# DYNAMICS OF YEAST CELL POPULATIONS

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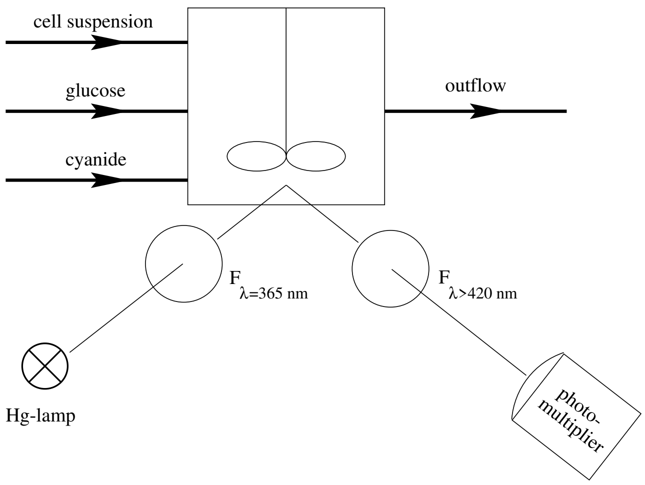
Sustained oscillations of the concentration of NADH have been observed in a stirred suspension of living yeast cells in a CSTR (Fig.(1)) with a continuous supply of glucose, yeast cells and cyanide [1]. The observations are due to oscillations in the metabolic conversion of glucose to ethanol within each cell. Due to the cell membrane the cells are chemically isolated from each other except for a few substances which are able to penetrate the membrane. The quantity observed is the average fluorescence of the NADH within the cells. The observed oscillations show that the oscillations of each cell must be at least partially synchronized possibly by exchange of species between the interior of the cells and the extracellular fluid [2].

The behavior of the CSTR cell population has been investigated by standard bifurcation analysis and by response studies. The concentrations of the reservoir species glucose and cyanide in the extracellular medium have been controlled by changing the compositions and the rates of the individual flows and the dynamics have been investigated by instantaneous additions of potential signalling species to the extracellular medium. The results of these investigations can all be explained as the behavior of a system close to a supercritical Hopf bifurcation.

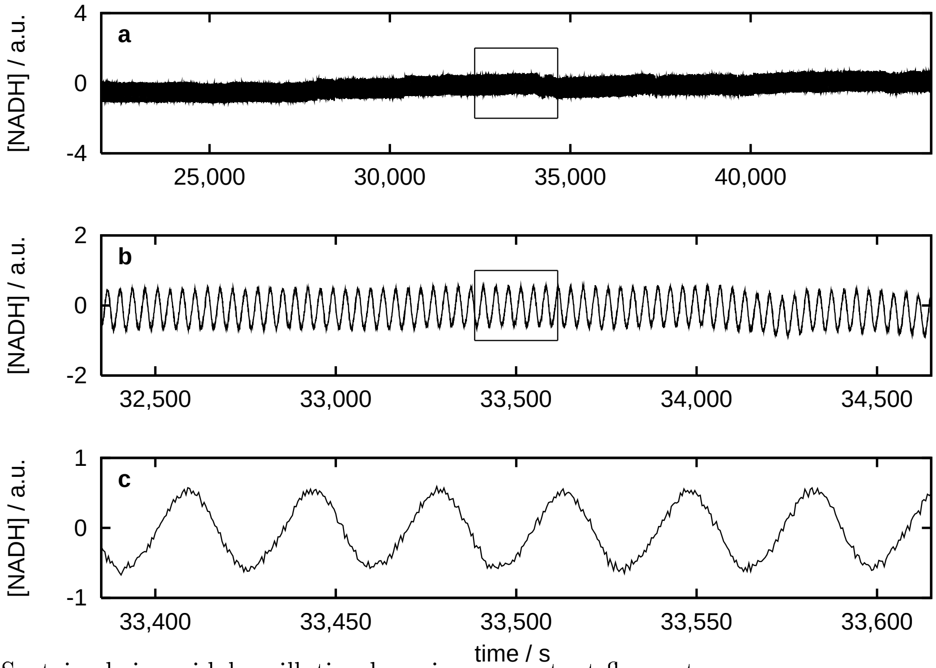
Damped oscillations were first observed by Duysen and Amesz in 1957 [3]. Longer transients of oscillations in a closed suspension of starved cells by addition of glucose were obtained by Ghosh and Chance in 1964 [4]. Semi-closed systems were studied by Hess and Boiteux in 1968 [5], and further studies on closed systems were done by Pye 1971 [2], by Winfree 1972 [6], and by Richard, Teusink, Bakker, Westerhoff and van Dam from 1993 [7].



