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'*IN SILICO*' SIMULATION OF BIOLOGICAL PROCESSES

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Contents

Symposium on 'In silico' simulation of biological processes, held at the Novartis Foundation, London, 27–29 November 2001

Editors: Gregory Bock (Organizer) and Jamie A. Goode

This symposium is based on a proposal made by Dr Paul Herrling

Making sense of complex phenomena in biology

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Abstract. The remarkable advances in biotechnology over the past two decades have resulted in the generation of a huge amount of experimental data. It is now recognized that, in many cases, to extract information from this data requires the development of computational models. Models can help gain insight on various mechanisms and can be used to process outcomes of complex biological interactions. To do the latter, models must become increasingly complex and, in many cases, they also become mathematically intractable. With the vast increase in computing power these models can now be numerically solved and can be made more and more sophisticated. A number of models can now successfully reproduce detailed observed biological phenomena and make important testable predictions. This naturally raises the question of what we mean by understanding a phenomenon by modelling it computationally. This paper briefly considers some selected examples of how simple mathematical models have provided deep insights into complicated chemical and biological phenomena and addresses the issue of what role, if any, mathematics has to play in computational biology.

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The enormous advances in molecular and cellular biology over the last two decades have led to an explosion of experimental data in the biomedical sciences. We now have the complete (or almost complete) mapping of the genome of a number of organisms and we can determine when in development certain genes are switched on; we can investigate at the molecular level complex interactions leading to cell differentiation and we can accurately follow the fate of single cells. However, we have to be careful not to fall into the practices of the 19th century, when biology was steeped in the mode of classification and there was a tremendous amount of list-making activity. This was recognized by D'Arcy Thompson, in his classic work *On growth and form*, first published in 1917 (see Thompson 1992 for the abridged version). He had the vision to realize that, although simply cataloguing different forms was an essential data-collecting exercise, it was also vitally important to develop theories as to how certain forms arose. Only then could one really comprehend the phenomenon under study.

Of course, the identification of a gene that causes a certain deformity, or affects an ion channel making an individual susceptible to certain diseases, has huge benefits for medicine. At the same time, one must recognize that collecting data is, in some sense, only the beginning. Knowing the spatiotemporal dynamics of the expression of a certain gene leads to the inevitable question of why that gene was switched on at that particular time and place. Genes contain the information to synthesize proteins. It is the physicochemical interactions of proteins and cells that lead to, for example, the development of structure and form in the early embryo. Cell fate can be determined by environmental factors as cells respond to signalling cues. Therefore, a study at the molecular level alone will not help us to understand how cells interact. Such interactions are highly non-linear, may be non-local, certainly involve multiple feedback loops and may even incorporate delays. Therefore they must be couched in a language that is able to compute the results of complex interactions. Presently, the best language we have for carrying out such calculations is mathematics. Mathematics has been extremely successful in helping us to understand physics. It is now becoming clear that mathematics and computation have a similar role to play in the life sciences.

Mathematics can play a number of important roles in making sense of complex phenomena. For example, in a phenomenon in which the microscopic elements are known in detail, the integration of interactions at this level to yield the observed macroscopic behaviour can be understood by capturing the essence of the whole process through focusing on the key elements, which form a small subset of the full microscopic system. Two examples of this are given in the next section. Mathematical analysis can show that several microscopic representations can give rise to the same macroscopic behaviour (see the third section), and that the behaviour at the macroscopic level may be greater than the sum of the individual microscopic parts (see the Turing model section).

Belousov–Zhabotinskii reaction

The phenomenon of temporal oscillations in chemical systems was first observed by Belousov in 1951 in the reaction now known as the Belousov–Zhabotinskii (BZ) reaction (for details see Field & Berger 1985). The classical BZ reaction consists of oxidation by bromate ions in an acidic medium catalysed by metal ion oxidants. For example, the oxidation of malonic acid in an acid medium by bromate ions, BrO_3^-, and catalysed by cerium, which has two states Ce^{3+} and Ce^{4+}. With other metal ion catalysts and appropriate dyes, the reaction can be followed by observing changes in colour. This system is capable of producing a spectacular array of spatiotemporal dynamics, including two-dimensional target patterns and outwardly rotating spiral waves, three-dimensional scroll waves and, most recently, two-dimensional inwardly rotating spirals (Vanag & Epstein 2001). All

