Biophysical Chemistry 245 (2019) 17–24

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: www.elsevier.com/locate/biophyschem

Robustness of frequency vs. amplitude coding of calcium oscillations during changing temperatures

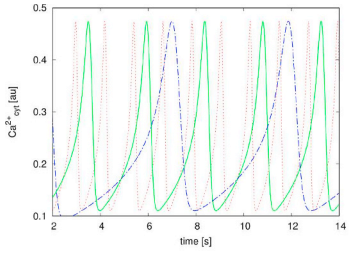
Luis Aguilera, Frank T. Bergmann, Giovanni Dalmasso, Sinan Elmas, Tobias Elsässer, Ruth Großeholz, Pascal Holzheu, Priyata Kalra, Ursula Kummer*, Sven Sahle, Nadine Veith

BioQuant/COS, Heidelberg University, Heidelberg, Germany

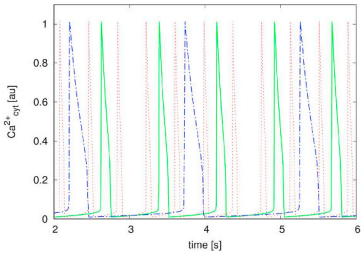
HIGHLIGHTS

- New methodology to fit oscillatory time series.
- Analysis of selectivity of decoding of frequency vs amplitude encoded calcium oscillations.
- Analysis of impact of varying temperatures during frequency vs amplitude encoded calcium oscillations.

GRAPHICAL ABSTRACT



Amplitude of calcium oscillations during varying temperatures



Frequencies of calcium oscillations during varying temperatures

ARTICLE INFO

Keywords:
Calcium oscillations
Decoding
Temperature dependence
Hepatocytes

ABSTRACT

Intracellular calcium oscillations have been widely studied. It is assumed that information is conveyed in the frequency, amplitude and shape of these oscillations. In particular, calcium signalling in mammalian liver cells has repeatedly been reported to display frequency coding so that an increasing amount of stimulus is translated into an increasing frequency of the oscillations. However, recently, we have shown that calcium oscillations in fish liver cells rather exhibit amplitude coding with increasing stimuli being translated into increasing amplitudes. Practical consequences of this difference are unknown so far. Here we investigated advantages and disadvantages of frequency vs. amplitude coding, in particular in environments with substantially changing temperatures (e.g. 10–20 degrees). For this purpose, we use computational modelling and a new approach to generate a calcium model exactly displaying a specific frequency and/or amplitude. We conclude that despite the advantages in flexibility that frequencies might offer for the transmission of information in the cell, amplitude coding is obviously more robust with respect to changes in environmental temperatures. This potentially explains the observed differences between two classes of organisms, one operating at constant temperatures whereas the other is not.

1. Introduction

Calcium signalling is one of the most widespread messenger systems. Information is transferred from extracellular agonists that bind to their specific receptors at the plasma membrane to intracellular targets like genes and proteins that are either directly activated by calcium or indirectly via calcium activated proteins. Many different types of agonists employ calcium as second messenger to convey their information. There is barely a cell type or organism that does not use calcium signalling as a central information processing unit. Calcium signalling is

* Corresponding author.
E-mail address: ursula.kummer@bioquant.uni-heidelberg.de (U. Kummer).

https://doi.org/10.1016/j.bpc.2018.11.003
Received 11 August 2018; Received in revised form 5 November 2018; Accepted 11 November 2018
Available online 26 November 2018

involved in diverse processes as organismic development, responses of immune cells, cellular stress response, muscle contraction or cell death (for a review see [1]).

The general machinery of calcium signalling, e.g. in liver cells is quite well understood. In hepatocytes calcium oscillations occur when agonists such as hormones or nucleotides bind to membrane-spanning receptor proteins on the surface of the cell. Upon binding of an agonist to the extracellular part of the receptor, the G_α subunit of the G-protein exchanges bound GDP with GTP and is thereby activated. The activated G_α subunit in turn activates phospholipase C (*PLC*), which again hydrolyses membrane bound phosphatidyl inositol 4,5-bisphosphate (PIP_2) to inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (*DAG*). IP_3 binds to the IP_3 receptor channel (IP_3R) of the endoplasmic reticulum (ER). Subsequently, the channel opens and releases calcium into the cytosol. In addition, there is calcium release from the mitochondria into the cytosol and calcium entry from the extracellular space through membrane channels. The calcium release into the cytosol further stimulates the opening of the IP_3 receptor channel, and thereby the release of calcium from the ER. Removal of Ca^{2+} from the cytosol is mediated by ATP-dependent Ca^{2+} pumps, that pump calcium back into the ER and out of the cell (for a review see e.g. [2]). A schematic representation of the most important processes in calcium signalling is depicted in Fig. 1.

Since the discovery of calcium oscillations, computational models have been an important and integral part of the research on this topic. They fundamentally helped in deciphering the exact mechanism behind the oscillations and in understanding which parts of the signal transduction machinery are crucial for which aspect of the oscillations [3].

However, one of the biggest and still not well answered question in this system is how one and the same messenger can carry such different information originating from so many different agonists. A partial explanation was offered when the oscillatory behaviour of calcium in response to many signals occurring in most cell types was discovered. On the encoding side of the signal, it was discovered that e.g. in mammalian liver cells, increasing amounts of stimulus are encoded by increasing frequencies of calcium oscillations [4]. In addition, different oscillation shapes occur in these cells depending on the exact nature of the stimulus [5–8]. Remarkably, recently, we observed that calcium oscillations in fish liver cells are amplitude encoded such that increasing amounts of stimulus lead to increasing amplitudes with a constant frequency [9].

