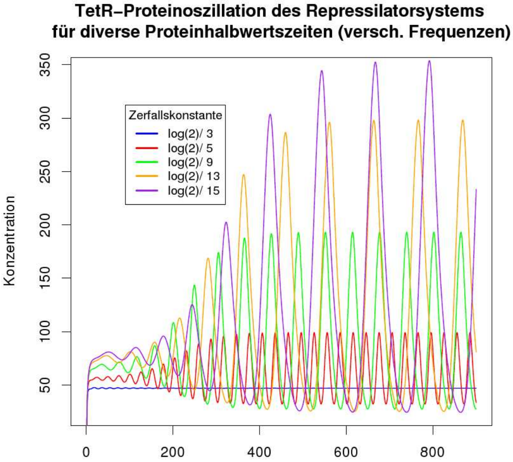
$\Longrightarrow$ How to vary frequency? Obtaining response curves

Example: Repressilator



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

Protein Half-Life Parameter Controls Frequency

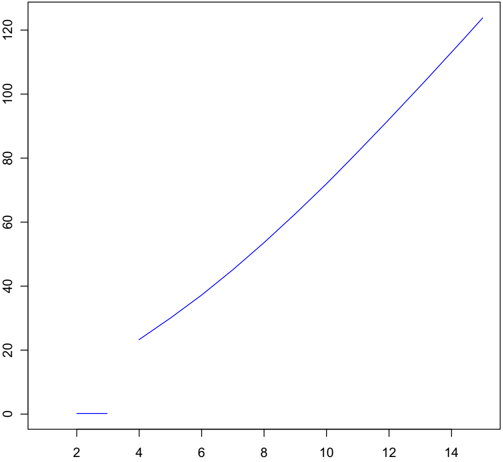


protein_hl $= 3, \ldots, 15$ influences protein-degradation rates (LacI, cI, TetR)

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)



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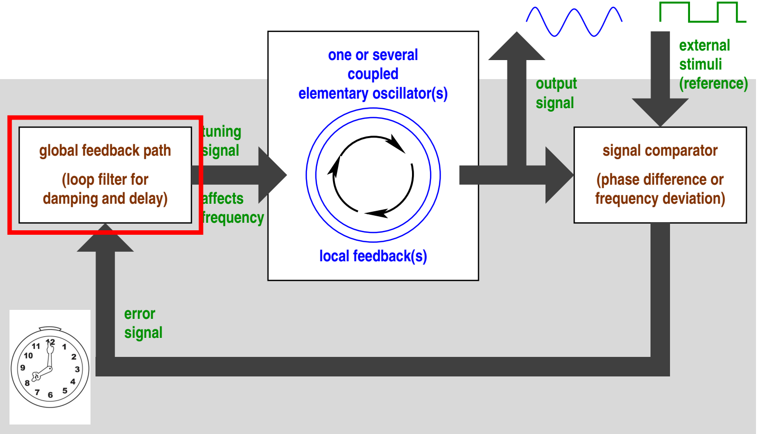
Considering Low-Pass Filters



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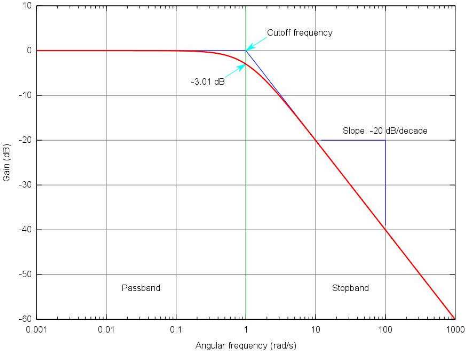
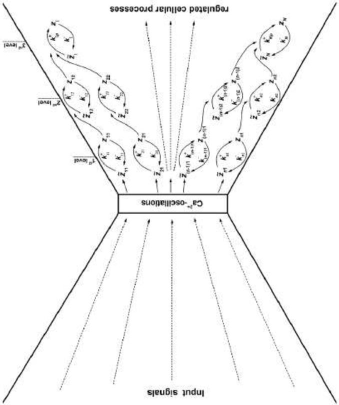
Effect of Low-Pass Filters to Oscillatory Signals