the steps in this reaction are still not fully determined and understood and, to date, there are of the order of about 50 reaction steps known. Detailed mathematical models have been written down for this reaction (see, for example, Field et al 1972) consisting of several coupled non-linear ordinary differential equations. Remarkably, a vast range of the dynamics of the full reaction can be understood by a simplified model consisting of only three coupled, non-linear differential equations, which can be further reduced to two equations. The reduction arises due to a mixture of caricaturizing certain complex interactions and using the fact that a number of reactions operate on different time scales, so that one can use a quasi-steady-state approach to reduce some differential equations to simpler algebraic equations, allowing for the elimination of certain variables.

A phase-plane analysis of the simplified model leads to an understanding of the essence of the pattern generator within the BZ reaction, namely the relaxation oscillator. This relies on the presence of a slow variable and a fast variable with certain characteristic dynamics (see, for example, Murray 1993). The introduction of diffusion into this model, leading to a system of coupled partial differential equations, allows for the model to capture a bewildering array of the spatiotemporal phenomena observed experimentally, such as propagating fronts, spiral waves, target patterns and toroidal scrolls.

These reduced models have proved to be an invaluable tool for the understanding of the essential mechanisms underlying the patterning processes in the BZ reaction in the way that the study of a detailed computational model would have been impossible. With over 50 reactions and a myriad of parameters (many unknown), the number of simulations required to carry out a full study would be astronomical.

Models for electrical activity

The problem of how a nerve impulse travels along an axon is central to the understanding of neural communication. The Hodgkin–Huxley model for electrical firing in the axon of the giant squid (see, for example, Cronin 1987) was a triumph of mathematical modelling in physiology and they later received the Nobel Prize for their work. The model, describing the temporal dynamics of a number of key ionic species which contribute to the transmembrane potential, consists of four complicated, highly non-linear coupled ordinary differential equations. A well-studied reduction of the model, the FitzHugh–Nagumo model, is a caricature and consists of only two equations (FitzHugh 1961, Nagumo et al 1962). Again, a phase-plane analysis of this model reveals the essential phenomenon of *excitability* by which a neuron 'fires' and determines the kinetic properties required to exhibit this behaviour.

Models for aggregation in *Dictyostelium discoideum*

The amoeba *Dictyostelium discoideum* is one of the most studied organisms in developmental biology from both experimental and theoretical aspects and serves as a model paradigm for development in higher organisms. In response to starvation conditions, these unicellular organisms chemically signal each other via cAMP leading to a multicellular aggregation in which the amoebae undergo differentiation into a stalk type and a spore type. The latter can survive for many years until conditions are favourable.

Intercellular signalling in this system, which involves relay and transduction, has been widely studied and modelled. For example, the Martiel & Goldbeter (1987) model consists of nine ordinary differential equations. By exploiting the different timescales on which reactions occur, this model can be reduced to simpler two- and three-variable systems which not only capture most of the experimental behaviour, but also allow one to determine under which parameter constraints certain phenomena arise (Goldbeter 1996). This model turns out to exhibit excitable behaviour, similar in essence to that observed in electrical propagation in nerves.

Such reduced, or caricature models, can then serve as 'modules' to be plugged in to behaviour at a higher level in a layered model to understand, for example, the phenomenon of cell streaming and aggregation in response to chemotactic signalling (Höfer et al 1995a,b, Höfer & Maini 1997). Assuming that the cells can be modelled as a continuum, it was shown that the resultant model could exhibit behaviour in agreement with experimental observations. Moreover, the model provided a simple (and counterintuitive) explanation for why the speed of wave propagation slows down with increasing wave number. More sophisticated computational models, in which cells are assumed to be discrete entities, have been shown to give rise to similar behaviour (Dallon & Othmer 1997). Such detailed models can be used to compare the movement of individual cells with experimental observations and therefore allow for a degree of verification that is impossible for models at the continuum level. However, the latter are mathematically tractable and therefore can be used to determine generic behaviours.

Several models, differing in their interpretation of the relay/transduction mechanism and/or details of the chemotactic response all exhibit very similar behaviour (Dallon et al 1997). In one sense this can be thought of as a failure because modelling has been unable to distinguish between different scenarios. On the other hand, these modelling efforts illustrate that the phenomenon of *D. discoideum* aggregation is very robust and has, at its heart, signal relay and chemotaxis.

