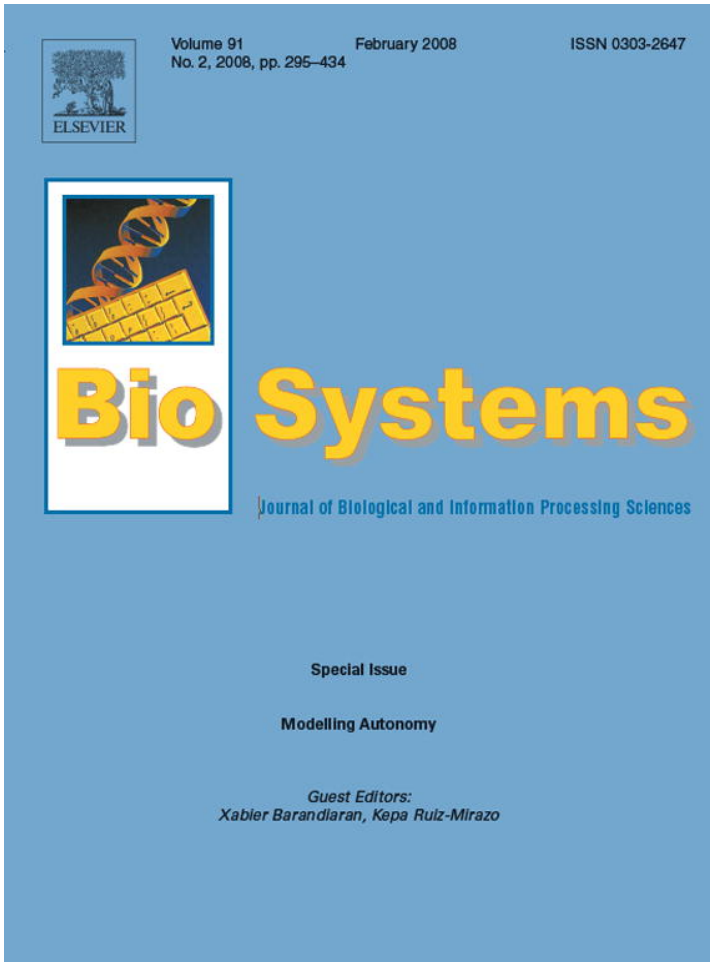

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On the way towards 'basic autonomous agents': Stochastic simulations of minimal lipid–peptide cells

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Abstract

In this paper, we apply a recently developed stochastic simulation platform to investigate the dynamic behaviour of minimal 'self-(re-)producing' cellular systems. In particular, we study a set of preliminary conditions for appearance of the simplest forms of *autonomy* in the context of lipid vesicles (more specifically, lipid–peptide vesicles) that enclose an autocatalytic/proto-metabolic reaction network. The problem is approached from a 'bottom-up' perspective, in the sense that we try to show how relatively simple cell components/processes could engage in a far-from-equilibrium dynamics, staying in those conditions thanks to a rudimentary but effective control of the matter-energy flow through it. In this general scenario, basic autonomy and, together with it, minimal *agent* systems would appear when (hypothetically pre-biological) cellular systems establish molecular trans-membrane mechanisms that allow them to couple internal chemical reactions with transport processes, in a way that they channel/transform external material-energetic resources into their own means and *actively* regulate boundary conditions (e.g., osmotic gradients, inflow/outflow of different compounds, . . .) that are critical for their constitution and persistence as proto-metabolic cells. The results of our simulations indicate that, before that stage is reached, there are a number of relevant issues that have to be carefully analysed and clarified: especially the immediate effects that the insertion of peptide chains (channel precursors) in the lipid bilayer may have in the structural properties of the membrane (elasticity, permeability, . . .) and in the overall dynamic behaviour of the cell.

Keywords: Minimal (lipid–peptide) cell; Self-assembly; Autocatalysis; Proto-metabolism; Stochastic kinetics (Gillespie method); Osmotic crisis; Reproduction; Artificial autonomous agents

1. Introduction

Maturana and Varela's work on autopoietic systems (Maturana and Varela, 1973; Varela et al., 1974) was a cornerstone in the construction of a new way of thinking about biological systems, in which the concepts of organization and autonomy played a central role (Varela, 1979). And it also signified a very important contribution to the development of Artificial Life as a discipline (Varela and Bourgine, 1991). However, most of their claims have somehow diffused away from the real practice of researchers in this field, and it is not clear whether their main message still holds or not. In this paper we retrieve the original autopoietic goal, but look into the problem with a rather different lens. Taking as a standpoint previous critical work on the theory of autopoiesis (Ruiz-Mirazo and Moreno, 2004), we propose a simulation model to study more realistically the origins of minimal autonomous cells.

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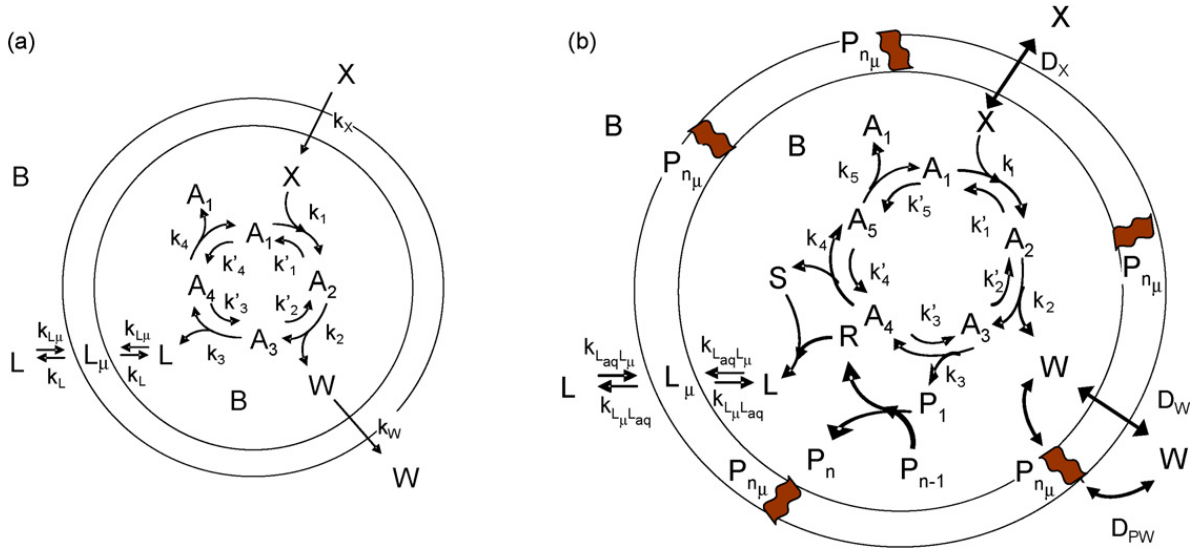

Fig. 1. Scheme drawings: (a) Scheme for the 'minimal lipid cell' scenario (analysed in (Mavelli and Ruiz-Mirazo, 2007)). (b) Scheme for the 'minimal lipid–peptide cell' scenario. *L* stands for lipid (amphiphilic molecule, in general); *B* for buffer; *X* and *S* for different lipid precursors; *W* for waste product; *R* for a byproduct of oligomerization processes required for internal lipid synthesis; P_1 is the aminoacid (monomer); P_n an oligomer; P_{n_μ} stands for a trans-membrane oligomer channel; the A_i stand for the minimal set of metabolites required in the internal autocatalytic cycle.