On the decoding side, several experimental studies showed over the years that different frequencies of calcium oscillations are able to elicit different responses on the level of gene expression and protein activity [10–15]. In addition, it was also demonstrated that different responses can be achieved when different amplitudes are applied [16]. The molecular mechansims behind these observations have been studied by mathematical models. These models either concentrate on the properties of one specific enzyme or on the generic decoding properties of calcium activated proteins which display cooperativity with respect to calcium binding. For the first case, one prominent example is the decoding of calcium oscillation frequencies by CaM kinase II which has been studies experimentally [11,17] and theoretically [18]. CaM kinase II, which is activated by calmodulin, plays a central role in many cellular processes. Another prominent example is the activation of the transcription factors NF-AT and NF-κB (for a review see [19]). In the more generic case, it has been shown that different frequencies and shapes of oscillations can be decoded by cooperative proteins [20–22]. In addition, decoding of two different simultanous signals has been studied in [22]. Finally, [23] studied the decoding of time-limited calcium oscillations. The generic differences in the decoding of frequencies versus amplitudes have so far only shortly been addressed in the study by Salazar et al. [24] showing that considering the cooperativity of calcium binding in a kinase/phosphatase mediated activation and at low levels of stimulation, calcium oscillations are more efficient in activating the target than a constant signal of same average concentration. Moreover, frequency coding exhibits a higher robustness towards noisy perturbation than amplitude coding [25]. The selectivity of amplitude versus frequency decoding has not been studied so far.

If information is encoded in the temporal dynamics of calcium oscillations, another question is how environmental changes affect this information processing. Thus, temperature changes in mammalian systems like human or rat/mouse might not play such a big role, since temperature is tightly controlled. However, in the absence of such strict regulation, it has been shown in cultured HEK-cells that the frequency of calcium oscillations can rise with increasing temperature [26]. So, how is information conserved? Moreover, fish with their changing body temperatures that typically ranges from 5 to 25 degrees Celsius must employ some sort of compensation, if the temporal order of calcium oscillation is meaningful in the cell. While temperature compensation is

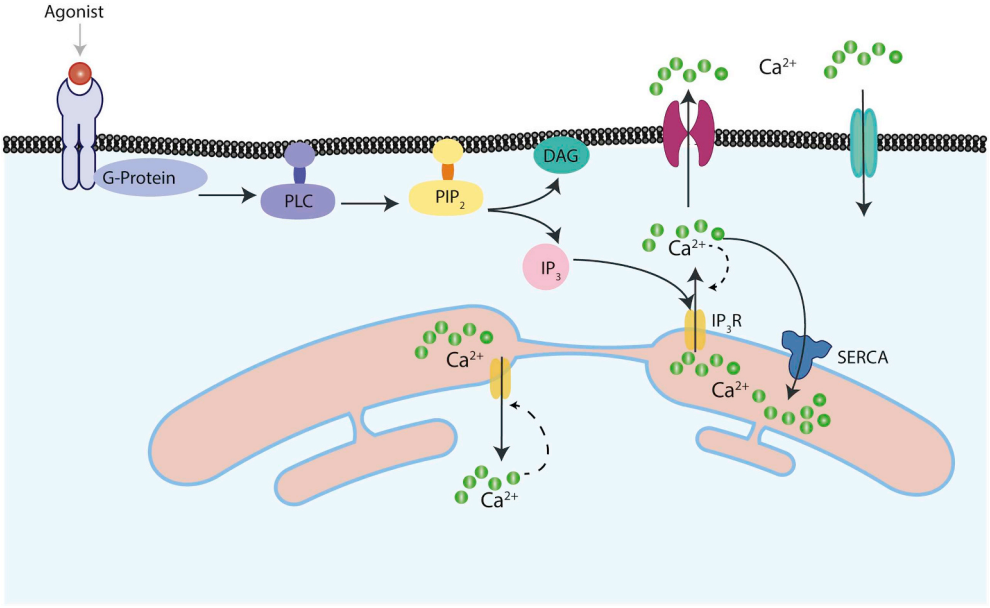


Fig. 1. Schematic representation of the processes involved in calcium signalling. SERCA denotes the calcium pump, PLC the phospholipase C, PIP2 phosphatidyl inositol 4,5-bisphosphate, DAG diacylglycerol and IP_3R the IP_3 receptor channel.

a well-known and active mechanism in the case of circadian clocks [27], this aspect has not been discussed in the literature in the context of calcium oscillations.

Here, we employ mathematical modelling to investigate the selectivity of amplitude vs. frequency encoded signals with respect to reliable decoding in the cell and their respective properties. We also study the decoding of signals when temperatures are changed. For this purpose, we use a simple model to produce calcium oscillations with specific properties. Moreover, we use optimisation and a new efficient approach to parameter fitting of oscillatory models. This new strategy allows to parameterise the model in such a way that predefined properties with respect to frequencies and amplitudes can be achieved or experimental data can be fitted. We take special care that the calcium oscillations are not simply mimicked by sinusoidal oscillations or let alone square pulses, but are of physiological shape (e.g. relaxation oscillations) resembling experimental data. We conclude that although frequency coding offers advantages with respect to achievable selectivity for decoding, it is inherently and obviously sensitive to changing temperatures. Thus, organisms with changing body temperatures might rather rely on amplitude coding which is more stable with respect to temperature changes in all studied models.

2. Methods and models

All models were set up in COPASI [28]. Deterministic simulations were carried out using the LSODA algorithm as implemented in this software. Sensitivity analysis and optimisation were also used as implemented in COPASI.

To generate different calcium signals and as input to decoding proteins we mainly used the simplest possible calcium model, namely the two-variable model published by Goldbeter and Dupont [29]. Fig. 2 summarises the essential structure of the model.

The model is represented by the following equations:

$$\frac{dY}{dt} = v_0 + v_1 \cdot \beta - v_2 + v_3 + k_f \cdot Y - k \cdot Z \tag{1}$$

$$\frac{dZ}{dt} = v_2 - v_3 - k_f \cdot Y \tag{2}$$

The two variables of the model are the concentration of free Ca^{2+} in the cytosol and Ca^{2+} in the endoplasmic reticulum; these variables are denoted by Z and Y, respectively. Parameter v_0 is the efflux of Ca^{2+} into and out of the cell and $v_1{}^{*}\beta$ represents a function triggered by agonist signal (β). The rest of the terms describe Ca^{2+} release and uptake from the endoplasmic reticulum, given by the following equations:

$$v_2 = V_{M2} \cdot \frac{Z^n}{K_2^n + Z^n} \tag{3}$$

$$v_3 = V_{M3} \cdot \frac{Y^m}{K_R^m + Y^m} \cdot \frac{Z^P}{K_A^P + Z^P} \tag{4}$$

In its original parameterisation this model displays frequency encoding, i.e. with increasing calcium entry the frequency of the oscillations increases whereas the amplitude stays constant. However, as explained in [9], a new parametrisation of the model that allows the rates of calcium release and uptake to be more symmetrical changes the bifurcation behaviour in such a way that the model now displays amplitude encoding as experimentally observed in fish liver cells.

