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Biochemical kinetics in changing volumes

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The need of taking into account the change of compartment volume when developing chemical kinetics analysis inside the living cell is discussed. Literature models of a single enzymatic Michaelis-Menten process, glycolytic oscillations, and mitotic cyclin oscillations were tested with appropriate theoretical extension in the direction of volume modification allowance. Linear and exponential type of volume increase regimes were compared. Due to the above, in a growing cell damping of the amplitude, phase shift, and time pattern deformation of the metabolic rhythms considered were detected, depending on the volume change character.

The perfomed computer simulations allow us to conclude that evolution of the cell volume can be an essential factor of the chemical kinetics in a growing cell. The phenomenon of additional metabolite oscillations caused by the periodic cell growth and division was theoretically predicted and mathematically described. Also, the hypothesis of the periodized state in the growing cell as the generalization of the steady-state was formulated.

As it was already shown almost three decades ago, changes in volume, when not negligible compared to the initial volume, cannot be omitted in chemical kinetics considerations (Gingold, 1974). The growing scientific interest in quantitative modeling of intracellular chemical phenomena provokes one to return to this question. Many theoretical concepts, e.g.: metabolic control analysis, MCA, (Kacser & Burns, 1973; Burns *et al.*, 1985), dynamic optimal metabolic control, DOMC, (Giuseppin & van Riel, 2000), flux-oriented theory (Crabtree *et al.*, 1997) and biochemical systems theory (Ni & Savageau, 1996), and software tools, represented by: Stochsim (Morton-Firth & Bray, 1998; Morton-Firth *et al.*, 1999), Gepasi (Mendes, 1997; Mendes & Kell, 1998), E-Cell (Tomita *et al.*, 1999), and

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including internet services like Virtual Cell (Schaff *et al.*, 1997), are oriented to complex mathematical models of the intracellular chemical reality. Based on the ideas of the classic chemical kinetics *in vitro*, they do not pay sufficient attention to the problem of varying volume compartments, arising when growing cells are considered. To be more specific, in the analytical considerations of the kinetics, in the differential equation describing the evolution of concentration A in time t, at the rate of the process R, and the varying compartment volume Ω:

$$\frac{d}{dt}A = R - \frac{A}{\Omega}\frac{d}{dt}\Omega \tag{1}$$

the "diluting" component $-\frac{A}{\Omega}\frac{d}{dt}\Omega$ is very often omitted. Such omission, which is certainly valid in the case of a constant volume, in other situations, as it is seen in the simple one-equation example with $R = const$, can lead to essential differences in the analytical solution, and a numeric discrepancy (Fig. 1a, b, c) in normal conditions exceeding 50%, and in the extreme ones reaching 100% approximation error (for details see Appendix 1).

Usually, of course, more sophisticated kinetics models are required than the one presented above. Below, three such examples are considered and the question of the potential significance of the cellular volume change for the theoretical prediction of the process kinetics is discussed.

EXAMPLE MODELS

The first basic example, and due to broad practice — a very important one, is Michaelis-Menten process (Chaplin, 1990; Lee, 1992) in a closed system. In a varying volume compartment it may be described by the following set of equations:

$$\frac{d}{dt}S = -k_{1+}SE + k_{1-}\{SE\} - \frac{S}{\Omega}\frac{d}{dt}\Omega \tag{2}$$

$$\frac{d}{dt}E = -k_{1+}SE + k_{1-}\{SE\} + k_{2+}\{SE\} - \frac{E}{\Omega}\frac{d}{dt}\Omega \tag{3}$$

$$\frac{d}{dt}\{SE\} = k_{1+}SE - k_{1-}\{SE\} - k_{2+}\{SE\} - \frac{\{SE\}}{\Omega}\frac{d}{dt}\Omega \tag{4}$$

$$\frac{d}{dt}P = k_{2+}\{SE\} - \frac{P}{\Omega}\frac{d}{dt}\Omega \tag{5}$$

where: S, E, $\{SE\}$, P are the concentrations of substrate, enzyme, complex and product, respectively, and k_{1-}, k_{1+}, k_{2+} are reaction rate coefficients.

As a second example a two-cell model of synchronization of the glycolytic oscillations in yeast was considered (Bier *et al.*, 2000). After incorporation of the "dilution" components, the equations of the extended model look as follows:

$$\frac{d}{dt}G_1 = V_{in} - k_1 G_1 T_1 - \frac{G_1}{\Omega}\frac{d}{dt}\Omega \tag{6}$$

$$\frac{d}{dt}T_1 = 2k_1 G_1 T_1 - \frac{k_p T_1}{K_m + T_1} + \varepsilon(T_2 - T_1) - \frac{T_1}{\Omega}\frac{d}{dt}\Omega \tag{7}$$

$$\frac{d}{dt}G_2 = V_{in} - k_1 G_2 T_2 - \frac{G_2}{\Omega}\frac{d}{dt}\Omega \tag{8}$$

$$\frac{d}{dt}T_2 = 2k_1 G_2 T_2 - \frac{k_p T_2}{K_m + T_2} - \varepsilon(T_2 - T_1) - \frac{T_2}{\Omega}\frac{d}{dt}\Omega \tag{9}$$

where: G_1, G_2, T_1, T_2 denote the concentrations of glucose and ATP, respectively, V_{in}, k_1, k_p, K_m, ε are parameters of the model describing glucose inflow, phosphofructokinase activity, Michaelis-Menten kinetics of ATP disintegration, and coupling.