The work here reported is an elaboration on a model for minimal cell dynamics (Mavelli and Ruiz-Mirazo, 2007), in which the self-assembly processes of simple lipid molecules (amphiphiles, like fatty acids) were coupled with different reaction schemes for their synthesis, bringing about vesicles that show, when taken away from equilibrium, quite interesting time behaviours (homeostasis, growth, division ...). The most complex case analysed in that previous work was a model cell that, by means of an internal reaction cycle, is able to produce the main lipid component of its membrane (see Fig. 1a). Although this would be, in itself, quite an achievement (in fact, still not realised *in vitro*), we consider that 'basic or minimal autonomy' requires further steps (in particular, an *active control* of the matter-energy flow through the system), so we now propose a more comprehensive scheme, with amino acids and oligopeptides as additional components of the cell (see Fig. 1b) and explore the consequences that this has on its dynamic and functional behaviour.

The main motivation behind this work was to develop a model that could suitably address the issue of how short peptide chains come to interact with lipid vesicles in a way that the latter may profit from the interaction to become more elaborate – and biologically relevant – compartments. In order to host a proto-metabolic reaction network, primitive vesicles would need to establish channels and other transport mechanisms that help them overcome problems like the accessibility of certain substrates to the interior (and the disposal of some others) or the regulation of osmotic imbalances. As we will argue more extensively below, when these mechanisms are internally produced and sustained (doing work, i.e. against the thermodynamic drift) it is possible to speak about the development of the first 'agent systems' (in a minimal, chemical sense (Kauffman, 2003)). In this context, although the present paper still constitutes a rather simplified portray of the situation, at least we hope it contributes to solve the difficult but prebiotically very intriguing question of how could simple cellular systems begin coupling internal chemical reactions with trans-membrane processes.

Our approach is a step forward with regard to previous models of proto-metabolic cells (e.g.: (Varela et al., 1974; McMullin and Varela, 1997; Dyson, 1982; Csendes, 1984; Fernando and Di Paolo, 2004; Munteanu and Solé, 2006; Ono and Ikegami, 1999; Madina et al., 2003; Segré and Lancet, 2000; Macía and Solé, 2007)) precisely because it tries to capture the active role and dynamic properties of the cellular compartment itself (the membrane), as a bilayer made of amphiphilic molecules (with specific properties – e.g.: volume, head area, etc. –) plus other compounds (like peptide chains), enclosing an 'aqueous core' where different reactions take place. As the reader may notice, our model cell shares some features with Ganti's 'chemoton' (Ganti, 1975, 2002, 2003), but also keeps important differences. For instance, diffusion and transport processes are here explicitly taken into account, as well as the possibility of the membrane to change its composition and functional properties. In addition, the original 'template' subsystem of the chemoton model is substituted by a more general

'polymer-production' subsystem (which, in a first approximation to the problem, is not made, in itself, autocatalytic—as Ganti would require). This is more coherent with a 'peptide-first' prebiotic hypothesis and does not require the inclusion of an already formed polymer/oligomer chain (like a catalytic template—primitive rybozime) within the system from the very beginning (i.e., chains of different lengths will be progressively built up, starting just from the monomers produced by the internal reaction network).

Actually, the major assumption behind this work is that lipid–peptide cells had to become minimal autonomous agents, solving basic problems like the control on concentration gradients, or the harvesting of energy resources, before they could establish template-replication mechanisms, with which their reproduction and hereditary properties became much more reliable, opening the way towards full-fledged living systems.

2. Is It Reasonable to Search for Minimal (infra-biological) Autonomous Agents?

The prototypical example of a minimal autonomous agent is a bacterial cell swimming upstream in a glucose gradient (Hoffmeyer, 1998; Kauffman, 2000, 2003). In this case, it is quite safe to say that the system is 'acting on its own behalf': indeed, it is investing a good deal of its energy budget to move to an area with higher nutrient concentration, somehow anticipating the overall metabolic reward it will get from that action. It is also a good, uncontroversial example because the capacity for movement of a system in an environment is tightly linked to our intuitive notion of agency. Nevertheless, a deeper and more rigorous analysis of the notion (Ruiz-Mirazo and Moreno, 2000; Ruiz-Mirazo, 2001) leads one to conceive of an agent as a system that exerts an action that contributes to its own maintenance (i.e., a *functional* action, as it will be here understood) in which the environment or, more precisely, one/some of the variables that define its *relationship* with the environment is/are changed. So, in a more condensed phrase, one can define an agent as *a system that actively modifies its boundary conditions in a functional way*.[1]

[1] Strictly speaking, it should say *potentially* functional, since the contribution to self-maintenance is not immediate, but via the external medium, so there is a causal gap between the source of the action and the functional reward (hence the anticipatory aspect of the action—and also the possibility that it does not have the expected effect back on the system). However, in the most basic or limit case (our interest here), that gap is reduced to its minimal expression, in a way that the term 'potentially' could be dropped.

This is a broad conception that, of course, includes movement (change in space-related boundary conditions). But it also includes other more basic capacities, at the cellular level, than those provided by chemotactic mechanisms. Take an active transport mechanism, like a proton-pump: there the cell is also investing part of its energy resources to carry out a non-spontaneous process (transport of an ion against the gradient) in order to regulate electrochemical potential differences across the membrane. The outcome of that action could be either to avoid an osmotic collapse of the compartment or to contribute to the production of an 'energy currency' (Skulachev, 1992) (precisely, maintaining that gradient in electrochemical potential, which is being used somewhere else to drive other fundamental cell processes), or both at the same time. In any case, a functional outcome that involves an active change/regulation of the boundary conditions of the system.

The question that immediately rises is how far down we should go to find really minimal autonomous agents: do they have to be full-fledged living systems or could they belong somewhere 'in-between'? Is it necessary for that type of system to be as complex as a genetically instructed metabolism, subject to Darwinian evolution? Could cellular systems with a rudimentary but active transport mechanism develop without macromolecules like proteins, RNA or DNA? These are questions at present open to scientific (experimental and theoretical) research, and this paper is our first attempt to start answering them.

What is clear to us right from the beginning is that standard physico-chemical self-organizing systems, like the classical 'dissipative structures' (Nicolis and Prigogine, 1977), cannot accomplish the goal (as far as they have no control on the flow of matter-energy through them); and, more significantly, that non-cellular complex chemical systems (reaction networks without a proper, self-generated boundary) cannot either. First, because without a semipermeable boundary (a membrane) it is in practice – in the physico-chemical domain – impossible to establish a neat inside–outside distinction and, therefore, an asymmetry between the system (ultimate responsible for – and beneficiary of – the functional action) and the environment (the external set of compounds and processes by means of which that action proceeds). Second, because in order to make a change or regulate the relationship (i.e., the boundary conditions: matter and energy exchanges) between system and environment it is critical that the membrane actually belongs to the system, in the sense that its composition and other dynamic properties are defined (and, if needed, redefined) from within.

Accordingly, we consider that the 'minimal cell' research program (Luisi et al., 2006; Szathmary, 2005; Solé et al., 2007) is the right track to investigate at what stage in the process of origins of life could autonomous agent systems come about. Simulation models of chemical autonomous agents that do not take into account the question of the compartment (e.g.: (Daley et al., 2002)), although interesting in some respects (these authors, for instance, deal with the important issue of how endergonic and exergonic processes could get coupled) are simply not approaching the problem in a suitable, complete way.