We use the different parameterisations (parameters always listed) to produce the calcium transients needed for this study.

Subsequently we integrated an additional equation describing an enzyme that cooperatively binds calcium to be activated. This enzyme decodes the calcium signal.

The first and simplest case that often has been used in previous coputational studies of decoding e.g. [21,32] is cooperative calcium binding and deactivation of the enzyme by mass action kinetics:

$$\frac{\mathrm{d}Enz}{\mathrm{d}t} = \frac{k_{act} \cdot Ca_{cyt}^h}{K_M^h + Ca_{cyt}^h} \cdot Enz_i - k_{inact} \cdot Enz \tag{5}$$

Here, Ca_{cyt} refers to the cytosolic calcium concentration and therefore corresponds to Z in the Goldbeter model, Enz_i represents the inactive and Enz the active form of the enzyme. The sum of the latter two being one. Please note that the free calcium concentration in the Goldbeter model downloaded from BioModels is represented by the variable Z as decribed above and used as such in the implementation. Alternatively, the inactivation could more realistically follow a saturation kinetics. This is the simple decoder used below:

$$\frac{\mathrm{d}Enz}{\mathrm{d}t} = \frac{k_{act} \cdot Ca_{cyt}^h}{K_{M1}^h + Ca_{cyt}^h} \cdot Enz_i - \frac{k_{inact} \cdot Enz}{Enz + K_{M2}} \tag{6}$$

In addition, we tested a more complicated case where one effector protein is activated by the above calcium activated enzyme (denoted by A) and deactivated by another calcium dependent enzyme (denoted by B) which follows the same calcium activated kinetics (for A and B), but has a different parametrisation. This is still a relatively simple case

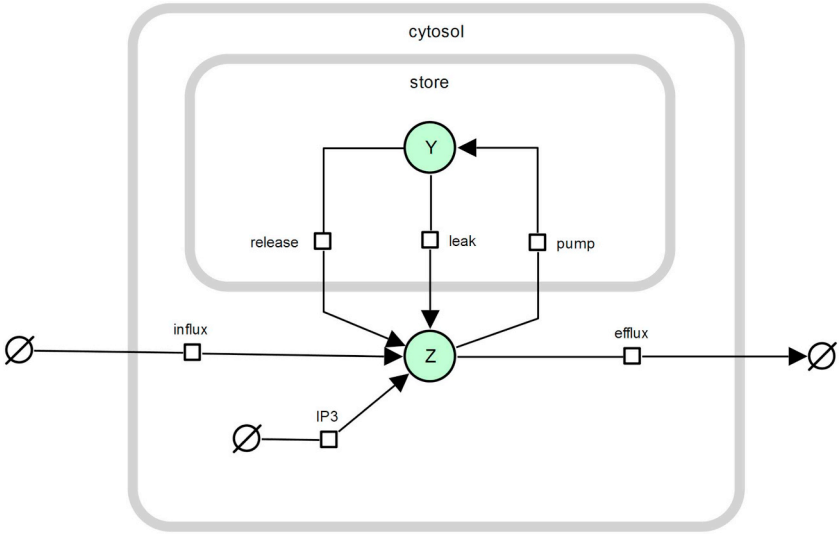


Fig. 2. Schematic representation of the reactions and processes represented by the simple calcium model by Goldbeter and Dupont [29]. The scheme was generated using VANTED [30] and adheres to the SBGN standard [31]

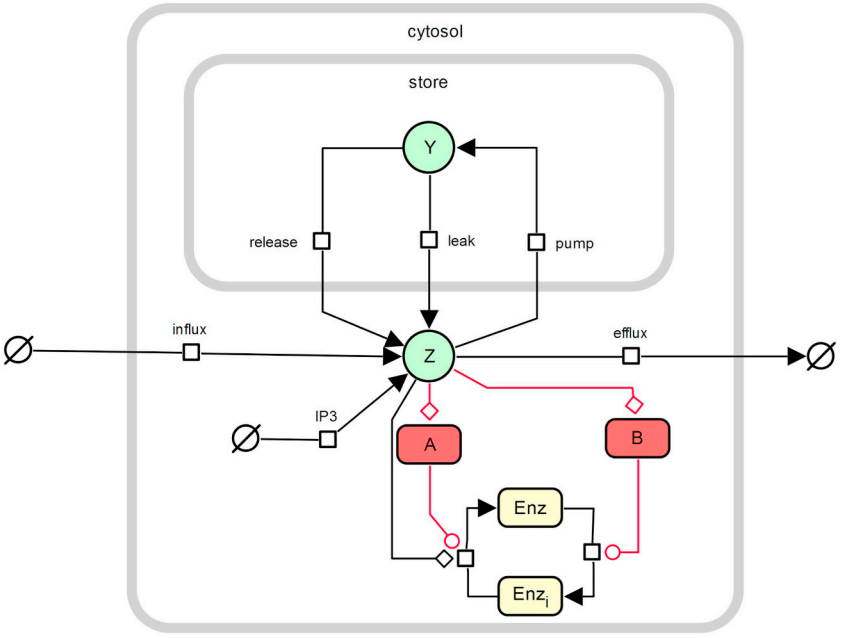


Fig. 3. Schematic representation of the decoder module used in our computations. The red arrow indicates alternative calcium dependent activation via protein A and inactivations via protein B as explained in the text. The scheme was generated using VANTED [30] and adheres to the SBGN standard [31]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

where e.g. proteins are activated by a calcium dependent kinase and inactivated by a calcium dependent phosphatase or gene expression is activated by a calcium dependent transcription factor and inactivated in a calcium dependent manner as well. It should be emphasized that lumping the corresponding equations condensating several processes in one will result in a narrowing of the possible solution space. Therefore, we can state with certainty that effects found in this simplified system will also be possible in a larger, more realistic scenario. The corresponding equation (complex decoder) becomes:

$$\frac{dEnz}{dt} = \frac{k_{act} \cdot Enz_i \cdot A}{K_A + Enz_i} - \frac{k_{inact} \cdot Enz \cdot B}{K_B + Enz} \quad (7)$$

All decoder alternatives are shown schematically in Fig. 3.

Finally, we also tested several of our results on more elaborate and realistic calcium models, all taken from the BioModels DB [33] as indicated in the text.