Discussion of additional modifications (due to the volume change) of partial process

rates, such as: $k_pT_1/(K_m+T_1)$, was omitted, which does not limit the universal applicability of the considerations.

As a third example, a minimal cascade model for the mitotic oscillator was investigated (Goldbeter, 1991). Supplemented by the "dilution" components, it my be written as:

$$\frac{d}{dt}C = v_i - v_d X \frac{C}{K_d + C} - k_d C - \frac{C}{\Omega}\frac{d}{dt}\Omega \quad (10)$$

$$\frac{d}{dt}M = V_1 \frac{(1-M)}{K_1 + (1-M)} - V_2 \frac{M}{K_2 + M} - \frac{M}{\Omega}\frac{d}{dt}\Omega \quad (11)$$

$$\frac{d}{dt}X = V_3 \frac{(1-X)}{K_3 + (1-X)} - V_4 \frac{X}{K_4 + X} - \frac{X}{\Omega}\frac{d}{dt}\Omega \quad (12)$$

with:

$$V_1 = \frac{C}{K_c + C} V_{M1} \quad (13)$$

$$V_3 = M V_{M3} \quad (14)$$

where: C is cyclin concentration, M and X, respectively, the fraction of active cdc2 kinase and cyclin protease, v_i, v_d, K_d, k_d, K_c, K_1, K_2, K_3, K_4, V_2, V_4, V_{M1}, V_{M3}, are parameters of the model characterizing cyclin synthesis, specific and non-specific cyclin degradation, cyclin activity, and the kinetics of the enzymes involved.

Here, additional modification of partial process rates was disregarded, too (as in the second example).

SIMULATIONS

Numerical simulations of the example models were done to investigate the role of the compartment volume change in the theoretical modeling of the kinetics of chemical processes.

The assumed volume change was described by three different regimes: constant, i.e. $\Omega = \Omega_0$, linear, i.e. $\Omega = \Omega_0(1+t/\tau)$, and exponential, i.e. $\Omega = \Omega_0 \exp(\ln 2\ t/\tau)$, with the arbitrary value of the initial volume Ω_0, and chosen time of volume doubling τ.

In the case of the Michaelis-Menten model, simulations of Eqns. 2–5 were done in the range of t = 0–5400 s. For the assumed modes of the volume growth, characterized by τ = 5400 s, two literature sets of kinetic parameters were applied describing a slow and a fast enzymatic processes. The parameters

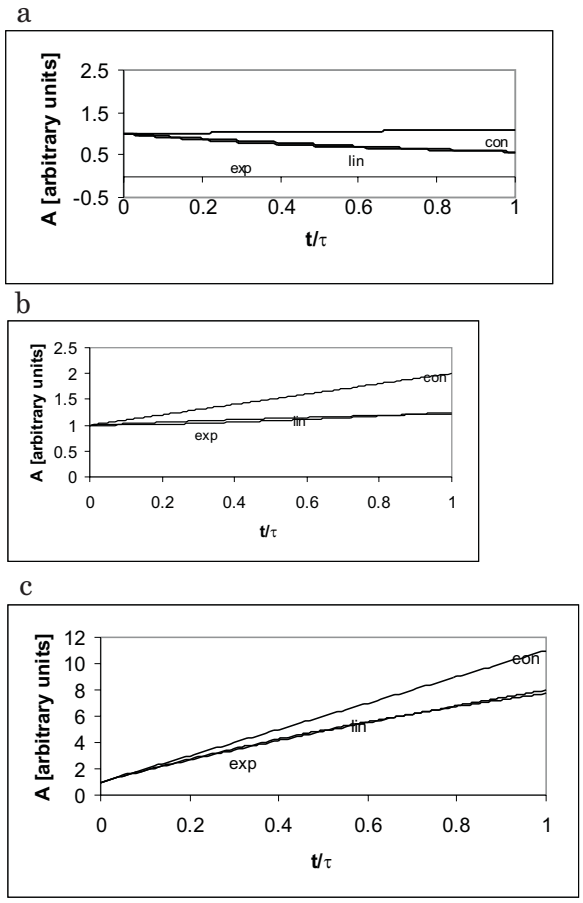


Figure 1. Evolution of concentration *A* of a compound processed by a constant rate chemical process in a varying volume compartment, predicted by Eqns. A.1.3–5, for initial concentration A_0 = 1 [*a.u.*] (typically, [μM] or [mM]) and chosen inflow $R\tau$. a: $R\tau$ = 0.1 [*a.u.*], b: $R\tau$ = 1 [*a.u.*], c: $R\tau$ = 10 [*a.u.*].

Curves were plotted for different volume Ω change regimes: con – constant, i.e. $\Omega = \Omega_0$, lin – linear, i.e. $\Omega = \Omega_0 (1+t/\tau)$, exp – exponential, i.e. $\Omega = \Omega_0 \exp(\ln 2\ t/\tau)$, with arbitrary values of the initial volume Ω_0, and characteristic time τ.

Table 1. Parameters of the simulation of the extended Michaelis-Menten model, with varying volume allowed (based on: Chaplin, 1990; Lee, 1992).