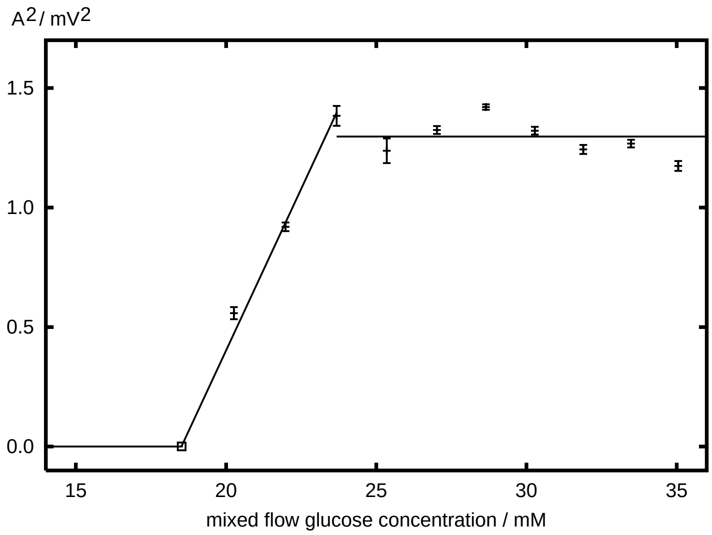

Fig(1). Experimental equipment. Dormant cells, glucose, and cyanide flow into the reactor. The concentration of NADH is monitored by observing the fluorescence of NADH from cells close to the cuvette surface.

**Experimental procedure and results**

The yeast cells are grown aerobically on glucose and yeast nitrogen base (YNB), harvested at the point of glucose depletion, starved and washed with buffer. They can be stored for a couple of days at 5°C. The experimental equipment is shown in Fig(1) and Fig(2) shows sustained oscillations in a CSTR. Fig(3) shows the bifurcation analysis, and Fig(4) the instantaneous quenching of the oscillations after the addition of a chemical species in an amount and at a phase which is characteristic for each chemical species.




Fig(2). Sustained sinusoidal oscillation by using a constant flow rate.



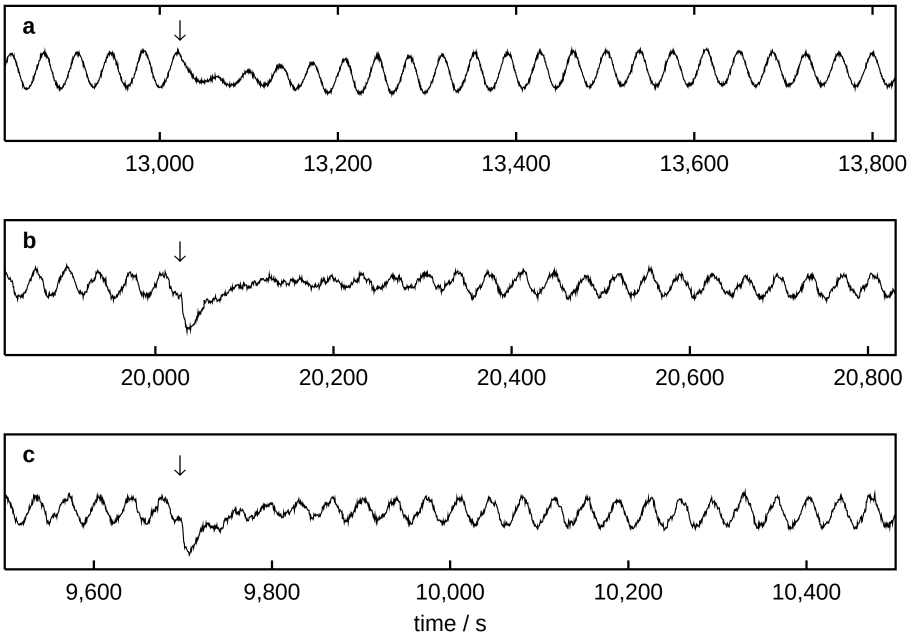

Fig(3). Square of the measured amplitude of the oscillations as a function of the mixed flow concentration of glucose. The transition point at 18.5mM correspond to an extracellular glucose concentration of 1.6mM. The kink at 23 mM can be explained by saturation of the membrane transporter.