The Turing model for pattern formation

Diffusion-driven instability was first proposed by Turing in a remarkable paper (Turing 1952), as a mechanism for generating self-organized spatial patterns. He considered a pair of chemicals reacting in such a way that the reaction kinetics were stabilizing, leading to a temporally stable, spatially uniform steady state in chemical concentrations. As we know, diffusion is a homogenizing process. Yet combined in the appropriate way, Turing showed mathematically that these two stabilizing influences could conspire to produce an instability resulting in spatially heterogeneous chemical profiles—a spatial pattern. This is an example of an *emergent property* and led to the general patterning principle of *short-range activation, long-range inhibition* (Gierer & Meinhardt 1972). Such patterns were later discovered in actual chemical systems and this mechanism has been proposed as a possible biological pattern generator (for a review, see Maini et al 1997, Murray 1993).

Turing's study raises a number of important points. It showed that one cannot justifiably follow a purely reductionist approach, as the whole may well be greater than the sum of the parts and that one rules out, at one's peril, the possibility of counterintuitive phenomena emerging as a consequence of collective behaviour. It also illustrates the power of the mathematical technique because, had these results been shown in a computational model without any mathematical backing, it would have been assumed that the instability (which is, after all, counterintuitive) could only have arisen due to a computational artefact. Not only did the mathematics show that the instability was a true reflection of the model behaviour, but also it specified exactly the properties the underlying interactions in the system must possess in order to exhibit the patterning phenomenon. Furthermore, mathematics served to enhance our intuitive understanding of a complex non-linear system.

Discussion

For models to be useful in processes such as drug design, they must necessarily incorporate a level of detail that, on the whole, makes the model mathematically intractable. The phenomenal increase in computing power over recent years now means that very sophisticated models involving the interaction of hundreds of variables in a complex three-dimensional geometry can be solved numerically. This naturally raises a number of questions. (1) How do we validate the model? Specifically, if the model exhibits a counterintuitive result, which is one of the most powerful uses of a model, how do we know that this is a faithful and generic outcome of the model and not simply the result of very special choice of model parameters, or an error in coding? (2) If we take

modelling to its ultimate extreme, we simply replace a biological system we do not understand by a computational model we do not understand. Although the latter is useful in that it can be used to compute the results of virtual experiments, can we say that the exercise has furthered our understanding? Moreover, since it is a model and therefore, by necessity, wrong in the strict sense of the word, how do we know that we are justified in using the model in a particular context?

In going from the gene to the whole organism, biological systems consist of an interaction of processes operating on a wide range of spatial and temporal scales. It is impossible to compute the effects of all the interactions at any level of this spatial hierarchy, even if they were all known. The approach to be taken, therefore, must involve a large degree of caricaturizing (based on experimental experience) and reduction (based on mathematical analysis). The degree to which one simplifies a model depends very much on the question one wishes to answer. For example, to understand in detail the effect of a particular element in the transduction pathway in *D. discoideum* will require a detailed model at that level. However, for understanding aspects of cell movement in response to the signal, it may be sufficient to consider a very simple model which represents the behaviour at the signal transduction level, allowing most of the analytical and computational effort to be spent on investigating cell movement. In this way, one can go from one spatial level to another by 'modularizing' processes at one level (or layer) to be plugged in to the next level. To do this, it is vital to make sure that the appropriate approximations have been made and the correct parameter space and spatiotemporal scales are used. This comes most naturally via a mathematical treatment. Eventually, this allows for a detailed mathematical validation of the process before one begins to expand the models to make them more realistic.

The particular examples considered in this article use the classical techniques of applied mathematics to help understand model behaviour. Much of the mathematical theory underlying dynamical systems and reaction–diffusion equations was motivated by problems in ecology, epidemiology, chemistry and biology. The excitement behind the Turing theory of pattern formation and other areas of non-linear dynamics was that very simple interactions could give rise to very complex behaviour. However, it is becoming increasingly clear that often in biology very complex interactions give rise to very simple behaviours. For example, complex biochemical networks are used to produce only a limited number of outcomes (von Dassow et al 2000). This suggests that it may be the interactions, not the parameter values, that determine system behaviour and, in particular, robustness. This requires perhaps the use of topological or graph theoretical ideas as tools for investigation. Hence it is clear that it will be necessary to incorporate tools from other branches of mathematics and to develop new mathematical approaches if we are to make sense of the mechanisms underlying the complexity of biological phenomena.