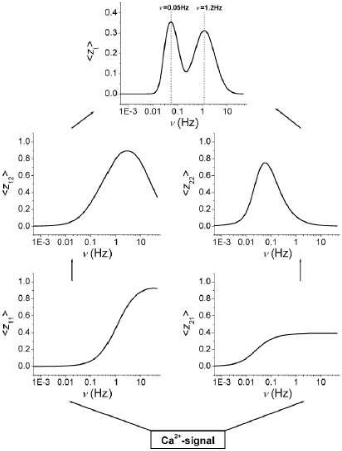
Frequency response – I/O mapping



- Low frequency oscillations pass through
- High frequency oscillations eliminated
- Signal smoothing, damping, and delay (desensibilise global feedback)

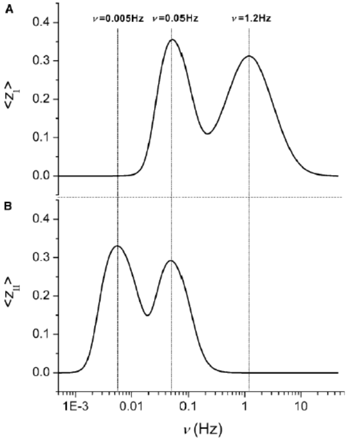
Signal Transduction Cascade Acts as Low-Pass Filter





M. Marhl, M. Perc, S. Schuster. Selective regulation of cellular processes via protein cascades acting as band-pass filters for time-limited oscillations. FEBS Letters 579(25):5461-5465, 2005

Frequency Response Depends on Cascade Topology



M. Marhl, M. Perc, S. Schuster. Selective regulation of cellular processes via protein cascades acting as band-pass filters for time-limited oscillations. FEBS Letters 579(25):5461-5465, 2005

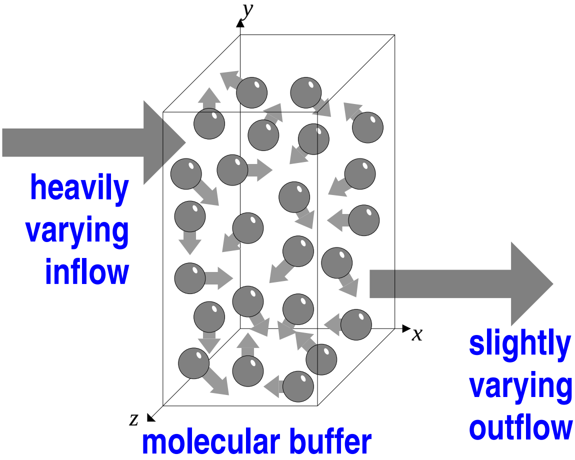
Low-Pass Filter by Moving Average Elements

Excursus: the DAX

www.ndr.de

- Common principle for smoothening oscillatory signals
- Length of average window determins frequency response
- Needs a buffer and produces a delay

Low-Pass Filter by Moving Average Elements



prototype of a diffusion system with delay

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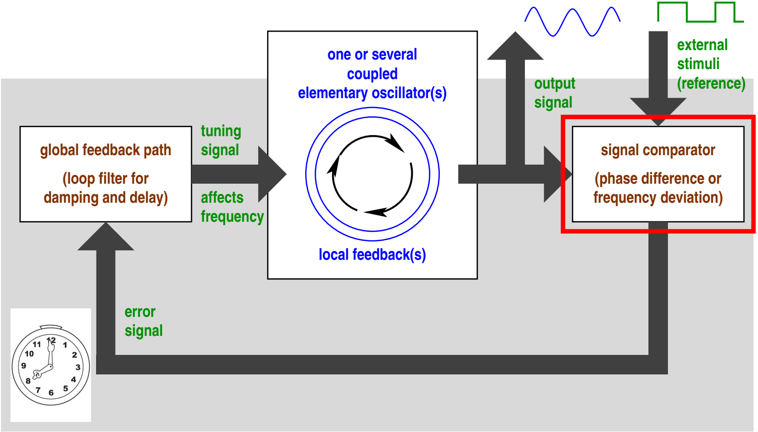
Considering Signal Comparators



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local feedback(s)

global feedback path

(loop filter for damping and delay)