Parameter	Slow process	Fast process
$S(0)$	0.01 M	0.1 M
$E(0)$	$1E-7$ M	0.01 M
$\{SE\}(0)$	0	0
$P(0)$	0	0
k_{1+}	$1E+6$ [1/Ms]	40 [1/Ms]
k_{1-}	1000 [1/s]	5 [1/s]
k_{2+}	10 [1/s]	0.5 [1/s]
Ω_0	*arbitrary value*	*arbitrary value*
τ	5400 s	5400 s

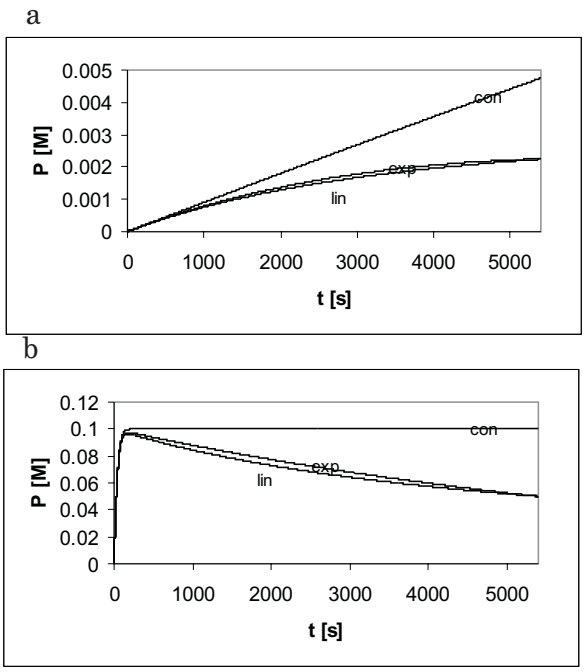


Figure 2. Time pattern of product P production by a relatively a: — slow, and b: — fast, Michaelis-Menten process in a varying volume compartment (Eqns. 2–5).

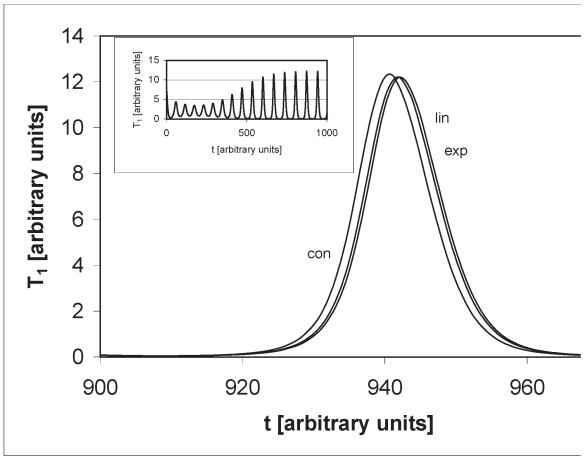
Curves were plotted for different volume Ω change regimes: con — constant, i.e. $\Omega = \Omega_0$, lin — linear, i.e. $\Omega = \Omega_0 (1+t/\tau)$, exp — exponential, i.e. $\Omega = \Omega_0 \exp(\ln 2\, t/\tau)$, with an arbitrary value of the initial volume Ω_0, and characteristic time τ = 5400 s. Other parameters are presented in Table 1.

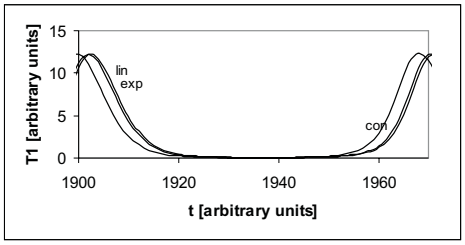
used are presented in Table 1. The results are shown in Fig. 2.

In the case of the synchronization model, simulations of Eqns. 6–9 were done in the range of t = 0–5400 *a.u.* (arbitrary units). For the assumed modes of volume growth, characterized by τ = 5400 *a.u.*, three different values of inflow rate: V_{in} = 0.001, 0.01, and 0.36 *a.u.* were checked to observe the effects of the modified frequency of oscillations, and for the literature value V_{in} = 0.36 *a.u.*, chosen regions of time: t = 900–970, 1900–1970, 5330–5400 *a.u.* were analyzed in more detail. The parameters applied are presented in Table 2. In practice, units *a.u.* mean second, mM, or their combinations. The results are shown in Figs. 3–5.

In the case of the mitotic model, simulations of Eqns. 10–14 were performed in the range of t = 0–100 min. The assumed volume regimes were characterized by τ = 25 min, and they were cyclically repeated every 25 min. Two sets of conditions were applied: the literature standard with v_i = 0.025 μM/min and k_d = 0.01 [1/min], or a test set with v_i = 0.037 μM/min and k_d = 0 [1/min] — almost completely removing the observed effect of exponential volume variation. The parameters applied are presented in Table 3. The results are shown in Fig. 6.

In all simulations the Gepasi 3.21 (Mendes, 1993) software was used.





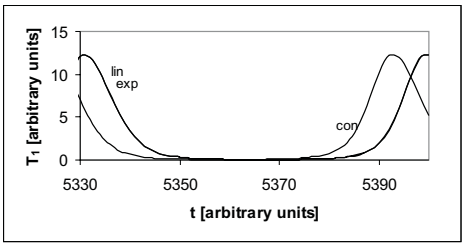


Figure 3. Fast ATP (T_1) oscillations predicted by the synchronization model in a varying volume compartment (Eqns. 6–9), for V_{in} = 0.36 [*a.u.*], and different intervals of time — a: t = 900–970 [*a.u.*], b: t = 1900–1970 [*a.u.*], c: t = 5330–5400 [*a.u.*].