The square root dependence of the amplitude of the oscillations shown in Fig(3) can be explained in two ways.

A: The cells oscillate in almost identical states. At the bifurcation point each cell passes a supercritical Hopf bifurcation. The point of saturation corresponds to a saturation of the glucose transporter.

B: Each cell is oscillating in its own limit cycle below as well as above the transition point. In a neighborhood of the transition point the state distribution is always very broad. Below the transition point the cells oscillate with random phases and with a constant average. Above the transition point the number of synchronized cells increase gradually. After the saturation point all cells are synchronized and the amplitude of the average concentration is almost constant.

We will use the modelling to investigate these two alternatives.




Fig(4) Quenching experiments (a) by addition of the optimal amount of glucose at the optimal phase and (b) by addition of the optimal amount of acetaldehyde at the optimal phase, and (c) addition of optimal amount of acetaldehyde 8° after the optimal phase.

## Modelling of the yeast cell population

We consider $N$ identical yeast cells with cytoplamic volume $\omega$ in an extracellular medium with volume $\Omega$. Metabolite concentration is $\mathbf{c}_i(t)$ in cell $i$ and $\mathbf{c}(t)$ in the medium. By neglecting the concentration changes caused by the flow of cells the kinetic equations for this system in a CSTR with specific flow rate $j$ and mixed flow concentrations $\mathbf{c}^f$ are

$$\begin{aligned}\dot{\mathbf{c}}_i &= \mathbf{F}(\mathbf{c}_i) + \mathbf{G}(\mathbf{c}_i, \mathbf{c}) \\ \dot{\mathbf{c}} &= -\frac{\omega}{\Omega}\sum_i \mathbf{G}(\mathbf{c}_i, \mathbf{c}) + \mathbf{E}(\mathbf{c}) + j(\mathbf{c}^f - \mathbf{c})\end{aligned}$$

where $\mathbf{F}(\mathbf{c}_i)$,$\mathbf{G}(\mathbf{c}_i, \mathbf{c})$ and $\mathbf{E}(\mathbf{c})$ describe the glycolytic reactions, the transport across the cellular membrane, and reactions in the extracellular medium respectively.

$\mathbf{G}$ is expanded to first order from the stationary state $(\mathbf{c}_I^s, \mathbf{c}^s)$ of a system where all $\mathbf{c}_i$ are identical to $\mathbf{c}_I^s$. The stationary state is defined by

$$\begin{aligned} \mathbf{F}(\mathbf{c}_I^s) + \mathbf{G}(\mathbf{c}_I^s, \mathbf{c}^s) &= \mathbf{0} \\ -\frac{N\omega}{\Omega}\mathbf{G}(\mathbf{c}_I^s, \mathbf{c}_s) + \mathbf{E}(\mathbf{c}^s) + j(\mathbf{c}^f - \mathbf{c}^s) &= \mathbf{0}. \end{aligned}$$

and the expansion gives

$$\begin{aligned} \mathbf{G}(\mathbf{c}_i, \mathbf{c}) &= \mathbf{G}(\mathbf{c}_I^s, \mathbf{c}^s) + \left.\frac{\partial \mathbf{G}}{\partial \mathbf{c}_i}\right|_{(\mathbf{c}_I^s, \mathbf{c}^s)} (\mathbf{c}_i - \mathbf{c}_I^s) + \left.\frac{\partial \mathbf{G}}{\partial \mathbf{c}}\right|_{(\mathbf{c}_I^s, \mathbf{c}^s)} (\mathbf{c} - \mathbf{c}^s) \\ &= \mathbf{G}(\mathbf{c}_I^s, \mathbf{c}^s) - \mathbf{D}_I(\mathbf{c}_i - \mathbf{c}_I^s) + \mathbf{D}_E(\mathbf{c} - \mathbf{c}^s). \end{aligned}$$