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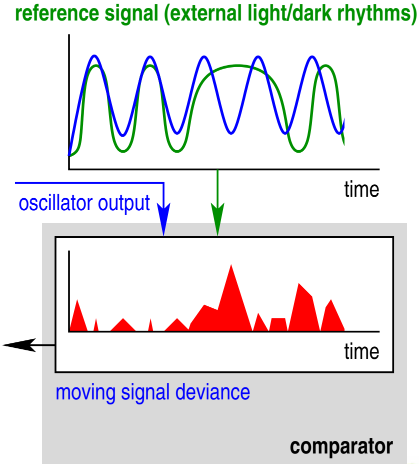
Collaboration with C. Bodenstein and B. Schau, FSU Jena

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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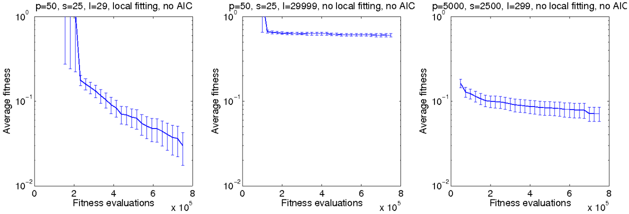
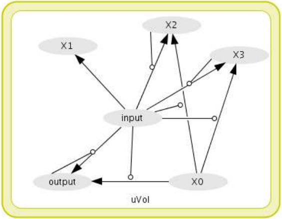
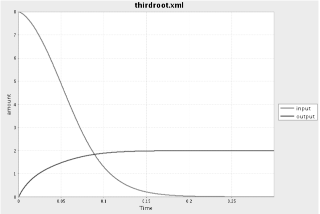
Third Root Network (achieved by SBMLevolver)

initial conc. of input species ↦ steady state conc. of output species

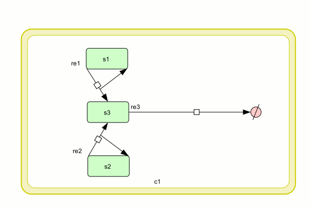


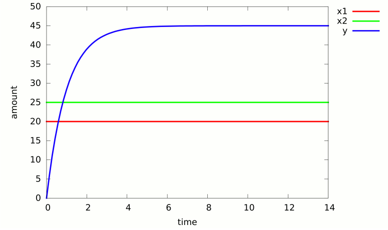




T. Lenser, T. Hinze, B. Ibrahim, P. Dittrich. Towards Evolutionary Network Reconstruction Tools for Systems Biology. In E. Marchiori, J.H. Moore, J.C. Rajapakse (Eds.), Proceedings Fifth European Conference on Evolutionary Computation, Machine Learning and Data Mining in Bioinformatics, Springer LNCS 4447:132-142, 2007

Addition





$$\frac{dx_1}{dt} = 0 \qquad \frac{dx_2}{dt} = 0 \qquad \frac{dy}{dt} = k_1 x_1 + k_2 x_2 - k_3 y$$

Let $k_1 = k_2 = k_3 > 0$.

Steady state:

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

B. Schau, T. Hinze, T. Lenser, I. Heiland, S. Schuster. Control System-Based Reverse Engineering of Circadian Oscillators. In I. Grosse, S. Neumann, S. Posch, F. Schreiber, P. Stadler (Eds.), Proceedings German Conference on Bioinformatics (GCB2009), p. 126-127, Martin-Luther University Halle-Wittenberg, 2009

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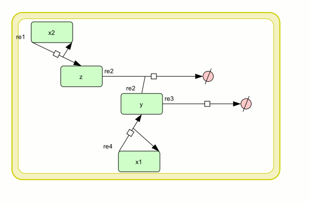
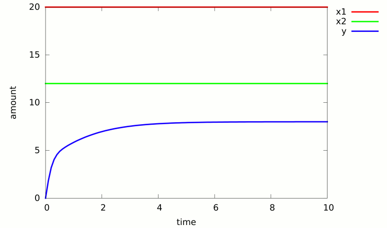
Non-Negative Subtraction





$$\frac{dx_1}{dt} = 0 \qquad \frac{dx_2}{dt} = 0$$

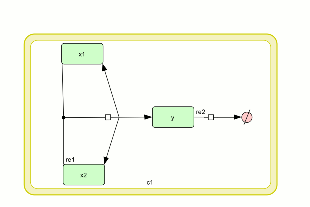
$$\frac{dy}{dt} = -k_2yz - k_1y + k_1x_1 \qquad \frac{dz}{dt} = k_1x_2 - k_2yz$$

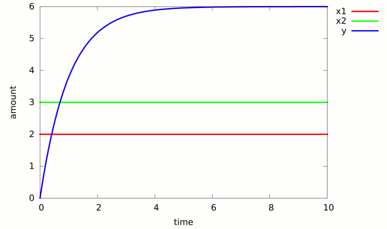
Let $k_1 > 0$ and $k_2 > 0$.