Curves were plotted for different volume Ω change regimes: con — constant, i.e. $\Omega = \Omega_0$, lin — linear, i.e. $\Omega = \Omega_0(1+t/\tau)$, exp — exponential, i.e. $\Omega = \Omega_0 \exp(\ln 2\, t/\tau)$, with an arbitrary value of the initial volume Ω_0, and characteristic time τ = 5400 [*a.u.*]. In the inset (Fig. 2a) the range t = 0–1000 [*a.u.*] is shown. Other parameters are presented in Table 2.

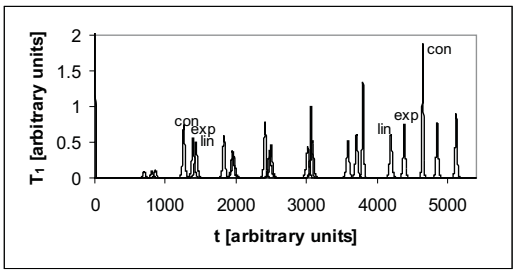


Figure 4. Slow ATP (T_1) oscillations predicted by the synchronization model in a varying volume compartment (Eqns. 6–9), in the range t = 0–5400 [*a.u.*], for V_{in} = 0.01 [*a.u.*].

Curves were plotted for different volume Ω change regimes: con — constant, i.e. $\Omega = \Omega_0$, lin — linear, i.e. $\Omega = \Omega_0(1+t/\tau)$, exp — exponential, i.e. $\Omega = \Omega_0 \exp(\ln 2\, t/\tau)$, with an arbitrary value of the initial volume Ω_0, and characteristic time τ = 5400 [*a.u.*]. Other parameters are presented in Table 2.

RESULTS

The simple case of a constant rate chemical process shows that variation of the compartment volume can quantitatively modify the results of simulations (Fig. 1a, b, c). After the time in the order of half of that needed for volume doubling, the observed decrease in the process production is in the order of tens of percentage points, independently of the inflow magnitude, and the volume growth type. Moreover, the effect increases with time. The case presented in Fig. 1b is probably not far from the cell reality, when the rate of the process balances volume growth and the related effect of dilution, so that the concentration of the products remains approximately constant.

Also in the case of a single Michaelis-Menten process a significant discrepancy is seen between the numeric results obtained for the simulations with and without volume change. Such a difference arises both in the case of a relatively slow process (Fig. 2a), and a fast one (Fig. 2b). The assumed value of τ = 5400 s (90 min) was inspired by the yeast cell volume growth data (Woldringh *et al*, 1993). So it is seen, for example, that an enzymatic processes lasting half of the cellular lifetime can really "feel" the cell volume expansion, which will be manifested as a serious (20%) decrease in the product concentration. The effect weakly depends on the volume change regime.

The theoretically predicted long term oscillations of glucose and ATP (Fig. 3a, b, c) at

Table 2. Parameters of the simulation of the extended two-cell model of synchronization of the glycolytic oscillations in yeast, with varying volume allowed (based on: Bier *et al.*, 2000).

Parameter	Value [*a.u.*]
$G_1(0)$	6.6
$G_2(0)$	10.3
$T_1(0)$	7.6
$T_2(0)$	0.41
V_{in}	0.001, 0.01, 0.36
k_1	0.02
k_p	6
K_m	13
ε	0.01
Ω_0	*arbitrary value*
τ	5400

the parameters taken from (Bier *et al.*, 2000) reveal a phase shift between the results of the simple (con) and extended (lin, or exp) model, but no significant change in the amplitude. The effect does not depend on the mode of volume growth.

Also at decreased V_{in} (Fig. 4), the decelerated and decreased oscillations predicted by the simple model require some phase shift to take into account the effect related to cell volume change. This effect depends on the type of volume change.

The lack of ATP at low V_{in} clearly shows (Fig. 5) the differences between the prognosis of creeping glucose accumulation in a non-growing (con) and growing cell (lin, exp). For those cases the type of volume change is not critical.

Another model tested (Fig. 6a, b) shows a strong influence of the type of volume growth on mitotic oscillations. Allowance for volume change, especially of the exponential type (exp), can influence the results so much that even damping of the oscillations can be observed, and a modification of the essential parameters may be needed to return close to the "literature" pattern (Fig. 6a) of the modeled processes (Fig. 6c).

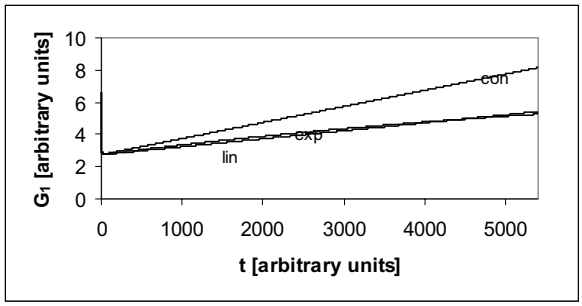


Figure 5. Glucose (G_1) creep predicted by the synchronization model in a varying volume compartment (eqns. 6–9), in the range t = 0–5400 [*a.u.*], for V_{in} = 0.001 *a.u.*.