3. Results and discussion

To systematically parameterise calcium models in such a way that specific, e.g. experimentally observed frequencies and/or amplitudes are generated, we employed different strategies. First, to explore, if a given model can show amplitude instead of frequency coding, we used a new approach to fit models of calcium oscillations to experimental data. This is faster and more reliable than the manually tweaking parameters as described earlier [9].

Subsequently, to tune the parameters to specific frequencies and amplitudes we used an optimisation approach. Both these approaches are described in the following.

In general, we preferred to use such approaches over simply using artificial square or sinusoidal oscillations as an input, because of the more physiological shape of the resulting calcium signals which might influence the outcome. In addition, we used relative frequency and amplitude changes which are also experimentally observed.

3.1. Parameter estimation for oscillatory systems

Estimating parameters for oscillatory systems faces the inherent problem of an objective function with numerous local minima that limits the performance of the optimization algorithms [34]. To solve this problem we implemented a custom genetic algorithm based search in Matlab [35]. Our optimisation strategy takes advantage of the information contained in the eigenvalues of the system's Jacobian. Oscillatory behaviour requires complex eigenvalues [36]. For this reason, the presence of complex eigenvalues in the system was used as a precondition to accept or reject parameter values. Eigenvalues were numerically calculated using the stability analysis as implemented in COPASI [28]. By rejecting parameter values that produce non-complex eigenvalues in the system the algorithms focuses on the finding of parameters that reproduce oscillatory dynamics. The parameter search was implemented in this genetic algorithm using a population size of 200 individuals for 50 generations. During each generation the progress in the fitting (reduction in the objective function value) showed a progressive agreement between experimental and simulated frequencies and amplitudes. At the end of the generations the best solution of the algorithm was selected as θ_{fit}. The pseudocode for the genetic algorithm for the fitting of oscillatory systems is given in supplementary file 1. Parallel computations were used allowing up to eight independent evaluations at the same time.

As an example, we fitted the simple Goldbeter model which exhibits frequency encoding in its original parameterisation to experimental data produced by Schweizer et al. [9]. The experimental data set shows amplitude encoding and describes time-courses of intracellular Ca^{2+} concentration in fish liver cells after the addition of hydrogen peroxide in increasing concentrations 20 µM, 40 µM and 80 µM.

To simulate the hydrogen peroxide stimuli we implemented three *events* in our model which double the values for parameter β that represents simulation at different time points mimicking the experimental set-up $\beta[0]$:

$$\beta = \begin{cases} \beta[0], & 0 \le time \le 460 \\ \beta[0] * 2, & 460 \le time \le 960 \\ \beta[0] * 4, & 960 \le time \le t_n \end{cases} \quad (8)$$

where, t_n is the total simulation time.

For the computation of the objective function, the measurements of intracellular Ca^{2+} (z_i) at each measurement time point (t_i, $i = 1, \ldots, n$) were made comparable with the corresponding observable chemical species in the model (Z_i) by the use of the following objective function:

$$F(\theta, z) = w_{tc} \sum_{i=1}^{N} (z_i - Z_i(\theta))^2 + w_a (a_e - a_s(\theta))^2 + w_f (f_e - f_s(\theta))^2. \quad (9)$$

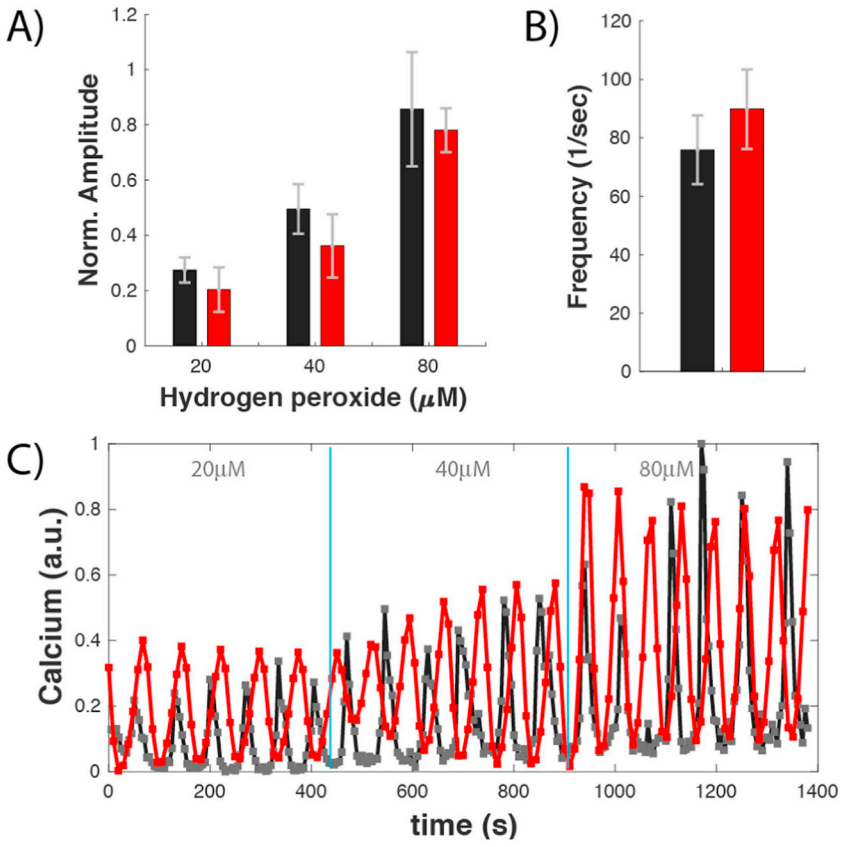


Fig. 4. Result of repeated parameter fitting: Experimental data of calcium concentration in individual RTL-W1 cells after addition of increasing hydrogen peroxide concentrations (20, 40 and 80 μM) displayed in black (reproduced from [9]), model results in red. A) Comparison between experimental amplitude (black bars) versus amplitudes obtained with the model (red bars). B) Comparison between experimental frequencies (black bars) versus simulated frequencies (red bars). C) Temporal dynamics for one exemplary parameter set (red lines) versus experimental calcium dynamics. The vertical blue dashed line represents the hydrogen peroxide stimuli. Parameters: v_0 0.988, v_1 6.8278, β 0.0169, k 5.4396, k_f 1.0296, V_{M2} 32.9863, K_2 1.0227, n 0.9666, V_{M3} 434.119, m 1.0559, K_R 1.825, P 1.4532, K_A 2.3453. For simulations results, error bars represent the standard deviation of 8 different fitted parameter sets. For the experimental data, error bars represent the standard deviation of the amplitude or frequency in the studied time course. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Eq. (9) compares the time courses for z_i and Z_i, the difference between simulated and experimental average amplitudes, (a_s and a_e, respectively), and the difference between the frequencies obtained by the model and the experimental frequencies (f_s and f_e, respectively). Constants w_{tc}, w_a and w_f represent weights for the time courses, amplitudes and frequencies, respectively. During the optimization here, all weights were set equal to 1 and since runs were successful, there was no need to adjust them. Frequencies and amplitudes in the data were determined using the function *PeakFinder* as encoded in Matlab [37].