There are different strategies to tackle the problem of the minimal cell: several research groups (Hutchinson et al., 1999; Gil et al., 2004; Castellanos et al., 2004) look into if from a strict top–down view; but most models and related *in vitro* experiments constitute 'semi-synthetic' approaches, in the sense that they combine self-assembled compartments (vesicles or liposomes) with biopolymers (genes, enzymes or other macromolecular machinery) extracted from extant living cells (see the review in (Luisi et al., 2006)). Finally, a significantly smaller amount of researchers is tackling the problem from a 'bottom-up' approach (Monnard and Deamer, 2001; Hanczyc et al., 2003; Chen et al., 2004; see also the review in Pohorille and Deamer, 2002), or looking into the basic *chemical* (rather than biochemical) logic of a minimal protocell (Morowitz et al., 1988).

Nevertheless, given the importance of the latter type of approach, the work here presented explores a scenario where relatively simple chemical components and processes bring about minimal self-(re-)producing cellular systems. Taking as our point of departure a case where the membrane compartment of the system is just made of endogenously produced lipidic/amphiphilic molecules (see Fig. 1a), we introduce in the scheme amino acid molecules (e.g., alanine), also produced from within, that can spontaneously polymerize to give short peptide chains; some of these chains may interact with the lipid bilayer, get inserted and form aggregates in it, and – if they are long enough – even span it (as illustrated in Fig. 1b). This transition from 'pure lipid' to 'lipid–peptide' protocells does not involve a significant increase in the complexity of the building blocks of the system (i.e., does not threaten the prebiotic plausibility of the model),[2] but can have important consequences for its dynamic behaviour, particularly due to the changes it is bound to provoke in the properties of the membrane (elasticity, permeability, ...). For instance, polyalanine and polyleucine embedded in lipid bilayers have already been shown to induce proton-conducting pathways (Oliver and Deamer, 1994). In any case, what is really significant is that, at these initial or rather elementary stages, we can assume that the cellular system would not require very specific peptide sequences (i.e., it is reasonable to begin with a single aminoacid, or combinations of just two different ones). This is further supported by the relatively simple structure and properties of trans-membrane segments in actual membrane proteins (Pohorille et al., 2005).

Other current bottom-up attempts to the construction of minimal lipid–peptide systems (Rasmussen et al., 2004) consider an even simpler protocell scenario: they just model the compartment as a different phase (a hydrophobic domain: a micelle or a droplet), where certain reactions (in particular, the polymerization of PNA) may be favoured. The contribution of Fernando and Rowe (2007) to this special issue, although it has the advantage of a more open artificial chemistry (in which evolutionary aspects can be more suitably addressed), involves a similar simplification regarding the compartment of the system. Instead, here we developed a somewhat more elaborate idea of minimal cell model, simulating a closed membrane that contains an aqueous core where reactions occur, because that cell topology is the key to provide a plausible and continuous account of the origin of biological cells. Even if the chemistry is fixed in advance, our simulations allow the exploration of how different reaction schemes affect the dynamic properties and organization of a cellular system.

In such conditions (proper cellular structure), while oligomerization processes are facilitated (probably in the water–lipid interphase), the vesicles may also benefit from the situation to become more elaborate and functional cell compartments, including, for instance, pores or rudimentary trans-membrane channels. The formation of these pores/channels has been proved instrumental to overcome the energetic-nutrient limitations of artificial cell bioreactors (Noireaux and Libchaber, 2004). It remains an open question whether this is also the case at earlier stages, when the molecular complexity of the system is strongly reduced. But that is precisely the hypothesis we would like to explore, convinced that the capturing and channelling of matter and energy resources into the system is pivotal for its autonomous construction and robust maintenance, and that the membrane must play central role in this task from very early stages (Ruiz-Mirazo and Moreno, 2004).

[2] The abiotic synthesis of both simple amphiphilic molecules, like fatty acids, and of aminoacids and short peptides is well established since long ago (Nooner and Oró, 1978; Miller, 1953). Oligomerization is not so easy experimentally, though.

3. Description of the Cell Dynamics Model

The programming platform we use for this work is an object-oriented (C++) environment that has been developed to simulate stochastically (by means of a Monte Carlo algorithm: the Gillespie method (Gillespie, 1976, 1977)) chemically reacting systems in non-homogeneous conditions (details to be found in (Mavelli, 2003; Mavelli and Ruiz-Mirazo, 2007)). The program considers these systems as a collection of different phases or reaction domains, each of which is assumed to be globally homogeneous. In this context, the dynamics of a cell can be modelled taking as a starting point the distinction of three fundamental phases or 'reaction domains' in the system: a general 'environment' (the common aqueous solution where all cells are contained), a hydrophobic or lipidic phase (the 'membrane') and a 'core' (the internal aqueous solution that belongs to each cell). A cell is made of a closed 'membrane' and a 'core', so the number of these phases may increase, if the initial cell divides. When desired, the program can follow the evolution of both the 'parent' and 'daughter' cells, monitoring the dynamics of cell populations.

In each domain a set of different chemical reactions (and associated kinetic constants) is defined and, as the simulation runs, the population/concentration of molecular species in it will vary following the stochastic algorithm. Moreover, the diverse domains can exchange molecules via diffusion processes, according to the permeability rules of the system, the concentration gradients and the specific value of the diffusion constants. Free flow of water is assumed between the aqueous solutions ('environment' and 'cell core' phases), to ensure the isotonic condition:

$$C_{\text{Total}} = \frac{\sum_{\text{i}}^{\text{Internal species}} n_i}{N_{\text{A}} V_{\text{Core}}} = \frac{\sum_{\text{j}}^{\text{External species}} n_{\text{j}}}{N_{\text{A}} V_{\text{Env}}} \quad (1)$$

that is, the fact that the global concentration of substrates inside and outside the membrane remains equal (NA stands for Avogadro's number). Throughout the simulation, at the end of every iteration, the core volume V_{core} of each cell is therefore rescaled in the following way:

$$V_{\text{Core}} = \frac{\sum_{i}^{\text{Internal species}} n_i}{\sum_{\text{j}}^{\text{External species}} n_{\text{j}}} V_{\text{Env}} \quad (2)$$

simulating an instantaneous flux of water to balance the osmotic pressure. For each cell, the membrane surface S_μ can be calculated as:

$$S_\mu = \frac{1}{2} \sum_{i}^{\text{Membrane species}} \alpha_i n_{i_\mu} \quad (3)$$

where α_{i} are the hydrophilic head areas of all the surface active molecules located on the membrane and the factor 0.5 takes into account the double molecular layer.

Therefore, while the volume of the environment remains fixed to its initial value, the volume of the core and the surface of the membrane are free to change (independently) during the simulation, provided that they satisfy some geometrical constrains. Although the initial shape of a cell will be assumed spherical (for the sake of standardizing initial conditions), our model does not assume that cells must stay spherical all the time or that they divide when they double their initial size (as it is usually done). Apart from being a bit artificial, those simplifying conditions lead to problems when it comes to share out the contents of a splitting cell (Munteanu and Solé, 2006), because the volume and the surface of a sphere do not grow to scale. Instead, here we will consider that the conditions for division or burst of a cell will depend on the relationship between the 'surface' of its membrane and the 'volume' of its core, which can be any within the following limits:

(1) The actual surface of the cell must be bigger than the theoretical spherical surface that corresponds to the actual volume at each iteration step; otherwise the cell bursts.
(2) The actual surface of the cell must be smaller than the theoretical surface that corresponds to two equal spheres of half the actual volume at each iteration step; otherwise the cell divides, giving rise to two statistically equivalent cells.