In this approximation the kinetic equations are

$$\begin{aligned} \dot{\mathbf{c}}_i &= \mathbf{F}(\mathbf{c}_i) + \mathbf{G}(\mathbf{c}_I^s, \mathbf{c}^s) - \mathbf{D}_I(\mathbf{c}_i - \mathbf{c}_I^s) + \mathbf{D}_E(\mathbf{c} - \mathbf{c}^s) \\ \dot{\mathbf{c}} &= \frac{\omega}{\Omega}\sum_i(-\mathbf{G}(\mathbf{c}_I^s, \mathbf{c}^s) + \mathbf{D}_I(\mathbf{c}_i - \mathbf{c}_I^s)) - \frac{N\omega}{\Omega}\mathbf{D}_E(\mathbf{c} - \mathbf{c}^s) + \mathbf{E}(\mathbf{c}) + j(\mathbf{c}^f - \mathbf{c}) \end{aligned}$$

The order of the system of kinetic equations is $(N+1)M$ where $M$ is the number of metabolites.

This order can be considerably reduced (to $2N+M$) by assuming that each cell embedded in its environment is oscillating close to a supercritical Hopf bifurcation, and that the state of each cell remains close to the oscillatory plane in the concentration space. Close to the Hopf point $\mu = 0$ the state of the cells can be described by complex amplitudes $z_i$ defined by

$$\mathbf{c}_i(t) = \mathbf{c}_I^s + z_i(t)\mathbf{u} + \overline{z_i}(t)\overline{\mathbf{u}}$$

where $\mathbf{u}$ and $\overline{\mathbf{u}}$ are the right eigenvectors of the Jacobian matrix at the bifurcation point with eigenvalues $\pm\imath\omega_0$. The corresponding left eigenvectors $\mathbf{u}^*$ and $\overline{\mathbf{u}}^*$ satisfy orthonormality relations $\mathbf{u}^* \cdot \mathbf{u} = 1$ and $\mathbf{u}^* \cdot \overline{\mathbf{u}} = 0$.

Defining $\mathbf{c} = \mathbf{c}^s + \mathbf{x}$, neglecting the extracellular reactions and flow and assuming that the transport across the membrane is passive with transport coefficient $\mathbf{D}$ a normal form representation of the kinetic equations becomes

$$\begin{aligned} \dot{z}_i &= (\imath\omega_0 + \sigma\mu)z_i + gz_i|z_i|^2 + \mathbf{u}^* \cdot \mathbf{D} \cdot (\mathbf{x} - \mathbf{x}_i) \\ &= (\imath\omega_0 + \sigma\mu)z_i + gz_i|z_i|^2 + \mathbf{u}^* \cdot \mathbf{D} \cdot (\mathbf{x} - \mathbf{u}z_i - \overline{\mathbf{u}}\overline{z_i}) \\ \dot{\mathbf{x}} &= \frac{N\omega}{\Omega}\mathbf{D}(\frac{1}{N}\sum_i \mathbf{x}_i - \mathbf{x}) \\ &= \frac{N\omega}{\Omega}\mathbf{D}\frac{1}{N}\sum_i(\mathbf{u}z_i + \overline{\mathbf{u}}\overline{z_i} - \mathbf{x}) \end{aligned}$$

where $\sigma$ and $g$ are complex, system dependent parameters. Together with the transformation from concentration space to amplitude space the system dependent coefficients can be calculated explicitly for a given kinetic model [8]. For the yeast system it is often assumed that acetaldehyde is the only synchronizing species. If there is only one synchronizing species W the diagonal matrix $\mathbf{D}$ describing the transport through the membrane has only one component $D_w$ in the diagonal and the equations can be simplified to

$$
\begin{aligned}
\dot{z}_i &= (\imath\omega_0 + \sigma\mu) z_i + g z_i |z_i|^2 + u_w^* D_w (w - w_i) \\
&= (\imath\omega_0 + \sigma\mu) z_i + g z_i |z_i|^2 + u_w^* D_w (w - u_w z_i - \overline{u_w z_i}) \\
\dot{w} &= \frac{N\omega}{\Omega} D_w \Big(\frac{1}{N} \sum_i w_i - w\Big) \\
&= \frac{N\omega}{\Omega} D_w \frac{1}{N} \sum_i (u_w z_i + \overline{u_w z_i} - w)
\end{aligned}
$$

