1. Two oscillators couple:
   One internal to one external:
   Arnold tongues or entrainment!

2. Biological oscillations: Cell cycle, circadian, calcium, embryos, proteins (DNA damage)
3. Oscillations of a protein density inside a cell: regulated by negative feed-back loops (NF-κB, p53, Wnt proteins):
   DNA damage, inflammation, embryo segmentation.

4. An external (cytokine or protein) oscillation coupled to internal oscillation: The cell ‘learns’ and get memory, it synchronizes (entrain)
   Arnold tongues → Overlap → Mode hopping

5. Pulsatile extracellular signaling in experiments (Chicago):
   When memory is short: Observe mode hopping.
   A way to control cell dynamics? Jump between genes?
   Chaotic motion!

6. Understand time correlations: One tongue dominates → stronger time correlations and memory (Poincare section).
Collaborators:

- Sandeep Krishna, Leo Kadanoff, Savas Tay, Mathias Heltberg, Ryan Kellogg, Namiko Mitarai, Uri Alon


Graphical Abstract

Cells hop between entrainment states

Periodic input (TNF) ↔ NF-κB

Entrainment

1:1

Intrinsic noise

Modehopping

Chaotic dynamics shows modehopping

Large amplitude (TNF) → Chaotic dynamics

Deterministic or stochastic

Modehopping

Modehopping enables gene switching

Modehopping

Downstream gene production

Multiplexing

NF-κB (TF) → Polymerase → Protein

Gene 1

Gene 2
Synchronization of two oscillators

Huygens’ clocks 1665
Three different non-linear dynamics
Two coupled oscillators: Arnold tongues

\[ \frac{\omega}{\Omega} = \frac{P}{Q} \]
Examples of Arnold tongues!
Examples of Arnold tongues!
Chicago basement convection!

Libchaber, Stavans, Glazier: External oscillating current!
Chicago basement convection!

Stavans, Heslot, Libchaber

Glazier, Jensen, Libchaber, Stavans
Semiconductors: Gwinn, Westervelt, Harvard
Sliding CDW’s at UCLA

Brown, Mozurkewich, Gruner
What about biology –
many oscillators!

- Cell cycles
- Circadian clocks
- Calcium oscillators
- Embryos
- Pace maker cells
- Protein oscillations (DNA damage)
- Population dynamics
Basic oscillator: Negative Feed-Back loops:
‘Typical’ Oscillating data: Hes1 - segmentation

(Hirata et al, 2002)
Simplest negative feed-back loop: Hes1

\[
\frac{d[mRNA]}{dt} = \alpha \cdot [o_{free}] - \frac{[mRNA(t)]}{\tau_{rna}}
\]

\[
\frac{d[Hes1]}{dt} = \beta \cdot [mRNA(t)] - \frac{[Hes1(t)\right]}{\tau_{hes1}}
\]

Jensen et al 2003
- Dashed curve [Hes1]
- Solid curve [mRNA]

- $\tau_{\text{rna}} = 24.1 \text{ min}$
- $\tau_{\text{hes1}} = 22.3 \text{ min}$
- $\tau = 24 \text{ min}$
- $\alpha = 20 \ [R]_0 \text{ min}^{-1}$
- $\beta = 1/20 \text{ min}^{-1}$
- $K_M = (0.1[R]_0)^n$
- $n = 4$
Simple models of ultradian oscillations

The NF-κB System in Mammalian Cells

- NF-κB family: dimeric transcription factors
- Regulates immune response, inflammation, apoptosis
- Over 150 triggering signals, over 150 targets
- Each NF-κB has a partner inhibitor IκB
- Fluorescence imaging of NF-κB and IκB in human S-type neuroblastoma cells.


How does the network produce oscillations?
Why does the cell need the oscillations?
Simple Model for Protein Oscillations

\[
\begin{align*}
\frac{dN_n}{dt} &= A \frac{(1 - N_n)}{\epsilon + I} - B \frac{IN_n}{\delta + N_n}, \\
\frac{dI_m}{dt} &= N_n^2 - I_m, \\
\frac{dI}{dt} &= I_m - C \frac{(1 - N_n)I}{\epsilon + I}.
\end{align*}
\]

\[
A = 0.007, \quad B = 954.5, \quad C = 0.035, \\
\delta = 0.029, \quad \epsilon = 2 \times 10^{-5}
\]
Oscillations of protein densities in a single cell

(M. Covert, Stanford, unpublished)
(Savas Tay, Chicago)
Embedded attractors: Chaos ??
Externally ‘forced’ NF-κB system

External modulation of TNF cytokine signal

Cells can ‘learn’ (memorize after transient) and synchronize their dynamics → Arnold tongues:

Maybe a way to control DNA damage/DNA repair

(S. Krishna, L. Kadanoff, MHJ)
Externally ‘forced’ NF-κB system

(S. Krishna, MHJ)
**NFκB model, driven by TNF:**

\[\begin{align*}
\text{NFκB} & \quad \frac{dN_n}{dt} = k_{Nin}(N_{tot} - N_n) \frac{K_l}{K_l + I} - k_{lin}I \frac{N_n}{K_N + N_n} \\
& \quad \frac{dI_m}{dt} = k_t N_n^2 - \gamma_m I_m \\
\text{IκBα} & \quad \frac{dI}{dt} = k_{il} I_m - \alpha [IKK]_a (N_{tot} - N_n) \frac{I}{K_l + I} \\
\text{IKK} & \quad \frac{d[IKK]_a}{dt} = k_a[TNF]([IKK]_{tot} - [IKK]_a - [IKK]_i) - k_i[IKK]_a \\
\text{TNF} & \quad \frac{d[IKK]_i}{dt} = k_i[IKK]_a - k_p[IKK]_i \frac{k_{A20}}{k_{A20} + [A20][TNF]}
\end{align*}\]