We are aware that this also entails a crude simplification, because all processes of division should not lead to equal 'daughter cells'. But it seems to be a first and easy way to avoid some further suppositions (e.g., constant spherical shape, division when initial size is doubled, ...). Besides, it is important to stress that, in these conditions, cell growth could be observed without ending up in a division process; or, alternatively, there could be cell division without growth (just by membrane deformation). In any case, if the overall population of the internal species increases too fast in relation to the growth of the membrane, this is bound to cause the breaking of the cell due to water inflow.

The limits above are the conditions for stability of a cell in our model; i.e., they establish the range of possible states in which it will not break or divide. In mathematical terms, defining the parameter Φ as the ratio between the actual cell surface S_μ and the surface of an ideal sphere with the actual volume of the core:

$$\Phi = \frac{S_\mu}{\sqrt[3]{36\pi V_{\text{core}}^2}} \tag{4}$$

the conditions for stability become $1 \leq \Phi \leq \sqrt[3]{2}$. Moreover, in order to take into account the lipid membrane elasticity and flexibility, the model also includes two 'tolerance parameters' that enlarge the range of stability of Φ as follows:

$$1 - \varepsilon \leq \Phi \leq (1 + \eta)\sqrt[3]{2} \tag{5}$$

where ε and η are the burst and fission tolerance, respectively. Both parameters may be fixed, or change as functions of the membrane composition. The initial and default value in all the simulation runs reported below was set equal to 0.1, so the stability range becomes $0.9 \leq \Phi \leq 1.386$. However, in those cases in which the membrane varies its composition and contains molecules with different contributions to elasticity (see TaP case below), these two parameters are recalculated according to the summation:

$$\varepsilon = \sum_{i=1}^{\text{Membrane species}} \varepsilon_i \chi_{i_\mu}, \quad \eta = \sum_{i=1}^{\text{Membrane species}} \eta_i \chi_{i_\mu} \tag{6}$$

where χ_{i_μ} is the relative molecular fraction of each species in the membrane.

All simulations were carried out with bilayered membranes composed of a generic lipid L, whose molecular volume and head surface area were equal to $1.0\,\text{nm}^3$ and $0.5\,\text{nm}^2$, respectively (taken as typical values for an amphiphilic molecule). The contribution of lipids to the burst tolerance was set to 0.1. The peptides spanning the membrane were assumed to be α-helixes of, at least, 20 residues, with a larger surface area ($5\,\text{nm}^2 \times 2$ since we suppose that the peptide cuts across the bilayer) and a total cylindrical volume of $20.0\,\text{nm}^3$ (approx. thickness of the bilayer: $\ell_\mu = 4.0\,\text{nm}$). The contribution of each peptide chain to the burst tolerance was normally set to 0.1, like a lipid, or alternatively to +1.0 (or −2.0), in the cases we wanted to simulate an increase (or decrease) of membrane elasticity due to the inserted peptides. Further modelling assumptions in relation to transport/diffusion processes and a calculation of the aqueous equilibrium concentration for lipid molecules ($[L]_{\text{eq}} = 0.004\,\text{M}$) are included in the appendix. The values of kinetic constants and other parameters that remained fixed in all simulations are shown in Fig. 2.

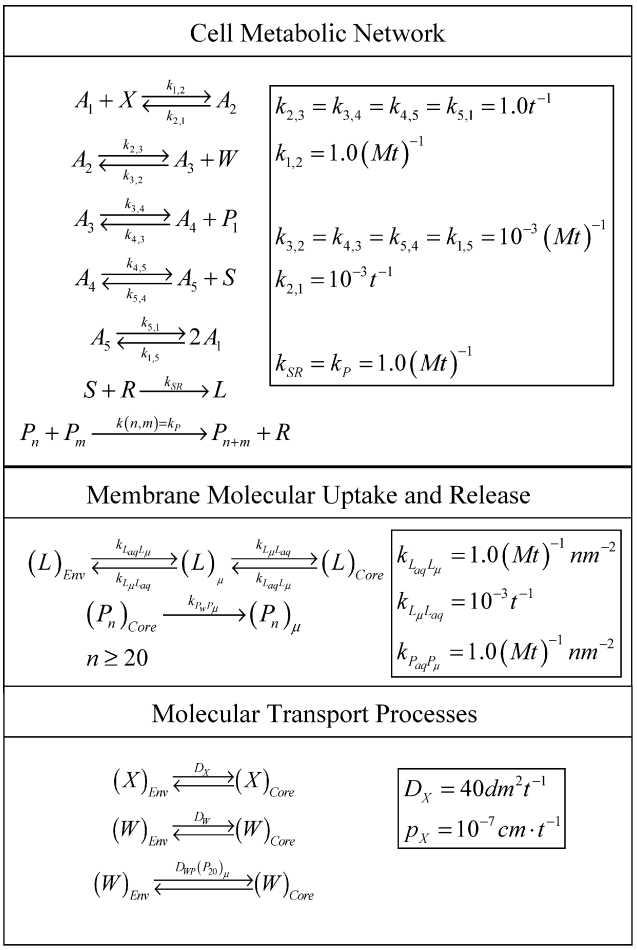

Fig. 2. Scheme table. Table of the complete cell reaction scheme with the kinetic and diffusion parameters kept constant in all reported simulations.

4. Results and Discussion

The previous general approach to cellular dynamics is applied in the present paper to study a particular problem of interest: the time behaviour of minimal 'self-(re-)producing' lipid–peptide cells as an important preliminary step towards the development of more elaborate models of autonomous *agent* cells. We focus on two main aspects that are relevant for the discussion: (i) the capacity of the internal network of reactions to determine the compositional properties of the boundary, and (ii) the dynamic consequences of the changes thereby induced in other system properties (membrane elasticity, permeability, . . .).

Thus, the first aspect we analysed with our simulation model was how the composition of the membrane (i.e., the ratio lipid-molecules/peptide-chains) is affected by the stoichiometric structure and reaction couplings of

the internal metabolic cycle. This analysis is important because it should demonstrate that, in our system, the type of boundary obtained is defined and can be modified, to a good extent, from *within*. This would also reflect part of the basic complementary relationship between compartment and internal network of reactions (in line with the original autopoietic claim (Maturana and Varela, 1973)).

4.1. CASE 1: Inert Polypeptides (IP)

We shall call 'inert polypeptides' those peptide chains that are not allowed to increase the osmotic tolerance (elasticity of the membrane) nor form channels for mediated molecular transport in the cell. These components can only contribute to increase the membrane surface, since they are assumed to be highly hydrophobic compounds, with a larger head area than the lipids. In such conditions, the accumulation of waste (W) during the cell cycle is crucial for cell survival. Fig. 3 shows the time course of the stability/viability factor (Φ) for cells with different W-permeability. Whereas, the permeability to the nutrient (D_x) did not alter substantially the dynamic behaviour of the system (it just caused an advance/delay

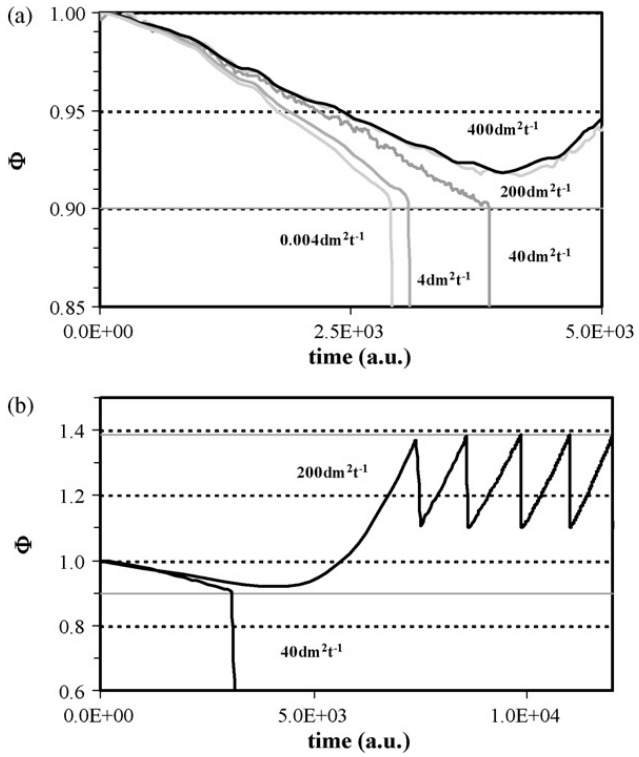

Fig. 3. Case 1: inert polypeptides (IP). Time course of the stability factor (Φ) for different values of the waste diffusion constant D_W. The upper plot shows a threshold around 40 $dm^2\,t^{-1}$. In the lower plot, a longer run for the case $D_W = 200\,dm^2\,t^{-1}$ is reported, showing the mother cell growing and splitting.