where $w_i$ is the W component of $\mathbf{x}_i$ and $w$ is the W component of $\mathbf{x}$. In the limit $\frac{N\omega}{\Omega} D_w \to \infty$ we have $\frac{1}{N}\sum_i (u_w z_i + \overline{u_w z_i} - w) \to 0$ so that $w = \frac{1}{N}\sum_i (u_w z_i + \overline{u_w z_i})$ after a short transient. This is the mean field limit as originally described by Kuramoto [9]. In this limit the concentrations of W inside each cell is equal to $w + w^s$. If furthermore the distribution is narrow the dynamics of the population corresponds to the dynamics of a simple supercritical Hopf bifurcation as described by the Stuart-Landau equations

$$
\dot{z} = (\imath\omega_0 + \sigma\mu) z + g z |z|^2.
$$

Using: $z = Re^{\imath\Theta}$ the solution to this equation is

$$
\begin{aligned}
R(t) &= \frac{R_s}{\sqrt{1 + (\frac{R_s^2}{R_0^2} - 1) e^{-2g' R_s^2 t}}} \\
\Theta(t) &= (\omega_{ss} - g'' R_s^2) t - \frac{g''}{2g'} \log\Big(1 + \Big(\frac{R_0^2}{R_s^2} - 1\Big)\big(1 - e^{-2g' R_s^2 t}\big)\Big)
\end{aligned}
$$

where $g = g' + \imath g''$, $R_s = R(\infty)$, $R_0 = R(0)$, $\Theta_0 = 0$ and $\omega_{ss} = \omega_0 + \mu\sigma''$.

In addition to the slow modes from the bifurcation a complex chemical system may have slow hyperbolic modes from the chemical mechanism which may contribute to the dynamics at the chosen operating point. Such modes may to lowest order be described by an amplitude $p$ satisfying an additional amplitude equation

$$
\dot{p} = \lambda p.
$$

To higher order, coupling terms between the $z$ and $p$ modes have to be taken into account [10].

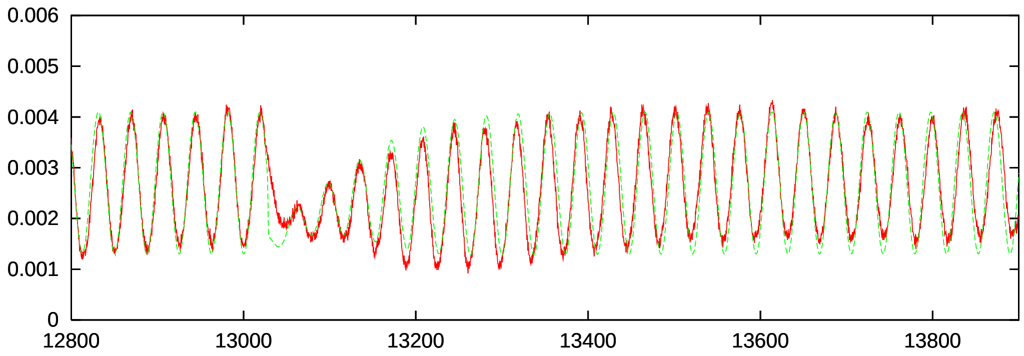
The canonical transformation from amplitude space to concentration space is

$$
\begin{aligned}
\mathbf{c} &= \mathbf{c}^s + \mathbf{u} z + \overline{\mathbf{u} z} + v r + \mathbf{h}_{110} |z|^2 + \mathbf{h}_{200} z^2 + \overline{\mathbf{h}_{200}}\,\overline{z}^2 \\
&+ \mathbf{h}_{120} |z|^2 z + \overline{\mathbf{h}_{120}} |z|^2 \overline{z} + \mathbf{h}_{300} z^3 + \overline{\mathbf{h}_{300}}\,\overline{z}^3 + \mathbf{b} w
\end{aligned}
$$

where $\mathbf{h}_{ijk}$ and $\mathbf{b}$ are $M$ dimensional vectors. By substituting $z = Re^{\imath\Theta}$ in this expression, keeping terms to second order and using a linear measuring fuction $y = \mathbf{h}^T \cdot \mathbf{c}$ the signal has the universal form

$$
y(t) = y_0 + R\cos(\Theta + \Theta_0) + aR^2 \cos(2(\Theta + \Theta_0) + \Theta_d) + b e^{\lambda t}
$$

By fitting the adjustable parameters $y_0, R_s, R_0, g', \omega_{ss}, g'', \Theta_0, a, \Theta_d, b$ and $\lambda$ to the fluorescence signal from an experimental quenching of the yeast cell population an estimate of some of the parameters in the amplitude equations can be obtained (Fig.(5)).