**IKK, TNF, A20:** Ashall, Rand, White, et al…. Science (2009)
Sinusoidally driven NF-kB oscillations

Jensen, Krishna (2012)
Sinusoidally driven NF-κB oscillations

![Graph showing frequency response and NF-κB dynamics](image)

Jensen, Krishna (2012)
Sinusoidally driven NF-κB oscillations

Jensen, Krishna (2012)
Sinusoidally driven NF-kB oscillations

Tongues overlap!

In phase space
Sinusoidally driven NF-kB oscillations

Jensen, Krishna (2012)
Sinusoidally driven NF-kB oscillations

Ryan Kellog, Savas Tay (2015)

Microfluidic chamber with mouse fibroblast cells

Can be driven by a periodic sawtooth shaped stimulation
Entrained NF-kB seems to aid expression of certain genes.
When tongues overlap:
Experimentally observed mode hopping between entrained states

B

1:1

TNF Period
90 min

1:2

NF-κB

3

Time

Input amplitude

1:1

TNF Period
180 min

1:2

NF-κB

3

Time

Input amplitude

C

1:1

TNF Period
150 min

1:2

Mode hopping

NF-κB

3

Time

Input amplitude

D

Single-cell NF-κB oscillations

Nuclear NF-κB

Time (min)

0 500 1000 1500

1:2

1:1

1:2

1:1

1:2

E

NF-κB period over time

Period length (min)

Time (min)

0 500 1000 1500

200

200

200

1:1

1:2

1:1

1:2
Stochastic Gillespie simulations: manifest as modehopping between entrained states
Deterministic chaos:
Mode hopping between several entrained states
Modehopping a way to switch between genes?: Multiplexing

(Cooperativity vs affinity)
Probability to be in different tongues:

Gillespie simulations of NF-kB network by Mathias Heltberg

Time Spent in Tongue, TNF = 1.8 times unperturbed

Time Spent in Tongue, TNF = 2.3 times unperturbed

driving frequency

NfkB Frequency

Time (min)

1:1 tongue

2:1 tongue

3:1 tongue

Time (min)

Freq.

Time (min)

2:1 tongue

3:1 tongue

Frequency
Gillespie simulations of NF-kB network by Mathias Hellberg

Probability to be in different tongues:

- 1/1
- 2/1

Time Spent in Tongue, TNF = 1.3 times unperturbed:
- 1/1
- 3/1

Time Spent in Tongue, TNF = 2.3 times unperturbed:
- 1/1
- 3/1

Driving frequency

Distribution of times in 2:1 tongue, TNF = 1.3 times unperturbed frequency:
- Stretched exponential
- Close to exponential

Long time correlations/memory

Memoryless?
To understand memory:

Simplify the NF-kB model: Overlap of two tongues

\[ \dot{N}_n = k_{Nin}(N_{tot} - N_n) \frac{K_I}{K_I + I} - k_{Iin}I \frac{N_n}{K_N + N_n} \]

\[ \dot{I}_{RNA} = k_t N_n^2 - \gamma_m I_{RNA} \]

\[ \dot{I} = k_{tl} I_{RNA} - \alpha I K K_a (N_{tot} - N_n) \frac{I}{K_I + I} \]

\[ I K K_a = k_a f(t)([I K K]_{tot} - I K K_a - I K K_i) - k_i I K K_a \]

\[ I K K_i = k_i I K K_a - k_p I K K_i \frac{k_{A20}}{k_{A20} + [A20] f(t)} \]

\[ f(t) = 0.5 + A \sin \left( \frac{2\pi}{T} t \right) \]
To simplify: Make a Poincare cut

Stochastic simulation: Jumps between the tongues!
Basin of attraction for the two tongues
Number of oscillations before leaving a tongue

Strongly time correlated (memory):
Stretched exponential – or sum of two exponentials
Two coupled oscillators: Arnold tongues

$\omega/\Omega = P/Q$
Only few examples Arnold tongues in other biological systems:
Cell cycle and circadian clock

Gerard and Goldbeter, May, 2012
Populations of genetic oscillators

Jeff Hasty et al, Science 2011
Cross, Charvin, Siggia: Budding yeast cell cycle: Experiments and Model (PNAS 2009)

FIG. 3. The daughter mass (solid line, arbitrary units) as a function of time for impulses of Cln3 (dashed) with periods \( T \), longer than the locking interval \( [T=160 \text{ min}, (a)] \), within the mode-locked interval \( [T=120 \text{ min}, (b)] \), and shorter than the mode-locked interval \( [T=90 \text{ min}, (c)] \). The amplitude of the perturbation is given by the first entry in Table I. Note that there is an extra cell cycle in (a) and an extra pulse in (c). The natural period of the cell is 138 min, which is the average period in (c).
Initial conditions in “phase space” to different tongues

Mathias Heltberg