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DISCUSSION

Noble: We will almost certainly revisit the question of levels and reduction versus integration at some stage during this meeting. But it's important to clarify here that you and your mathematical colleagues are using the term 'reduction' in a different sense to that which we biologists use. Let me clarify: when you 'reduce' the Hodgkin–Huxley equations to FitzHugh–Nagumo equations, you are not doing what would be regarded as reduction in biology, which would be to say that we can explain the Hodgkin–Huxley kinetics in terms of the molecular structure of the channels. You are asking whether we can use fewer differential equations, and whether as a result of that we get an understanding. It is extremely important to see those senses of reduction as being completely different.

Maini: I agree; that's an important point.

Noble: Does mathematical reduction always go that way? I was intrigued by the fact that even you, as a mathematician, said you had to understand how that graph worked, in order to understand the mathematics. I always had this naïve idea that mathematicians just understood! I take it there are different sorts of mathematicians, as well as different kinds of biologists, and some will be able to understand things from just the equations. Presumably, the question of understanding in maths is also an issue.

Maini: What I meant by 'understanding' is that we need to determine what are the crucial properties of the system that make it behave in the way that it does. The easiest method for doing that in this case is a phase-plane analysis. This tells us that the behaviour observed is generic for a wide class of interactions, enabling us to determine how accurately parameters must be measured. My talk focused on the differential equation approach to modelling. However, there may be cases where other forms of modelling and/or analysis — for example, graph theory, networks or topology — may be more appropriate. An issue here is how do we expose these problems to those communities?

Loew: I would assert that the kind of mathematical reduction you were talking about — basically, extending your mathematical insights to produce a minimal model — may provide insights to mathematicians, but in most cases it wouldn't be very useful to a biologist. This is because in creating the minimal model you have eliminated many of the parameters that may tie the model to the actual biology. In the BZ reaction you mentioned, you were able to list all of the individual reactions. A biologist would want to see this list of reactions, and see what happens if there is a mutant that behaves a little differently. What does this do to the overall behaviour? You wouldn't be able to use the model, at least as not as directly, if you had your minimal model instead. I feel that it takes us one step further away from biology if we produce these kinds of minimal models.

Maini: It depends what sort of reduction you do. If you use quasi-steady-state assumptions, the parameters in the reduced model are actually algebraically related to the parameters in the full model, so you can still follow through and compute the effects of changing parameters at the level of the full model. Very little information is lost. My concern about very detailed computational models is that one is replacing a complicated biological system one wishes to understand by a complicated computational model one does not understand. Of course, in the very detailed model one can see the outcome of changing a specific parameter, but how do you know whether the answer is correct if you cannot determine on what processes in the model the outcome depends?

Loew: I think it is important because of the issue Denis Noble raised at the beginning of the meeting: about whether there is the possibility for a theoretical biology. If you can produce minimal equations that you can somehow use in a useful way to describe a whole class of biology, this would be very important. I can see analogies in chemistry, where there are some people who like to do *ab initio* calculations in theoretical chemistry, trying to understand molecular structure in the greatest detail. But sometimes it is more useful to get a broader view of the patterns of behaviour and look at things in terms of interaction of orbitals. There it is very useful. Chemistry has found what you call the 'reductionist' approach very useful. It remains to be seen whether this will be useful in biology.

Maini: I would argue that it has already been shown in Kees Weijer's work that such an approach is very useful. He has beautiful models for *Dictyostelium*. He is an experimentalist, and works with mathematicians in the modelling. When it comes to looking at how the cells interact with each other, he will use reductions such as FitzHugh–Nagumo. His approach has resulted in a very detailed understanding of pattern formation processes in *Dictyostelium discoideum*.