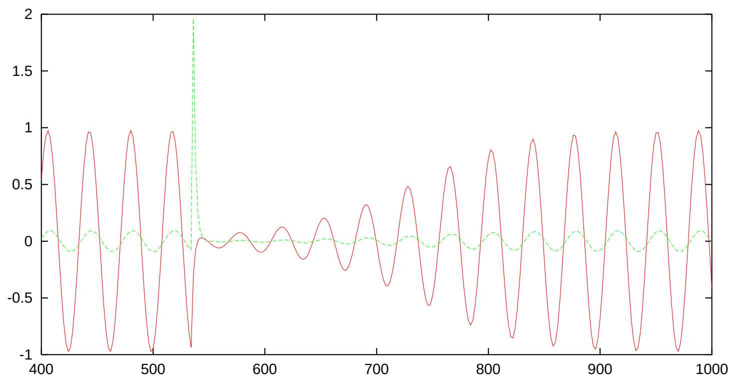



Fig(5) The full line shows the experimental signal and the dashed line the fitted signal.

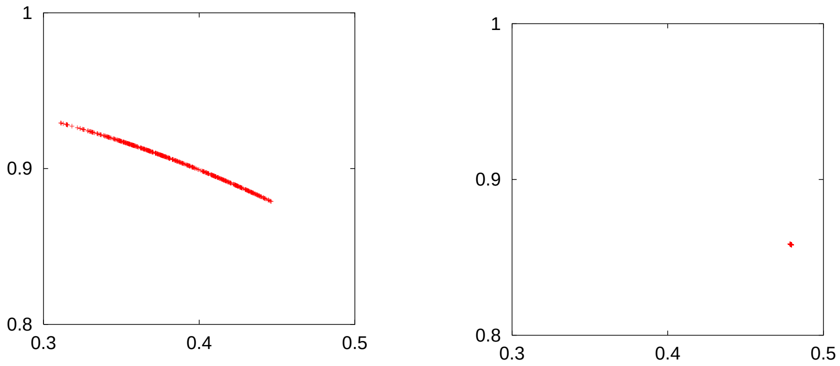
Setting $R(\infty) = 1$ the fittet values of the model parameters are $\omega_0 = 0.168\mathrm{s}^{-1}$, $g' = -0.014\mathrm{s}^{-1}$ and $g'' = 0.003\mathrm{s}^{-1}$. Except for a small interval just following the quenching the fit of the universal behavior of a system at a supercritical Hopf bifurcation to the experimental data is very good indicating that the cells to a large extent are synchronized with a narrow state distribution. In the mean field limit it can be shown that the state for which all cells oscillate with the same amplitude and phase is a stable solution. This is also true for the present system in spite of the fact that $\frac{N\omega}{\Omega}$ is small.

By using the fitted values of the parameters and the realistic value of $\frac{N\omega}{\Omega} = 0.05$ the dynamics of a yeast cell population can be simulated numerically for selected values of the remaining parameters. A quenching is simulated by an addition of the substance W to the extracellular medium at a phase and in an amount able to momentarily stop the oscillations.

Fig.(6) shows the real part of the order parameter during a quenching together with the value of $w$ for a simulation of a population of identical cells with the following parameter values: $u_w = 0.050$, $u_w^* = 0.01$ and $D_w = 10$. It demonstrates that a quenching similar to the ones observed in the experiments can be obtained; this is even possible for a system where the variance has not yet converged to zero (Fig.(7)). This explains why we have never observed any deviation from the Hopf behaviour even if the quenching was repeated before the variance of the state distribution has converged to zero.