Curves were plotted for different volume Ω change regimes: con – constant, i.e. $\Omega = \Omega_0$, lin – linear, i.e. $\Omega = \Omega_0(1+t/\tau)$, exp – exponential, i.e. $\Omega = \Omega_0 \exp(\ln 2\, t/\tau)$, with an arbitrary value of the initial volume Ω_0, and characteristic time t = 5400 *a.u.*. Other parameters are presented in Table 2.

DISCUSSION

Summing up, evidence is presented that volume change can give some quantitative amendment of the output of chemical kinetics investigations in a growing cell. Depending on the process type, damping of the ampli-

Table 3. Parameters of the simulation of the extended minimal cascade model for the mitotic oscillator, with varying volume allowed (based on: Goldbeter, 1991).

Parameter	Value
$C(0)$	0.01 μM
$M(0)$	0.01
$X(0)$	0.01
v_i	0.025 μM/min *, 0.037 μM/min **
v_d	0.25 μM/min
K_d	0.02 μM
k_d	0.01 [1/min] *, 0 **
K_c	0.5 μM
K_1, K_2, K_3, K_4	0.005
V_2	1.5
V_4	0.5
V_{M1}	3
V_{M3}	1
Ω_0	*arbitrary value*
τ	25 min

*Simulations for standard parameters; **simulation for modified parameters.

tude, phase shift, or time pattern deformation can be detected. The significance of the volume change regime is also dependent on the process class, especially for natural rhythms.

The most important theoretical consequence of incorporation of volume changes into kinetics considerations seems to be the phenomenon of volumetrically forced oscillations. The matter is presented below.

First, let us consider a simple "mathematical" cell (Fig. 7a), with one molecule of interest in the beginning (initial conditions). From time $t = 0$ two analyzed molecules (per lifetime, per initial volume) are synthesized. For simplicity assume that the lifetime of such a cell equals two time units. During the first unit of time our cell produces one new molecule. In the second temporal interval it produces another molecule, a portion of the "new volume" equal to the initial one, and yet another molecule, created in the new space. At the end of its life it has doubled its volume and contains four molecules. Then, the cell divides into halves, each with equal number of molecules – two.

If the process continues in the same manner, after the next division each cell should posses 2.5 "mathematical" molecules. The next cellular generations will have 2.75, 2.875 ... 3 molecules. Here, 3 is the limiting value. When this virtual experiment is repeated starting with a different initial number of molecules, in the appropriately late generations the cells will always contain three mole-

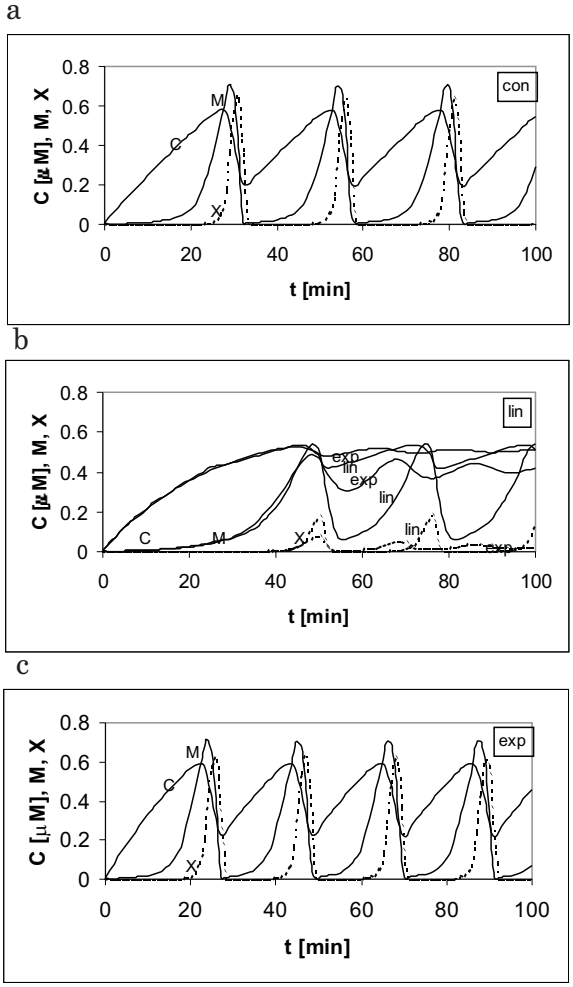


Figure 6. Oscillations in the mitotic model (Eqns. 10–14), for different volume change regimes — a: con — constant, i.e. $\Omega = \Omega_0$, b: lin — linear, i.e. $\Omega = \Omega_0(1+t/\tau)$, c: exp — exponential, i.e. $\Omega = \Omega_0 \exp(\ln 2\, t/\tau)$, with an arbitrary value of the initial volume Ω_0, and characteristic time τ = 25 [*min*], repeated every 25 minutes.

Other parameters with v_i = 0.025 μM/min and k_d = 001 1/min, for cases **a** and **b**, were taken from a literature standard (Goldbeter, 1991), but with v_i = 0.037 μM/min and k_d = 001 [1/min], for case **c**, were chosen by modification of the above to avoid amplitude decrease. C, M, X – mean cyclin concentration, fraction of active cdc2 kinase and cyclin protease, respectively. Other parameters are presented in Table 3.

cules after division. Of course, 3 is not a new mathematical constant. Simply this is the assumed number of molecules produced by a single cell during its life, and depends on the type of cells.

It is also easy to show that the number of molecules during the "mathematical cell-cycle" stabilizes too, and tends to 4 in the first half, and to 6 in the second, so stable oscillations of concentration, between 3 and 4 molecules per initial volume, should be expected asymptotically during the cell cycle (Fig. 7b).