Crampin: One of the things mathematics is useful for is to abstract phenomena from specific models to reveal general properties of particular types of system. For example, if you combine an excitable kinetic system with chemotaxis for cell movement, then you will always get the sorts of behaviour that Philip Maini is describing. In this respect, the biological details become unimportant. However, if you do start with a complicated model and use mathematical techniques to reduce the model to a mathematically tractable form, then you can keep track of where different parameters have gone. Some of the variables will turn out not to have very much bearing on what goes on. These you can eliminate happily, knowing that if the biologist goes away and does an experiment, then changing these parameters is not going to have a strong effect. But the important ones you will keep, and they will still appear in the final equations. You should be able to predict what effect varying these parameters in experiments will have. Reducing the mathematical complexity doesn't necessarily throw out all of the biology.

Hunter: If you accept that both approaches are needed (I think they are complementary), who is doing the process of linking the two? Having got the dispersion relation and the parameter range that leads to instability, how does one map this back to the biological system? And how do we deduce general ways of moving between the state space of 11 equations to the state space of two equations?

Maini: That's an issue we have been trying to tackle. There are certain approaches such as homogenization techniques for looking at these sorts of issues. But most of the homogenization techniques that I have seen in the materials context tend to be very specialized. I think it is a challenging problem. Most mathematicians are more interested in proving theorems and are not really interested in such messy applications. They will happily take the sort of equations that I wrote down and throw out a few more terms, so they can just prove some theorem, without caring where the equations arrived from. That is fine, because good mathematics may come out of it, but it is not mathematical biology. Perhaps it will be the physicists who will help to bridge the gap that exists.

Noble: There are obviously different demands here. Part of what you said in relation to helping the biologists was highly significant. It was determining where there was robustness, which I think is extremely important. This may correspond to part of what we call the logic of life. If, through comparing different reductions and the topology of different models, we can end up with a demonstration of robustness, then we have an insight that is biologically important whether or not anyone else goes on to use those mathematical reductions in any of their modelling. Another success is as follows. Where in our computationally heavy modelling we have come up with counterintuitive results, then going back to the mathematicians and asking them to look at it has proven extremely valuable. One example of this is in relation to investigating one of the transporters involved in ischaemic heart disease, where we came across what still seems to me to be a counterintuitive result when we down-regulated or up-regulated this transporter. We gave this problem to Rob Hinch, to see whether he could look at it mathematically. He demonstrated that it was a necessary feature of what it is that is being modelled. This is another respect in which mathematical reduction (as distinct from the biological kind) must be a help to us where we are puzzled by the behaviour of our more complicated models. So we have some unalloyed successes that we can chalk up, even if people don't go on to use the reductions in their modelling.

Hinch: The idea of all modelling, if it is to be useful and predictive, is for it to come up with some original ideas. If you have a very complex simulation model which comes up with a new idea, you do not know whether that is an artefact of the actual model, or if it is a real mechanism occurring. The power of mathematics and the mathematical analysis where these counterintuitive results come up, is that you

can pinpoint what is causing this novel behaviour to happen. This would be a much better way to direct the experimental work. The idea is that by having these reduced models we can understand the mechanism of this interesting behaviour, which will immediately make it much easier for an experimentalist to see whether this is a real phenomenon, or just an artefact of the modelling.

Crampin: In addition to what Philip Maini said, I want to draw a distinction between on the one hand this type of mathematical reduction (formal ways of moving between complicated models and simpler representations), and on the other hand the 'art' of modelling—using scientific insight to do that same process. I am not sure whether there will ever be general formal methods for taking a complicated model and generating a simpler one. In practice one uses a combination of approaches, both formally manipulating the equations and using knowledge of the system you are working on. There is also an interesting difference between simulation models and analytical models. The tradition in applied mathematics is that a model is developed to answer a specific question, just for that purpose. It is unlikely for people to expect that model to be used in all sorts of different contexts. In contrast, if we are talking about generating simulation tools, models must be sufficiently general to be applicable in all sorts of different areas, even if you are building computational tools where you can construct models on an *ad hoc* basis for each problem.

Noble: Yes, the modellers are building a jigsaw.

Loew: I certainly appreciate the value of producing a minimal model, both from the point of view of the mathematical insight that it provides, and also from the practical point of view of being able to use a reduced form of a model as a building block for a more complex model. This is certainly an important modelling technique. But the reason I was deliberately being provocative was because we need to be able to connect to the laboratory biologist. It is important not only to avoid just being mathematicians who prove theorems but also to always be practical about how the models are being used as aids for biology. If they get too abstract, then the biologists get very quickly turned off to what we are doing.