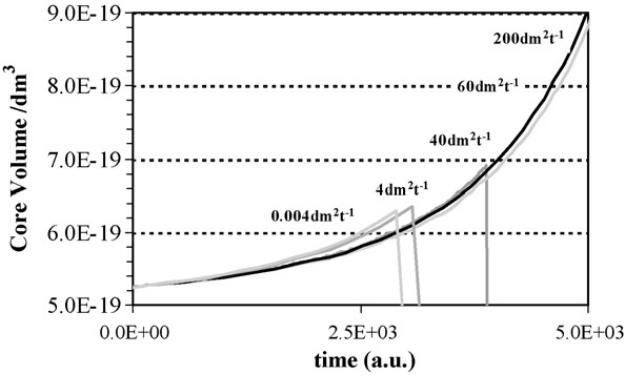

Fig. 4. Case 1: inert polypeptides (IP). Time course of the core volume for different values of the waste diffusion constant D_W.

in the first division time), we found a critical threshold value for D_w (around 40 $dm^2\,t^{-1}$). In fact, a higher W-permeability allows the cell to limit the increase of the core volume since it limits the growth of the internal concentrations and, thus, the flux of water from the environment. This is clear from Fig. 4, where the time course of the cell core volume (for different permeabilities) is displayed.

Focusing the attention on a relatively stable cell (the cell with $D_W = 200\,dm^2\,t^{-1}$), we investigated how the membrane composition changes over time. In the upper plot of Fig. 5, the time course of the relative lipid/polypeptide composition for the initial cell is reported, on the left-hand axis, while the membrane surface is on the right. As can be observed, that original mother cell splits seven times and, in spite of the change of its size, the membrane composition ratio keeps lower than the value 1/20 = 0.05, which is determined by the cell metabolic network stoichiometry. In fact, since R is at the same time a reactant for lipid synthesis and a byproduct of peptide condensation, for each P_{20} formed also 20 new L can be synthesized. Fig. 5(b) shows the internal concentrations of the species that can be exchanged between cell and environment. We observe that the concentration of substrate X in the aqueous cell core, set at 0.0 at the beginning, rapidly reaches a value slightly lower than the external (fixed) concentration 0.001 M, since X is continuously consumed by the metabolic cycle and fed from outside. On the other hand, since L is continuously produced, its internal concentration is slightly larger than the expected equilibrium value (0.004 M, see Appendix A). The concentration of waste in the core ($[W]_{core}$), instead, oscillates during the cell cycle. Actually, after each splitting, $[W]_{core}$ quickly increases, since the rate of W production remains constant, while the rate of its outgoing flux is halved, due to the membrane division. In Fig. 5(c) the time trend of some other compound concentrations is included. In

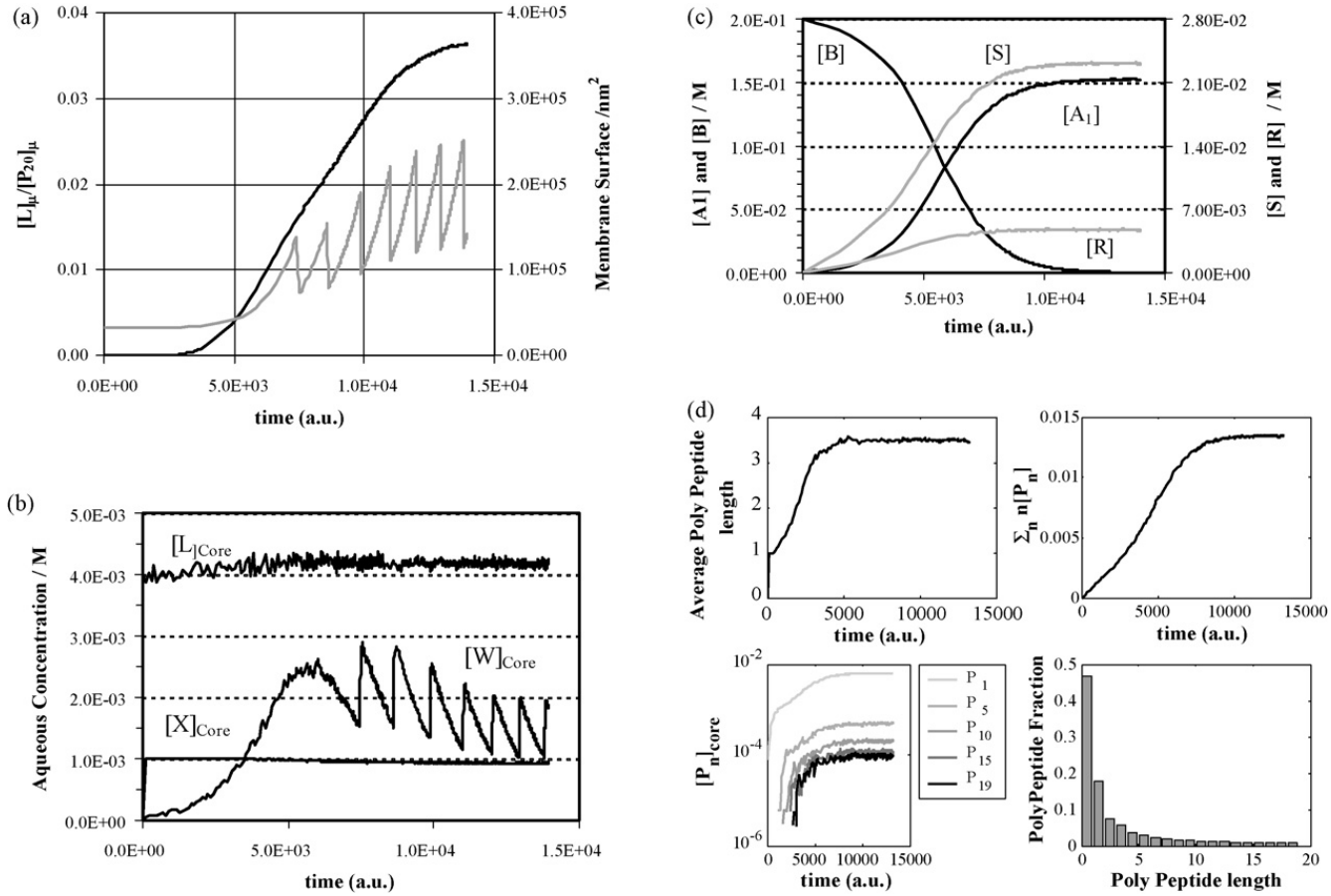

Fig. 5. Case 1: inert polypeptides (IP). Original mother cell long runs for the case $D_W = 200\,dm^2\,t^{-1}$: (a) the ratio between the lipid and the polypeptide membrane concentration is reported against time, on the left-hand axis, while the membrane surface variation is plotted on the right; (b) change in time of [*W*], [*L*] and [*X*] in the core of the cell; (c) change of other compound concentrations (d) analysis of polypeptides in the mother cell aqueous core: in the upper left corner average polypeptide size against time; in the upper right corner the overall aminoacid concentration in the core; in lower left corner concentration time courses of different size polypeptides, in the lower right corner stationary size distribution of polypeptides.

all of these a similar behaviour was observed, tending to constant asymptotic values (like for species A_i ($i = 2, \ldots 5$)—data here not shown). Only the buffer concentration continuously decreases in time for, at each splitting, the buffer molecules are divided between daughter cells and they are not synthesized any longer. The peptide population distribution and internal concentration profiles for different oligomers are shown in Fig. 5(d).