Now that we have both the objective function from Eq. (9) to compare the similarity between the experimental data and the model with a given set of parameters, θ, a parameter estimate, θ_{fit}, can be calculated by minimising this objective function, that is:

$$\theta_{fit} = \text{argmin}_{\theta} F(\theta, z). \tag{10}$$

One exemplary fit is shown in Fig. 4 together with the statistics on eight fits resulting from repeatedly running the fitting process.

3.2. Creating models with specific amplitudes and frequencies using optimisation

Often, during our studies, it was desirable to obtain models with specific amplitudes and frequencies within physiologically realistic boundaries, but for values for which we did not possess experimental data. For this purpose we employed the optimisation routine of the software COPASI [28].

First, we used the software to calculate amplitudes and frequencies in the course of a simulation. Periods of oscillations can be easily calculated by detecting the time points at which the rate of change for the concentration of calcium changes its sign from positive to negative. This is achieved by generating events with a corresponding trigger function in COPASI. The frequency of the oscillation can then be calculated as the inverse of the period. The amplitude on the other hand is calculated by also detecting minima of the time series, using an event thact is triggered when the rate of change switches its sign from negative to positive. Storing the respective calcium concentration at maxima and minima and calculating the difference results in the amplitude.

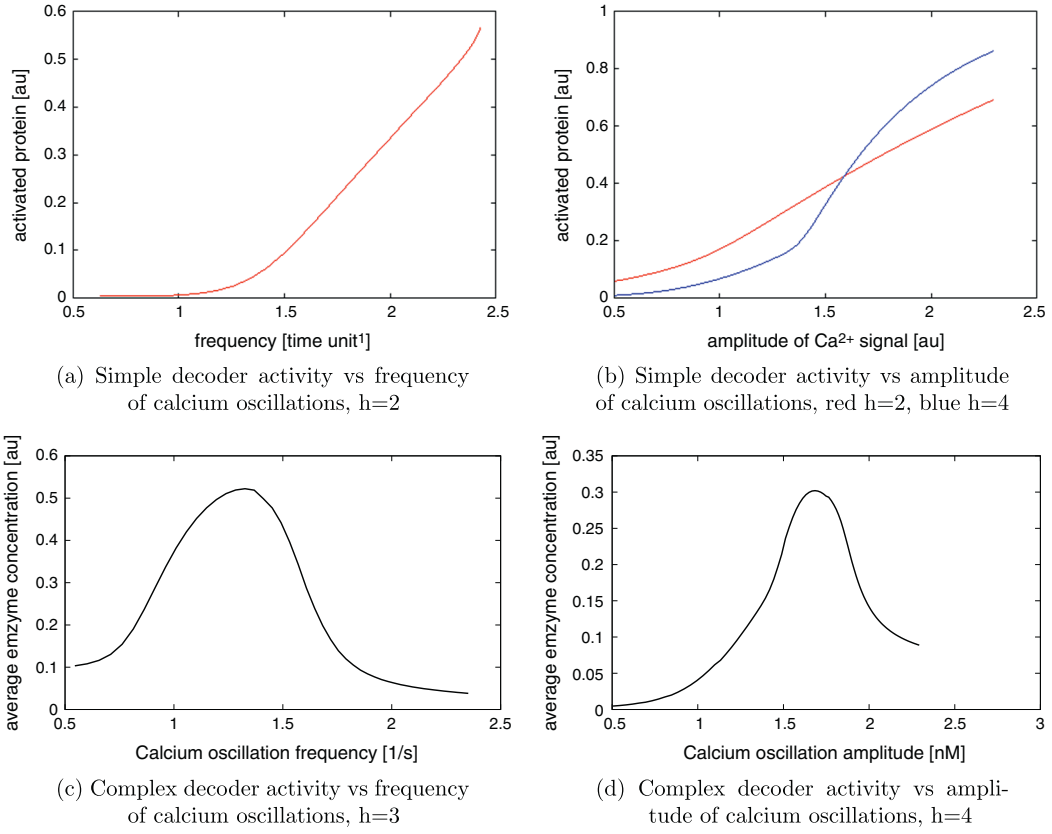
These two values - the frequency and the amplitude - can then be used in the optimisation routine of COPASI as target and set to a specific value which is achieved by parameter changes within user-defined parameter boundaries using a local or global optimisation routine. A COPASI file with this routine is available as part of the supplementary material.

3.3. Decoding amplitudes vs frequencies

As observed earlier, oscillations compared to simply increased steady state concentrations are more efficient in activating proteins, especially if these proteins display cooperative calcium binding. The response of the latter represented by the simple decoder (Eq. (6)) to increasing frequencies corresponds to a gradual increase in activation. However, if the frequency of the oscillations becomes so high that the activation of the decoder does not return to its basal activity in between calcium peaks, the dependency becomes much more nonlinear as also seen in Fig. 5a. Here, a moderate Hill coefficient of two with respect to calcium binding is sufficient to cause threshold behaviour meaning that the activation is very low below a certain calcium frequency and - after surpassing this frequency - the activation of the decoder rapidly rises. Such a behaviour is a prerequisite for being able to switch on certain calcium-dependent proteins and/or genes once a certain value is surpassed.

Similarly, amplitude encoded oscillations can be decoded by cooperative calcium binding as shown in Fig. 5b, again employing the simple decoder module (Eq. (6)). Due to the cooperative dependency of the enzyme activity on calcium and depending on the Hill coefficient, threshold behaviour can be observed as well. In that way, the decoding of amplitudes and frequencies qualitatively can be quite similar.