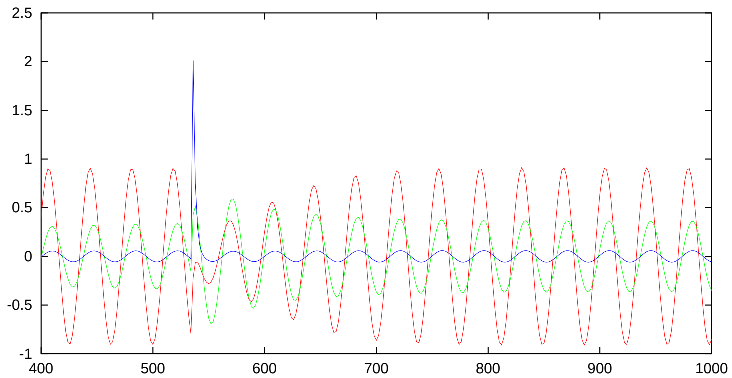

Fig(6) Simulation of a quenching experiment done by adding W to the external medium at the appropriate phase of the oscillation. The full line shows the real part of the order parameter $\frac{1}{N}\sum_i z_i$ and the dashed line shows the concentration af W in the external medium. The sharp peak at $t = 534$s shows the external addition of W.




Fig(7) Distribution of states of individual cells in $z$ space for the system from Fig(6) at $t = 500$s before the quenching and at $t = 1000$s after the return of the order parameter to the limit cycle. At this time the variance of the distribution is very small. The simulation demonstrates that a quenching will also look normal for a system with a small but nonvanishing width of the state distribution.

An elongated distribution will only exist as a transient for a population of identical cells, but in a real population the individual cells are not identical and small variations in size and chemical composition have to be taken into account. In this case the final state distribution is synchronized and with an elongation which is increasing with the variance in the population. If such a distribution is perturbed in a way so that the curved segment is moved to a position which includes the origo, the state of the cells remains distributed along the whole limit cycle for a long time during which the amplitude of the order parameter oscillations remain small. Such behavior has never been observed in our experiments and this indicates that the state distribution in our experiments remain quite narrow.

Close to a supercritical Hopf bifurcation the distance of each cell from the bifurcation point is the most important factor determining the cell dynamics. This distance is reflected in the value of $\mu_i$. By giving each cell its own $\mu$ the final attractor is a curved segment in state space circulating around the origo. Fig(8) shows the oscillations of the real part of $z_i$ in two cells with extreme values of $\mu$. The distribution of values of $\mu$ is flat in the range $-0.1g_r$ to $1.1g_r$ such that 10% of the cells are nonoscillatory. Nevertheless, these cells are seen to oscillate when all the cells are coupled.




Fig(8) Time series for oscillations of $\Re z_i$ in a population of nonidentical cells during a perturbation with W in the extracellular medium. The full line shows w. The dashed line shows $\Re z_i$ in a cell with $\mu = 1.1$ and the dotted line shows $\Re z_i$ for a cell with , $\mu = -0.1$. Note that the cell with $\mu = -0.1$ which by itself is nonoscillatory nevertheless is oscillating driven by the oscillations of $w$.

## Conclusion and perspectives

NONLINEAR DYNAMICS: We have made an experimental realization of a biological system with persistent Hopf dynamics which can be modelled as a large ($10^9$) population of Stuart-Landau oscillators. By the modelling we have confirmed our suggestion that the cells are tightly coupled at the experimental conditions and that the disappearence of the oscillations in the experiments is probably not a desynchronization phenomena. By simulations we have shown that such systems under different experimental conditions may desynchronize but then the quenching response is quite different and no experimental evidence of this has yet been obtained. An experimental challenge is to find a method to measure directly the oscillations of the individual yeast cells in the CSTR.

BIOLOGY: The realization of Hopf dynamics for a biological system implies that a rigorous connection exists between the measurable dynamic properties of the system, the structure of the kinetic network, and the values of the kinetic constants of the organism. This experimental method is a new way of studying the kinetic details of glycolysis as it actually takes place in a living cell. The synchronization of the cells shows that yeast cells have a hidden potential for cell communication which is expressed in this unnatural experimental environment, but might emerge spontaneously under natural stress conditions. It provides a potential for an evolutionary path to multicellular behavior for yeast cells and possibly also for other unicellular organisms.

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