Can real cells exhibit any similarities to such "mathematical" cells? The answer is yes, and the argument is the dynamical stability of their composition.

The proposed periodic regime of stabilization is in opposition to the well known "steady-state behavior", for example of Michaelis-Menten type processes (Schulz, 1994). It is very easy to establish "steady-state" conditions *in vitro* but may be hard to find them in the interior of growing and dividing cells. It applies not only when the observation time is of the order or shorter than one of the characteristic times of reaching the enzymatic equilibrium, but also when it is of the order of the lifetime of the single cell. The basic reason is the change of the volume of the reaction compartment with time and the related virtual time-dependent outflow $\left(-\frac{A}{\Omega}\frac{d}{dt}\Omega\right)$ of chemical components. In such a case the levels of all reactants cannot stabilize simultaneously and the idea of the steady-state should be verified.

Although examples of oscillating biological or chemical processes, i.e. systems not operating in the steady-state mode, are already well known, such as: Belousov-Zhabotinsky reaction (Cross *et al.*, 1997), or theoretical models: Lotka-Volterra, Brusselator, and Oregonator (Pojman, 1999), it is reasonable to consider the oscillations forced by periodic volume changes separately. This is because the driving force of the oscillations in the above cited classic periodic processes is autocatalysis, which differs strongly from the periodicity caused by volume growth and division, which is a non-autocatalytic effect.

An analytical introduction to the discussed concept of periodical stabilization of reagent concentrations in subsequent generations of varying volume cells, further called perio-

dization, may be simply presented in the case of a Michaelis-Menten process.

By addition of Eqns. 3 and 4, and incorporation of the external enzyme inflow q_E, one obtains:

$$\frac{d}{dt}E^* = q_E - \frac{E^*}{\Omega}\frac{d}{dt}\Omega \qquad (15)$$

where:

$$E^* = E + \{SE\} \qquad (16)$$

is the total enzyme concentration.

It is clearly seen from Eqn. 15 that even if the flux q_E of enzyme is zero, the concentration E^* changes in time due to the change in volume Ω.

The solution of Eqn. 15 gives

$$E^*(t) = \frac{\int_0^t q_E(\xi)\Omega(\xi)d\xi + \Omega(0)E^*(0)}{\Omega(t)} \qquad (17)$$

with initial values $\Omega(0)$ and $E^*(0)$ of the volume and the total enzyme concentration; ξ is an integration variable.

Let us imagine a cell (single compartment) with the lifetime τ, and the volume (which contains an enzyme of the total concentration governed by Eqn. 17) increasing from $\Omega(0)$ to

$$\Omega(\tau) = 2\Omega(0) \qquad (18)$$

then dividing into halves. When considering future generations of such cells, all obeying Eqns. 17 and 18, we may suppose that daughter cells inherit from the parent cells the concentrations of reactants. In other words, the final concentration in the parent cell becomes the initial concentration in the daughter cells. In agreement with this heritability law and using the net time t' in the range $(0–\tau)$ for each generation, one can write down the recurrence:

$$E^*_{n+1}(0) = E^*_n(\tau) \qquad (19)$$

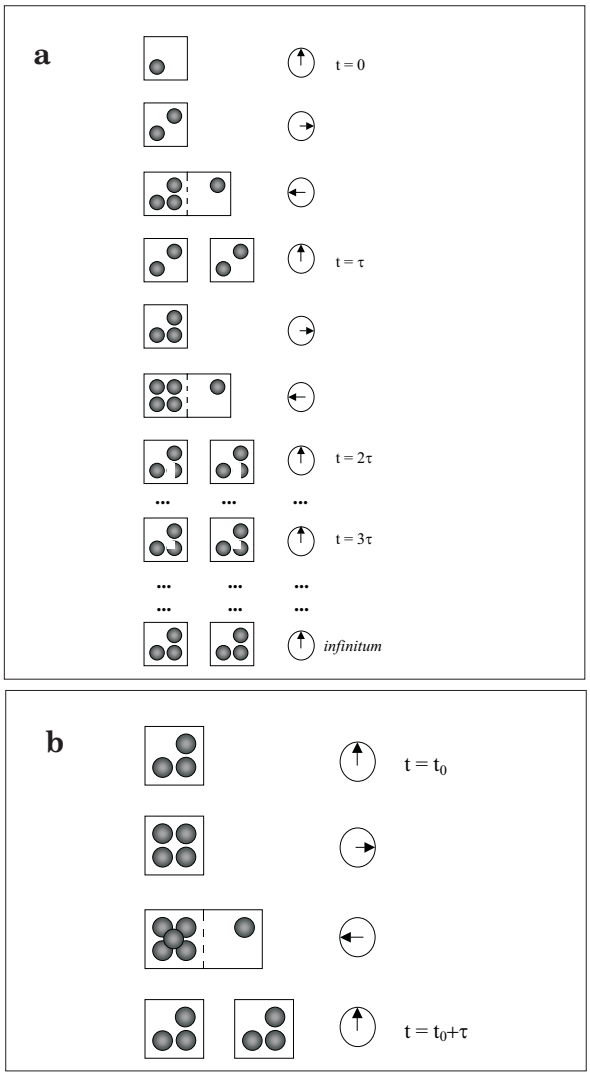


Figure 7. "Mathematical cell-cycle".

a: The cell has one molecule of interest in the beginning, at time $t = 0$, then switches its metabolism from an arbitrary preliminary mode to the given regime in which two analyzed molecules are synthesized per lifetime τ, per initial volume. The molecules are synthesized uniformly. Doubling of the volume takes place during the second half of the cycle. **b**: Asymptotical repetitive behavior.

with n = 1,2,3... numbering the cellular generations.