However, quantitatively, there is a big difference between these two



(a) Simple decoder activity vs frequency of calcium oscillations, h=2

(b) Simple decoder activity vs amplitude of calcium oscillations, red h=2, blue h=4

(c) Complex decoder activity vs frequency of calcium oscillations, h=3

(d) Complex decoder activity vs amplitude of calcium oscillations, h=4

Fig. 5. Simulation of decoder activities depending on frequencies and amplitude of calcium oscillations. Parameters for frequency encoding (panels a and c): ν_0 0.1439, ν_1 1, β 0.5–2.5 (scan parameter), k 11.02, k_f 23.37, V_{M2} 391.283, K_2 0.2246, n 1.072, V_{M3} 14,056.4, m 1, K_R 2.239 P 1.7, K_A 0.784. Parameters for amplitude encoding (panels b and d): ν_0 0.1439, ν_1 1, β 1.8–3 (scan parameter), k 3.199, k_f 3.073, V_{M2} 23.235, K_2 0.0745, n 1.072, V_{M3} 1374.19, m 1, K_R 50.159 P 1.7, K_A 4.72. Decoder parameters were computationally optimised to allow for threshold activation and - in the case of the complex decoder - threshold activation and deactivation. Parameters for the decoder in Panel (a): k_{act} 1.4, K_{M1} 0.2375, h 2, k_{inact} 0.2, K_{M2} 0.01. Parameters for the decoder in Panel (b): k_{act} 1296.52, K_{M1} 57.356, h 2 or 4, k_{inact} 0.2, K_{M2} 0.01. Parameter for the decoder in Panel (c): Enzyme A: k_{act} 2, K_{M1} 0.742, h 3, k_{inact} 0.063, K_{M2} 0.01, Enzyme B: k_{act} 2, K_{M1} 0.1535, h 3, k_{inact} 0.358, K_{M2} 0.01, final decoder: k_{act} 5.4649, K_A 0.07, k_{inact} 36.571, K_B 0.1414. Parameter for the decoder in Panel (d): Enzyme A: k_{act} 900, K_{M1} 8.5, h 4, k_{inact} 0.2, K_{M2} 0.01, Enzyme B: k_{act} 800, K_{M1} 10, h 4, k_{inact} 0.2, K_{M2} 0.01, Decoder: k_{act} 0.05, K_A 1, k_{inact} 1, K_B 1.

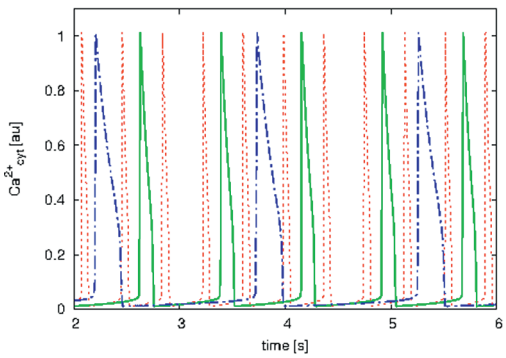
modes of encoding and decoding, namely in the magnitude of the Hill coefficient required to display threshold behaviour. To compare this property quantitatively, we used the simple decoder model and computationally optimised the threshold behaviour in the model allowing the few parameters of the simple decoder to be varied. The optimising function was defined such that a fast switch from minimal to maximal behaviour was desirable. In all cases, the deactivation kinetics was set to the same value to restrict degrees of freedom and make the results comparable. From these optimisation runs, it became obvious that frequencies offer a stronger threshold behaviour and thus sensitivity with very moderate Hill coefficients compared to amplitudes (Fig. 5a and b). Thus, a Hill coefficient of two is already sufficient to generate specificity in the response when employing frequencies, whereas higher Hill coefficients are necessary in the amplitude case. This is important for information processing. Experimental results showed that certain genes and proteins are activated by specific frequencies [10–15]. To achieve such a selectivity, threshold behaviour is a prerequisite. The fact, that such behaviour is easier to achieve with frequencies compared to amplitude decoding and that smaller Hill coefficients are needed, is a potential advantage for this form of information processing, since generating large Hill coefficients requires multiple binding sites and large structural rearrangements upon binding.

Apart from creating threshold behaviour with using the non-linearities created by the cooperativity of binding and the inabilities of oscillations to return to base levels with higher frequencies or amplitudes, the combination of two such dependencies can lead to the activation of the decoder not only above a certain minimal value of frequency and amplitude, but also below a maximal value. This is shown using the more complex decoder module (Eq. (7)) as depicted in Fig. 5c and d and a realistic range of frequencies/amplitudes. Again, it is straightforward and simple to achieve this for frequency decoding at moderate Hill coefficients and harder for amplitude decoding. In fact, we were only able to generate this behaviour for amplitude decoding for Hill coefficients equal or larger than four.

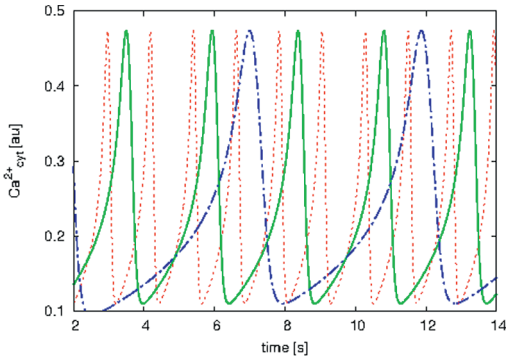
At the time of writing the manuscript, we learned that Schoch and Pahle were independently investigating the information processing capabilities of decoder modules using frequencies and overlapping with the results of our study. Particularly, their study focuses on identifying optimality criteria for the selectivity in the decoding of calcium frequencies. We therefore would like to refer to their manuscript appearing in the same journal 2018 for more detailed analyses.