Finally, the increase of the overall cell population in these conditions is also reported (Fig. 6a). We analysed the size distribution of the final cell population and found that it is Gaussian (Fig. 6b). It is not surprising to observe a continuous growth of the cell population, because the environment works as an unlimited source of nutrients. Nevertheless, that continuous growth in number does not correspond to a continuous cell size increase, as clearly shown by the time course of the membrane area of the original cell in Fig. 5(a). In fact, each cell reaches a steady state where all internal concentration of substances are constant except for the waste that oscillates continuously, see Fig. 5(b). These oscillations, due to the unbalance between the production and the outwards flux of waste molecules, are then responsible for the timing of the cell cycle and the steady size of the cell, since they affect the flux of water from the outside and, thus, the core volume increase. As a consequence of this, the cell could regulate its size and the splitting time if it were able to finely tune the rate of waste flux.

4.2. CASE 2: Tolerance-altering Polypeptides (TaP)

In the second scenario explored, we consider that the polypeptides inserted in the lipid bilayer may have an effect in its elastic properties, in the sense that they can increase (or decrease) the flexibility of the membrane (i.e., its 'tolerance'). This is interesting not only because it is more realistic, but also because it gives the system the possibility to change the conditions for its own stability or survival in our model (i.e., the range of values in which no burst or division process will take place). The

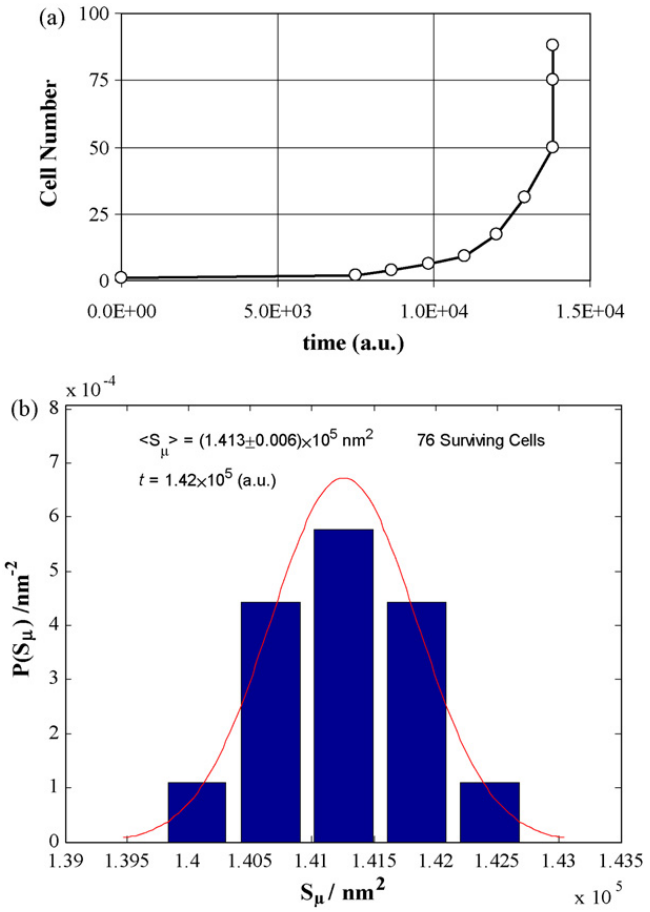

Fig. 6. Case 1: inert polypetides (IP). (a) Long runs for the case $D_W = 200\ dm^2\ t^{-1}$: total number of cells is reported vs. time. In the lower graph (b), the size distribution of the final population of surviving cells is portrayed (in terms of the total surface area of the membrane), showing that it follows a Gaussian trend.

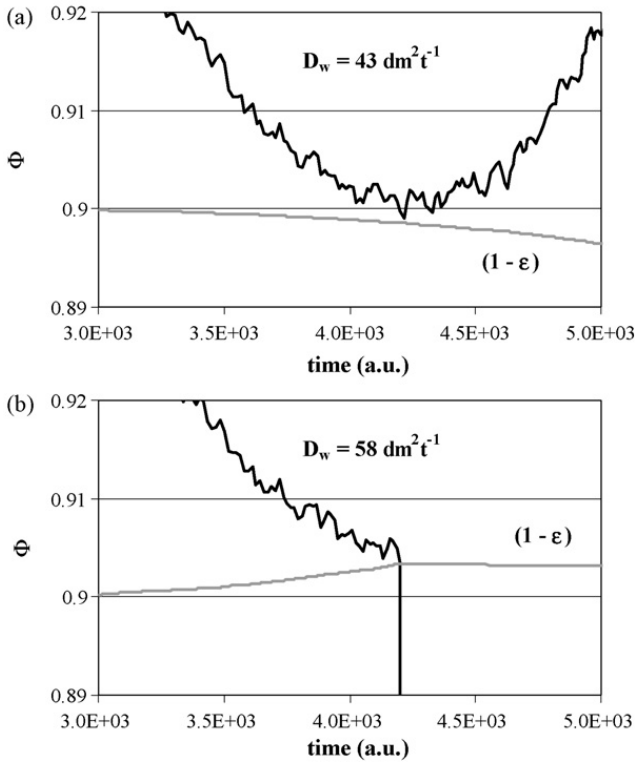

Fig. 7. Case 2: osmotic tolerance-altering polypeptides (TaP). Time course of the stability factor (Φ) for peptides with positive and negative contribution to the tolerance. (a) In the upper graph $\varepsilon_P = \eta_P = +1.0$ and $D_W = 43\ dm^2\ t^{-1}$: a small decrease in the D_W threshold was found (i.e., if the cell gains elasticity it can cope with lower permeability values without undergoing an osmotic burst); (b) in the lower graph, $\varepsilon_P = \eta_P = -2.0$ and $D_W = 58\ dm^2\ t^{-1}$: the plot shows the increase in the D_W threshold (i.e., if the cell becomes 'more rigid' it cannot cope with similarly low permeability values).

modification in the viability range happens as the simulation is run, in so far as the composition of the membrane – or lipid–peptide ratio – does not reach its asymptotic value. In present simulations we tried two different cases: one in which the elasticity contribution of the polypeptides was positive (i.e., the range of stability widened, until new limits were eventually established in the system) and a second one in which it was negative, making the cell more rigid (i.e., leading to a narrower stability range in comparison with the previous, 'inert' case). In the two cases, both the 'osmotic' and 'burst' tolerance parameters were altered, by setting $\varepsilon_{P_n} = \eta_{P_n} = +1.0$ (or, alternatively, $=-2.0$).