With the assumption that in each cellular generation the time pattern of the flux $q_E(\xi)$ is the same, Eqns. 17, 18 and 19 lead to the following series of initial and final values:

$$E^*_{n+1}(0) = \frac{\int_0^\tau q_E(\xi)\Omega(\xi)d\xi}{2\Omega(0)}\left[1 + \left(\frac{1}{2}\right)^1 + \ldots + \left(\frac{1}{2}\right)^{n-1}\right] + \left(\frac{1}{2}\right)^n E^*_1(0) \qquad (20)$$

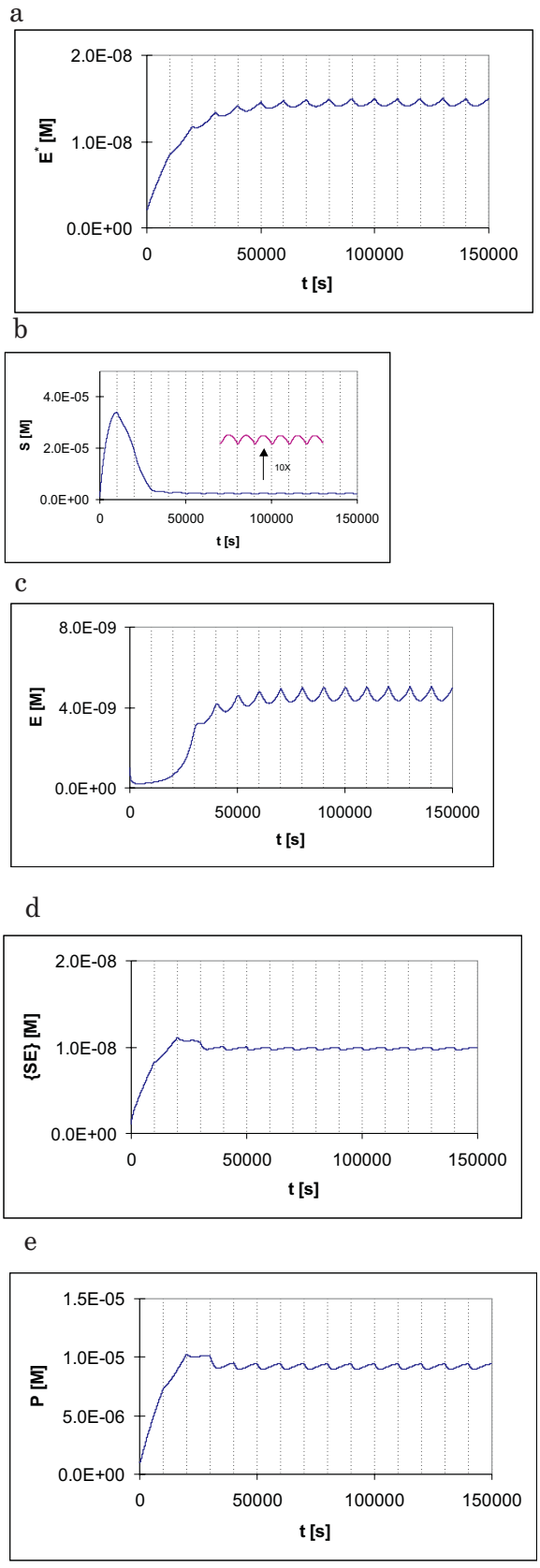


Figure 8. Periodization. Global time *t* patterns of a Michaelis-Menten reaction in 15 successive generations of cells, calculated by using Eqns. 2–5 with definition 16, final concentration heritability law, and Euler algorithm.

$$E^*_{n+1}(\tau)=\frac{\int_0^{\tau} q_E(\xi)\Omega(\xi)d\xi}{2\Omega(0)}\left[1+\left(\frac{1}{2}\right)^1+\ldots+\left(\frac{1}{2}\right)^n\right]+\left(\frac{1}{2}\right)^{n+1}E^*_1(0) \tag{21}$$

which at $n \to \infty$ reach the limit

$$E^*_\infty(0)=E^*_\infty(\tau)=\frac{\int_0^{\tau} q_E(\xi)\Omega(\xi)d\xi}{\Omega(0)} \tag{22}$$

It means that at an invariant time pattern of enzyme flux the dependence of the total enzyme concentration on time tends in the successive generations to the invariant periodic course from $E^*_\infty(0)$ to $E^*_\infty(\tau)=E^*_\infty(0)$ described by the expression

$$E^*_\infty(t')=\frac{\int_0^{\tau} q_E(\xi)\Omega(\xi)d\xi}{\Omega(t')}\left[1+\frac{\int_0^{t'} q_E(\xi)\Omega(\xi)d\xi}{\int_0^{\tau} q_E(\xi)\Omega(\xi)d\xi}\right] \tag{23}$$

which is not dependent on the starting value $E^*_1(0)$. This phenomenon we call periodization. Of course, $E^*(t')$ would be periodical from the beginning (first generation) if it starts at $E^*_1(0)=E^*_\infty(0)$. This predicted behavior is clearly confirmed when one simulates Eqns. 2–5 with incorporation of substrate

Figure 8. Continued.