3.4. Temperature effects

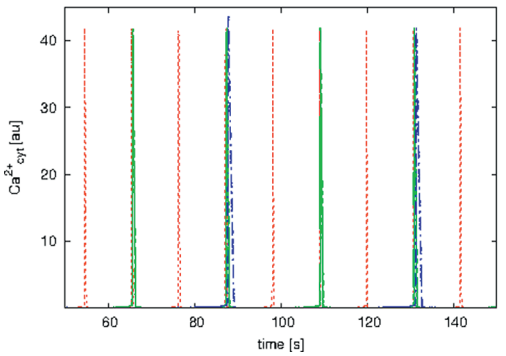
Within the above discussed context, the question remains why fish liver cells use amplitude encoding of calcium oscillations rather than frequency encoding employed by mammalian liver cells. One of the fundamental differences between mammalian organisms and fish is the constancy of temperature in mammalian organisms, whereas fish change their body temperature with the environment. How does this influence information processed in calcium signalling? Increasing temperatures usually lead to increased rates of chemical reactions,



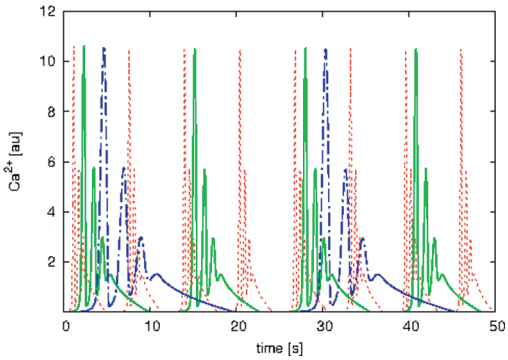
(a) Goldbeter model with original parameters



(b) Goldbeter model with parameters allowing for amplitude encoding (as in Fig.5b)



(c) Larsen model (Biomodels 331)



(d) Kummer model (Biomodels 329)

Fig. 6. Simulation of two steps of temperature change (each by 10 K) in different models of calcium oscillations (a-d). All kinetic parameters as published in BioModelsDB. Temperature steps are simulated by increasing all reactions rates two-fold. Green: Original parameters, blue: decrease by 10 K, red: increase by 10 K. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

including enzymatic reactions and diffusion speeds. According to the Arrhenius relation, chemical reactions approximately double their speed when increasing the temperature by 10 K (at least for the range of temperature considered in this study). Published data for diverse enzymes indicate that the speed increases at the uppermost twice with an increase in temperature of 10 K. Diffusion speeds of calcium in the cell have been measured to increase twofold with a temperature increase of 10 K [38].

If rates are increased by equal factors, not surprisingly, the frequency of the oscillations in the simple Goldbeter model increases while amplitudes remain unchanged (Fig. 6). This holds for both parametrisations of the model, the original one with frequency encoding and the one that allows for amplitude encoding. This effect can also be seen in more elaborate models which we tested (BioModels 329 and 331). Interestingly, one of the few experiments done on the temperature dependence of calcium oscillations shows exactly this effect: Calcium oscillations in HEK cells increase their frequency with increasing temperature [26].

Since we do not know the exact temperature dependence for processes that are not dominated by calcium diffusion, e.g. calcium pumps, we also randomly assigned factors (between 1.75 and 2) to describe different temperature dependencies to all of these. Again, amplitudes were fairly stable whereas frequencies changed drastically. As an example 30 repeats with randomly sampled temperature dependency for the original parameter set and a simulated increase of 10 K in the Goldbeter model resulted in the following: Original period 2.08, after temperature shift 0.8 ± 0.108, original amplitude 1.12, after temperature shift 1.029 ± 0.075. 30 repeats for the Goldbeter model with parameters allowing for amplitude encoding: Original period 2.46, after temperature shift 1.026 ± 0.057, original amplitude 0.43, after temperature shift 0.49 ± 0.158. It should be noted that changing the pumping rate very differently compared to the diffusion rate can impact the relative balance of uptake and release fluxes drastically and therefore, quite a large variation is observed.

If information is encoded in the frequency of calcium oscillations as suggested for mammalian liver cells, it is certainly not beneficial to change the frequency without changing the actual information, namely the actual agonist and its amount. However, as shown above, an amplitude encoded oscillation does not easily lose its main information, namely the amplitude with changing temperatures and is therefore more robust with respect to changing environments.

A long-term adaptation to changing temperatures can be achieved by changing expression levels of proteins. Thus, decreasing temperatures can be compensated by increasing expression levels leading to roughly the same reaction rates. However, such a long-term adaptation is much too slow to compensate rapid changes in temperatures like those expected to occur in natural conditions for fish. Here, employing a information processing mechanism that is more robust towards temperature changes could be a beneficial strategy.

4. Conclusion

Calcium oscillations offer many possibilities to convey information to downstream targets in calcium signalling. In most systems studied so

far, frequency coding of the strength of the signal (amount of stimulus) has been observed. However, calcium signalling in fish liver cells has been reported to employ amplitude coding. As indicated above, on one side frequency encoding offers selectivity in the decoding with moderate Hill coefficients compared to amplitude encoding. On the other side, frequencies are intrinsically sensitive to temperature changes. Fish which have largely changing body temperatures would need a sophisticated temperature compensation mechanism to rely on frequency coding. Amplitude coding on the other hand is relatively insensitive to changes in temperature. This is a potential explanation for the observed difference between information transfer in mammalian versus fish cells.

Acknowledgements

The authors would like to thank the Klaus Tschira Foundation for funding this project. We also would like to thank Katrin Hübner and Paula Marin for participating in our discussions.