Steady state:

$$y = \begin{cases} x_1 - x_2 \text{ iff } x_1 > x_2 \\ 0 \text{ otherwise} \end{cases}$$

Multiplication





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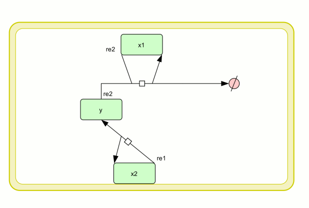
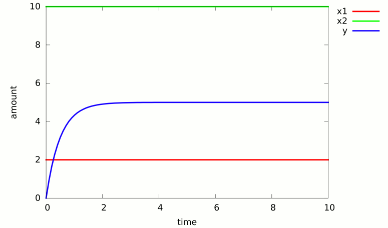
Division





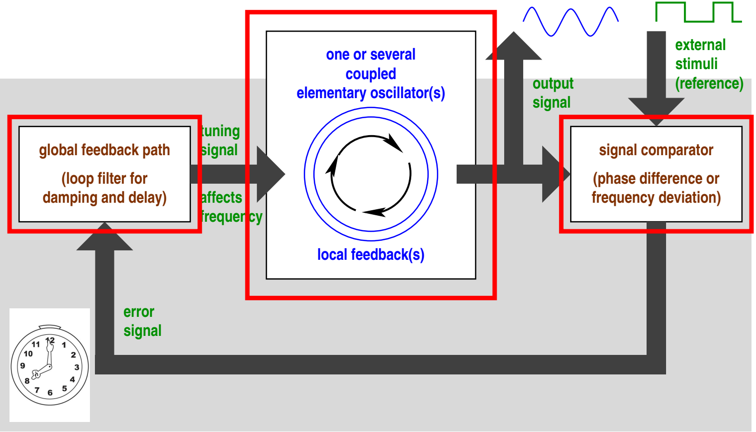
$$\frac{dx_1}{dt} = 0 \qquad \frac{dx_2}{dt} = 0 \qquad \frac{dy}{dt} = k_2 x_2 - k_1 x_1 y$$

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

$$y = \begin{cases} \lim\limits_{t\to\infty} \left(1 - \mathrm{e}^{-k_1 t}\right) \cdot \frac{x_2}{x_1} & \text{iff } x_1 > 0 \\ \lim\limits_{t\to\infty} \int k_2 x_2 dt & \text{otherwise} \end{cases}$$

$$= \begin{cases} \frac{x_2}{x_1} & \text{iff } x_1 > 0 \\ \to \infty & \text{iff } x_1 = 0 \text{ and } x_2 > 0 \\ 0 & \text{iff } x_1 = 0 \text{ and } x_2 = 0 \end{cases}$$

Considering Phase-Locked Loops



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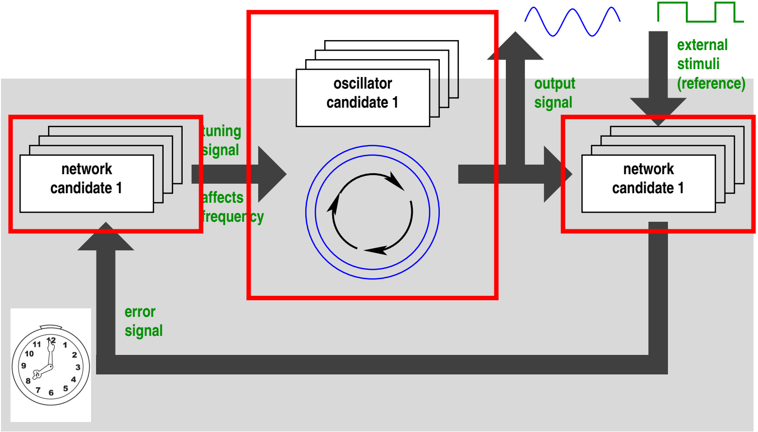
Combine Modular Components



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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)

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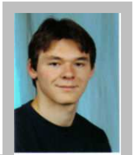
Special Thanks go to . . .

... my coworkers



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Bundesministerium
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... you for your attention. Questions?

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