Winslow: There is another sense in which model reduction can be performed. It doesn't involve reducing the number of equations used to describe a system, but rather involves using computational techniques to study the generic properties of those equations. These approaches have been used with some success. One example is bifurcation theory to understand the generic behaviours of non-linear systems subject to parameter variation. This kind of model reduction is where a complex, oscillating cell may be equivalent to a much simpler oscillating system by virtue of the way in which it undergoes oscillation, perhaps by a half-bifurcation. There is no reduction in the number of equations here, but lumping of systems into those that share these general dynamical properties.

Paterson: Les Loew, you commented that for the lab biologist, we need to present models in a form they see as relevant. There is a whole branch of biology that looks at people as opposed to cells! I have people on my staff who you can show gene expression data until you are blue in the face, but they want to understand a complex disease state such as diabetes where there are huge unanswered questions of integrated physiology that can only be answered by investigations at the clinical level. In terms of tying models to the biology you are right, and for bench scientists working with high-throughput *in vitro* data, I think the types of very detailed models we are talking about are very necessary. But in terms of tying it to extremely relevant data at the clinical level, for understanding the manifestation of disease states, you can't afford to build a model at the gene expression level for a complicated disease state such as diabetes. While gene expression data in key pathways may be relevant, clinical data of the diverse phenotype must be linked as well. How this relates to Peter Hunter's point about the transition, is that biology gives us a wonderful stepping stone—the cell. There is a tremendous amount of detail within the cell. I would be interested to hear estimates of the fraction of the proteins coded by the genome that actually participate in communication outside the cell membrane. My guess is that it is an extremely small fraction. If you look at the cell as a highly self-organized information and resource-processing entity, and consider that it is participating in many different activities taking place in the organism, then there are opportunities to operate at a more highly aggregated level where you are looking at aggregated cellular functions that link up to clinical data. Then you go into the more detailed cellular models to link into *invitro* and gene expression data. In this way you can have your cake and eat it too. The fact that the cell represents a nice bridging point between these two extremes can help us provide multiple modelling domains that are relevant to molecular cell biologists and clinical biologists.

Cassman: Philip Maini, what did you mean by the term 'robustness'? This is another term that is thrown around a lot. It usually means that the output is insensitive to the actual parameterization of the model. I'm not sure this is what you meant.

Maini: What I meant in this particular context is that in some of these models you could change the parameter values by several orders of magnitude and it would not qualitatively change the outcome.

Noble: There's another possible sense, which I regard as extremely important. Between the different models we determine what is essential, and, having done the mathematical analysis, we can say that the robustness lies within a certain domain and these models are inside it, but another model is outside it.

Berridge: For those of us who are simple-minded biologists, when we come across something like *Dictyostelium* with five or six models all capable of explaining the same phenomenon but apparently slightly different, which one are

we going to adopt? There needs to be some kind of seal of approval so we know which one to opt for.

Crampin: To turn that on its head, as a modeller reading the primary experimental literature, I often find completely conflicting results!

Berridge: One of the nice things about Philip Maini's paper was that he was able to explain this very complicated behaviour of cells aggregating, including complex spiral waves, using just two ideas. One was the excitable medium idea, and the other one was chemotaxis. While he used chemotaxis as part of the model, I don't think there is anything in the model that actually explains the phenomenon of chemotaxis. This is a complex phenomenon, for which I don't think there is a mathematical model. How is it that a cell can detect a minute gradient between its front end and back end? While those working on eukaryotes don't have a good model, people working on bacteria do. This is where we really need some help from the mathematicians, to give us a clue as to the sorts of parameters a cell might use to detect minute gradients and move in the right direction.

Maini: There are mathematicians trying to model the movement of individual cells.

Berridge: It's not the movement I'm referring to, but the actual detection of the gradient.

Shimizu: The gradient-sensing mechanism is very well understood in bacteria. The cell compares the concentration that is being detected at present to the concentration that was detected a few seconds ago in the past. So in bacteria, it is by temporal comparisons that the gradient is measured. This is different from the spatial comparisons that *Dictyostelium* makes.

Berridge: I understand the bacterial system; it is the eukaryotic cell where it isn't clear. There isn't a model that adequately explains how this is done.