Appendix A. Supplemetary data

References

[1] M.J. Berridge, P. Lipp, M.D. Bootman, The versatility and universality of calcium signalling, Nat. Rev. Mol. Cell Biol. 1 (2000) 11.
[2] E. Carafoli, L. Santella, D. Branca, M. Brini, Generation, control, and processing of cellular calcium signals, Crit. Rev. Biochem. Mol. Biol. 36 (2001) 107–260.
[3] G. Dupont, M. Falcke, V. Kirk, J. Sneyd, Models of Calcium Signalling, (2016).
[4] N. Woods, K. Cuthbertson, P. Cobbold, Repetitive transient rises in cytoplasmic free calcium in hormone-stimulated hepatocytes, Nature 0319 (1986) 600–602.
[5] M. Nash, K. Young, R. Challiss, S. Nahorski, Receptor-specific messenger oscillations, Nature 413 (2001) 381–382.
[6] U. Kummer, L. Olsen, C. Dixon, A. Green, E. Bornberg-Bauer, G. Baier, Switching from simple to complex oscillations in calcium signaling, Biophys. J. 79 (2000) 1188–1195.
[7] J.-Y. Chatton, H. Liu, J. Stucki, Modulation of hormone-induced calcium oscillations by intracellular ph in rat hepatocytes, A. J. Physiol. Gastrointest. Liver Physiol. 272 (1997) G954–G961.
[8] A.K. Green, C.J. Dixon, A.G. McLennan, P.H. Cobbold, M.J. Fisher, Adenine dinucleotide-mediated cytosolic free ca2+ oscillations in single hepatocytes, FEBS Lett. 322 (1993) 197–200.
[9] N. Schweizer, U. Kummer, H. Hercht, T. Braunbeck, Amplitudeencoded calcium oscillations in fish cells, Biophys. Chem. 159 (2011) 294–302.
[10] R. Dolmetsch, K. Xu, R. Lewis, Calcium oscillations increase the efficiency and specificity of gene expression, Nature 392 (1998) 933–936.
[11] F. Eshete, R.D. Fields, Spike frequency decoding and autonomous activation of ca2+ −calmodulin-dependent protein kinase ii in dorsal root ganglion neurons, J. Neurosci. 21 (2001) 6694–6705.
[12] W.-H. Li, J. Llopis, M. Whitney, G. Zlokarnik, R. Tsien, Cell-permeant caged $InsP_3$ ester shows that Ca^{2+} spike frequency can optimize gene expression, Nature 392 (1998) 936–941.
[13] E. Oancea, T. Meyer, Protein kinase C as a molecular machine for decoding calcium and diacylglycerol signals, Cell 95 (1998) 307–318.
[14] L. Robb-Gaspers, G. Rutter, P. Burnett, G. Hajnóczky, R. Denton, A. Thomas, Coupling between cytosolic and mitochondrial calcium oscillations: role in the regulation of hepatic metabolism, Biochimica et Biophysica Acta (BBA) 1366 (1998) 17–32.
[15] P. Tompa, R. Töth-Boconádi, P. Friedrich, Frequency decoding of fast calcium oscillations by calpain, Cell Calcium 29 (2001) 161–170.
[16] R. Dolmetsch, R. Lewis, C. Goodnow, J. Healy, Differential activation of transcription factors induced by ca2+ response amplitude and duration, Nature 386 (1997) 855–858.
[17] P. De Koninck, H. Schulman, Sensitivity of cam kinase ii to the frequency of ca^{2+} oscillations, Science 279 (1998) 227–230.
[18] G. Dupont, G. Houart, P. De Koninck, Sensitivity of CaM kinase II to the frequency of Ca2+ oscillations: a simple model, Cell Calcium 34 (2003) 485–497.
[19] E. Smedler, P. Uhlén, Frequency decoding of calcium oscillations, Biochimica Et Biophysica Acta (BBA) 1840 (2014) 964–969.
[20] A. Larsen, U. Kummer, Information processing in calcium signal transduction, in: M. Falcke, D. Malchow (Eds.), Understanding Calcium Dynamics, Vol. 623 Springer Verlag, Berlin, 2003, pp. 153–178.
[21] A. Larsen, L. Olsen, U. Kummer, On the encoding and decoding of calcium signals in hepatocytes, Biophys. Chem. 107 (2004) 83–99.
[22] S. Schuster, B. Knoke, M. Marhl, Differential regulation of proteins by bursting calcium oscillations - a theoretical study, Biosystems 81 (2005) 49–63.
[23] M. Marhl, M. Perc, S. Schuster, A minimal model for decoding of timelimited ca2+ oscillations, Biophys. Chem. 120 (2006) 161–167.
[24] C. Salazar, A. Politi, T. Höfer, Decoding of calcium oscillations by phosphorylation cycles: Analytic results, Biophys. J. 94 (2008) 1203.
[25] S. Schuster, M. Marhl, T. Höfer, Modelling of simple and complex calcium oscillations-From single-cell responses to intercellular signalling, Eur. J. Biochem. 269 (2002) 1333–1355.
[26] D. Szekely, S.C. Brennan, H.-C. Mun, A.D. Conigrave, P.W. Kuchel, Effectors of the frequency of calcium oscillations in hek-293 cells: wavelet analysis and a computer model, Eur. Biophys. J. 39 (2009) 149.
[27] P. Kidd, M. Young, E. Siggia, Temperature compensation and temperature sensation in the circadian clock, Proc. Natl. Acad. Sci. 112 (2015) E6284.
[28] S. Hoops, S. Sahle, R. Gauges, C. Lee, J. Pahle, N. Simus, M. Singhal, L. Xu, P. Mendes, U. Kummer, Copasi - a complex pathway simulator, Bioinformatics 22 (2006) 3067–3074.
[29] A. Goldbeter, G. Dupont, M.J. Berridge, Minimal model for signalinduced ca2+ oscillations and for their frequency encoding through protein phosphorylation, Proc. Natl. Acad. Sci. 87 (1990) 1461–1465.
[30] B.H. Junker, C. Klukas, F. Schreiber, Vanted: a system for advanced data analysis and visualization in the context of biological networks, BMC Bioinformatics 7 (2006) 109.
[31] N. Le Novère, M. Hucka, H. Mi, S. Moodie, F. Schreiber, A. Sorokin, E. Demir, K. Wegner, M.I. Aladjem, S.M. Wimalaratne, et al., The systems biology graphical notation, Nat. Biotechnol. 27 (2009) 735–741.
[32] J. Pahle, A. Green, C. Dixon, U. Kummer, Information transfer in signaling pathways: a study using coupled simulated and experimental data, BMC Bioinformatics 9 (2008) 139.
[33] N. Le Novere, B. Bornstein, A. Broicher, M. Courtot, M. Donizelli, H. Dharuri, L. Li, H. Sauro, M. Schilstra, B. Shapiro, et al., Biomodels database: a free, centralized database of curated, published, quantitative kinetic models of biochemical and cellular systems, Nucleic Acids Res. 34 (2006) D689–D691.
[34] S. Nabavi, C.M. Williams, A novel cost function to estimate parameters of oscillatory biochemical systems, EURASIP J. Bioinforma. Syst. Biol. 2012 (1) (2012).
[35] The Mathworks Inc, MATLAB Version 7.10.0 (R2010a), (2010).
[36] S.H. Strogatz, Nonlinear Dynamics and Chaos with Applications to Physics, Biology, Chemistry, and Engineering, Westview Press, 2000.
[37] N. Yoder, Peakfinder, MATLAB Central File Exchange, 2009.
[38] B.D. Sidell, J.R. Hazel, Temperature affects the diffusion of small molecules through cytosol of fish muscle, J. Exp. Biol. 129 (1987) 191–203.