Linearly changing volume $\Omega(t')=\Omega(0)\left(1+\frac{t'}{\tau}\right)$ with the net time $t'=t-n\tau$, for the n = 1,2,3,...15th generation was assumed. Substrate and enzyme external inflows are incorporated: q_s, q_E, and proportional product outflow $-\alpha P$. The starting values and other constant parameters are: $S_1(0)$ = 1*E*–6 M, $E_1(0)$ = 1*E*–9 M, $\{SE\}_1(0)$ = 1*E*–9 M, $P_1(0)$ = 1*E*–6 M, k_{1-} = 1*E*–1 [1/s], k_{1+} = 1*E*+6 [1/Ms], k_{2+} = 1 [1/s], τ = 10000 s, and α = 1*E*–3 [1/s], q_s = 1*E*–8 M/s, q_E = 1*E*–12 M/s. On separate charts: **a**. Concentration of the total enzyme E^*. **b**. Concentration of the substrate *S*. **c**. Concentration of the free enzyme *E*. **d**. Concentration of the complex $\{SE\}$. **e**. Concentration of the product *P*.

and enzyme external inflows, a proportional product outflow, def. 16, and the defined parameters (Fig. 8a). As it can be seen, similarly to the total enzyme also the substrate, free enzyme, complex and product undergo periodization (Fig. 8b–e).

Some of the oscillations already described as those of NADH (Pye, 1971) or Ca^{2+} (Cuthbertson & Cobbold, 1985) are more frequent, and higher in amplitude than the volumetrically forced ones. Some small periodicity can be seen in a report of individually measured internal pH along the cell cycle in *Schizosaccharomyces pombe* (Karagiannis & Young, 2001).

A separate question is: are volumetrically forced oscillations desired in the intracellular system? If not, how can the cell avoid them? From the mathematical point of view, the easiest way to reach such a goal is to expand exponentially. Then the term $(d\Omega/dt)/\Omega$ remains constant, not producing periodic disturbance. So, if a given cell volume growths exponentially, the cell exhibits no periodization. Despite the above, constant volumetric dilution should still be considered.

According to our hypothesis the concept presented above may be applied, instead of the idea of the steady-state, for processes occurring in a repetitively varying volume compartment, which are very common in cells. Of course it needs further development to take into account more complex processes including transport and regulation. Both of them playing an important physiological role, obviously moderating the simple picture of periodization sketched above. Studying these procesess requires, however, different computational approach (Brownian dynamics and stochastic simulations, respectively). The model presented here applies only to cases which can be treated macroscopically.

A future investigation area related with periodization and cell volume variations may concentrate around the mechanisms and signaling of cell volume regulation and the effects of cell volume changes on cellular functions.

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APPENDIX 1

Let us consider a kinetic equation, describing the evolution of concentration A of a compound processed by a constant rate chemical process (R = *const*) in a varying volume compartment (Ω):

$$\frac{d}{dt}A = R - \frac{A}{\Omega}\frac{d}{dt}\Omega \qquad \text{(A.1.1)}$$

The solution of problem A.1.1 with the initial concentration A_0 can be written as:

$$A = \frac{A_0\Omega_0}{\Omega(t)} + R\frac{\int_0^t \Omega(\xi)d\xi}{\Omega(t)} \qquad \text{(A.1.2)}$$

where Ω_0 is the initial volume, ξ is an integration variable.

For special cases of constant volume, and linear or exponential growth, at the chosen characteristic period τ for the volume doubling, the above solution can be written in the distinct forms:

$$A_{con} = A_0 + R\tau\frac{t}{\tau} \qquad / \ \Omega = \Omega_0 / \qquad \text{(A.1.3)}$$

$$A_{lin} = \frac{A_0}{1+\frac{t}{\tau}} + R\tau \frac{\frac{t}{\tau} + \frac{1}{2}\left(\frac{t}{\tau}\right)^2}{1+\frac{t}{\tau}} \qquad /\Omega = \Omega_0(1+\frac{t}{\tau})/ \tag{A.1.4}$$

$$A_{\exp} = A_0 \exp(-\ln 2 \frac{t}{\tau}) + \frac{R\tau}{\ln 2}[1-\exp(-\ln 2 \frac{t}{\tau})]$$

$$/\Omega = \Omega_0 \exp(\ln 2 \frac{t}{\tau})/ \tag{A.1.5}$$

Defining the relative errors of approximation of the compound concentration at the volume change omission, e_{lin} and $e_{\exp}$, as:

$$e_{lin} = \frac{A_{con} - A_{lin}}{A_{lin}} \tag{A.1.6}$$

$$e_{\exp} = \frac{A_{con} - A_{\exp}}{A_{\exp}} \tag{A.1.7}$$

it is easy to calculate that for $t/\tau = 1$, and for the extreme rate conditions:

$$\left.\begin{aligned} e_{lin} &= 1 \\ e_{\exp} &= 1 \end{aligned}\right\} \qquad /R\tau << A_0/ \tag{A.1.8}$$

and

$$\left.\begin{aligned} e_{lin} &= 1/3 \\ e_{\exp} &= 2\ln 2 - 1 \end{aligned}\right\} \qquad /R\tau >> A_0/ \tag{A.1.9}$$