These changes have different types of effect on the dynamics and properties of the system, depending on the time window of the analysis. At relatively short times, we can report a small but noticeable effect on the threshold for D_W, as shown in Fig. 7(a and b). In fact, in the critical time range (around 4000 t) in which the initial 'mother' cell gets dangerously close to the osmotic crisis limit, the membrane fraction of P_{20} is still rather low, but just big enough to start having positive/negative effects on the cell stability. On the other hand, at much longer times, after the initial cell has undergone a series of divisions and produced a collection of similar cells, we can analyse the statistical differences found in the population in each case. Fig. 8 shows a comparative study of the three cases explored: inert (IP), positive (+1) and negative (−2) tolerance effect. We chose a higher value in the negative case to push cells closer to their absolute limit for viability, the spherical shape ($\phi = 1$). In the upper plot of the graph, the time courses of the membrane surface area standard deviations σ_{S_μ} are reported for large values of time. It is very clear, given that spikes in the σ_{S_μ} time trends correspond to splitting events, that 'rigid' cells split faster than 'more elastic' ones, since they reach the division condition earlier (see Fig. 8b). The maintained difference between standard deviations in each of the cases (notice the arrows in Fig. 8a, for instance) reflect the fact that the distribution of sizes in the population is

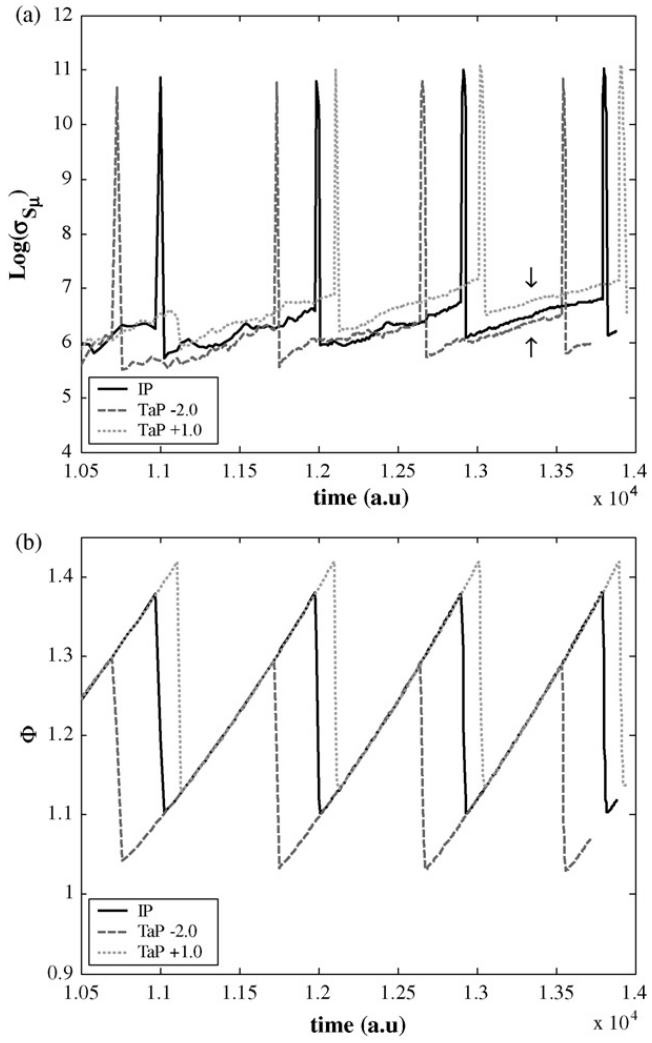

Fig. 8. Case 2: osmotic tolerance-altering polypeptides (TaP). Comparison between TaP and IP cases. (a) In the upper graph the time courses of the membrane surface area standard deviations σ_{S_μ} are reported for large values of time. All the simulations were carried out setting $D_W = 200\,dm^2\,t^{-1}$. Spikes in the σ_{S_μ} time trends correspond to splitting events. The difference in the values of the standard deviation at a certain point in time (marked with the arrows in the graph) show, as expected, that the distribution of sizes is wider in the 'elastic' case (+1) than in the 'rigid' one (−2). We can also say (data not shown) that in the former case vesicles reach larger sizes than in the latter. (b) In the lower graph the time courses of the membrane viability coefficient Φ are reported for long times, once reproduction cycles are regularized.

wider in the elastic case than in the rigid one, as it is to be expected. Results not shown here also indicate that the mean size of the elastic vesicles is larger than that of the rigid ones.

4.3. CASE 3: Permeability-altering Polypeptides (PaP)

Finally, we studied how the presence of polypeptides in the lipid bilayer could change its permeability to a certain compound that is critical for the osmotic stability of the system. Given our previous results in CASE 1 (the scenario with inert peptides), according to which the rate of disposal of the waste product from the proto-metabolic cycle (W) was crucial in that sense, we analysed the possibility that peptide chains could assist in the passive transport of that byproduct. So we assume here that polypeptides of a certain critical length (20 residues) are able to span the bilayer and form selective channels (or pathways, more strictly—since channels would require the self-assembly of different α-helices) for waste molecules, while the background or intrinsic permeability of the lipidic membrane to W is set very low ($D_W = 0.004\,dm^2\,t^{-1}$), in order to observe more clearly the peptide effect. Also in this case, a threshold was found in the values of the aided or mediated diffusion coefficient: only if $D_{WP}/dm^2\,t^{-1} \geq 1.6$ could the original mother cell remain stable after 4000 t, as shown in the upper plot of Fig. 9(a). The threshold now is lower than in the IP scenario because, in this case, the probability for diffusion processes is multiplied by the number of channels present in the membrane.

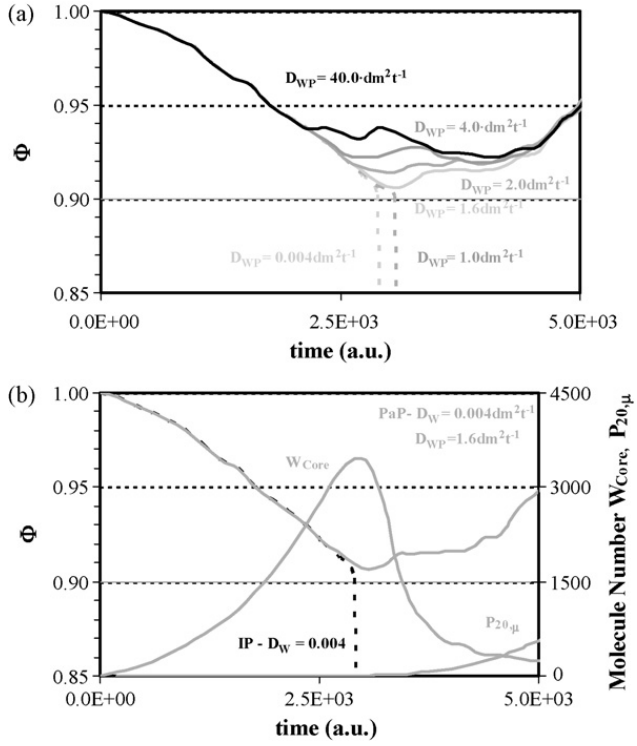

Fig. 9. Case 3: permeability-altering polypeptides (PaP). Time course of the stability factor Φ for different waste diffusion constants (D_{PW}): (a) the upper plot shows a threshold below $1.6\,dm^2\,t^{-1}$. (b) In the lower plot, a comparison between the IP cell ($D_W = 0.004\,dm^2\,t^{-1}$, black line) and the PaP cell ($D_W = 0.004\,dm^2\,t^{-1}$, $D_{PW} = 1.6\,dm^2\,t^{-1}$, grey line) is reported on the left axis; the time evolution of the number of molecules of W and P_{20} in the core volume and in the membrane are reported, on the right axis (grey lines).

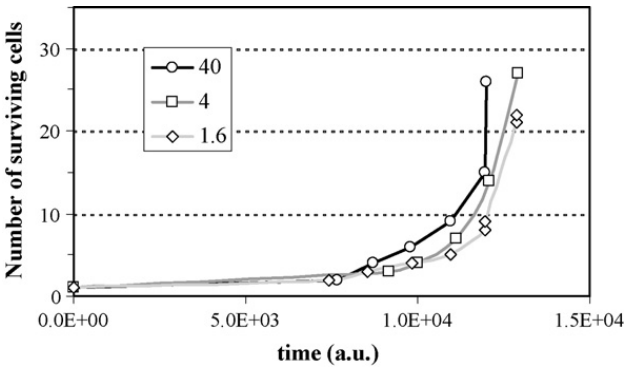

Fig. 10. Case 3: permeability-altering polypeptides (PaP). Time course of the number of surviving cells for different waste diffusion constants (D_{PW}) above the threshold: $D_{PW} = 40.0\,dm^2\,t^{-1}$ (circles), $D_{PW} = 4.0\,dm^2\,t^{-1}$ (squares) and $D_{PW} = 1.6\,dm^2\,t^{-1}$ (diamonds).

A comparison between IP and PaP cases is reported in Fig. 9(b). Up to the incorporation of peptides into membrane, the time courses of the stability coefficient (Φ) are completely equal for both. But as soon as alternative W transport pathways are formed in the membrane, the amount of waste in the cell aqueous core rapidly decreases. Accordingly, the core volume growth rate is decreased. Therefore, if a cell is able to produce the peptide chains fast enough so that their insertion in the membrane induces a relatively quick change in the release of the waste product, an eventual osmotic crisis can be avoided. Within a population of diverse lipid and lipid–peptide cells, this could confer an obvious evolutionary advantage to those cellular systems that manage to produce the channels rapidly or effectively enough. Quite interestingly, in these conditions it is not the cells that grow more quickly that have the advantage (see results in Fig. 10): stability or robustness as a cellular entity is crucial (and implies, precisely, control on growth and division rates). So our approach also allows to investigate (pre-Darwinian) evolutionary dynamics in which the capacity for robust 'self-maintenance' or 'self-production' of the systems involved (i.e., their organizational structure) may still be more critical than their reproductive success. Further work along these lines (instead of following the traditional approaches of 'molecular replicator' models) should be carried out to provide a more complete understanding of the origins of biological evolution mechanisms.

5. Final Remarks and Perspectives

The main issue we were exploring in this paper is to what extent the insertion of internally produced peptide chains in the membrane of a cell could have immediate advantages for its viability/stability. From our simulation results, we can conclude that this is the case. When peptide chains are so that they alter the permeability of the boundary to certain compounds (in particular, the waste product of the metabolic cycle) the cell as a whole gains the possibility to modify or control – even if it is in a very simple way – its growth, standard size, splitting time, . . . avoiding an eventual osmotic burst. In the scenario of the tolerance altering polypeptide (TaP), we can even say that the system is dynamically changing the conditions that define its own viability (the limit values for its stability coefficient). So the different effects here analysed could be interpreted as examples of endogenous changes in the nature of the boundary conditions of the system leading to more robust or efficient self-maintenance; in other words, as candidates for emergent 'functional actions' that could be at the roots of basic autonomous behaviour.

However, it is not clear to us whether this type of changes, even if they are internally produced, can be said to be 'actions' in a proper sense. Somehow, the changes follow from the new organizational structure of the system and the thermodynamic drive to arrive at the corresponding stationary state. Even if the cell is able to keep in a non-equilibrium state, the changes provoked in its boundary conditions do not involve the carrying out of non-spontaneous (endergonic) processes. For instance, it would be very interesting to investigate the possibility that those peptide chains become mechanisms of *active* transport, where it is more obvious that the cell is performing an action (working against the gradient).

The implementation of this kind of transport mechanisms (pumps) involves a more complex situation, in which energy transduction processes have to be taken into account. But it would allow the system to have a more flexible and selective control on concentration gradients (inflow/outflow of different compounds). In addition, it would open the way to explore primitive versions of 'bioenergetic' cells (cells with chemical and chemiosmotic energy currencies (Skulachev, 1992; Harold, 2001)), as well as to reconsider the need to uptake an externally provided high-free-energy compound, X (which implies a strong heterotrophic requirement in our present scheme).

Therefore, further elaborations of the present model would be needed to give an account of the appearance and development of basic autonomous agents. Nevertheless, the scenario and conditions here analysed are prerequisites for that transition. All together, we consider that this new simulation platform and approach to minimal cell dynamics constitutes a suitable tool

to advance in our present and future efforts of understanding the most elementary forms of autonomy and agency.

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Appendix A. Modelling Assumptions for Transport/Diffusion Processes

The probability for the uptake of a generic molecule Z from an aqueous domain (the environment or the core) to the lipid bilayer was calculated as the product of a constant $k_{Z_{aq}Z_\mu}$ times the aqueous molecule concentration $[Z]_{aq}$ (where aq can be Env or Core) multiplied by the membrane surface area S_μ. On the other hand, the backward process probability (i.e., the release of a membrane component to an aqueous domain) was assumed proportional to the corresponding constant $k_{Z_\mu Z_{aq}}$ times the number of molecules of that component in the bilayer. In the case of the exchange of lipids from and to a membrane made by lipids and peptides, the aqueous equilibrium concentration of the lipid molecules can be obtained by equating the forward and backward process probability ($k_{L_{aq}L_\mu}[L]^{Eq}_{aq}S_\mu = k_{L_\mu L_{aq}}n_{L_\mu}$):

$$[L]^{Eq}_{aq} = 2\left(\frac{k_{L_\mu L_{aq}}}{k_{L_{aq}L_\mu}}\right)\frac{n_{L_\mu}}{n_{L_\mu}\alpha_L + n_{P_\mu}\alpha_P} = 2\left(\frac{k_{L_\mu L_{aq}}}{k_{L_{aq}L_\mu}}\right)\frac{1-\chi_{P_\mu}}{\alpha_L + (\alpha_P - \alpha_L)\chi_{P_\mu}}$$

where χ_{P_μ} is the peptide molecular fraction and $S_\mu = (\alpha_L n_{L_\mu} + \alpha_P n_{P_\mu})/2$. Since we typically set $k_{L_{aq}L_\mu} = 1.0\,M^{-1}\,t^{-1}\,nm^{-2}$ and $k_{L_\mu L_{aq}} = 0.001t^{-1}$, the lipid equilibrium concentration $[L]^{Eq}_{aq}$, both in the cell core and the external environment, can be estimated equal to $2k_{L_\mu L_{aq}}/(k_{L_{aq}L_\mu}\alpha_L) = 0.004\,M$ for a pure lipid membrane, or when χ_{P_μ} is negligible. We should also mention that, while all the reported concentrations are molar concentrations, the measure unit of time was left undefined and, in principle, all simulations should be reported against time/$k_{L_\mu L_{aq}}$ (although in the plots we preferred reporting the arbitrary unit notation (a.u.)).

The uptake of peptide chains from the core to the membrane is assumed to be an irreversible process, since peptides with a length greater than 20 are supposed to be highly hydrophobic compounds (this is quite reasonable if we assume that the aminoacid is itself hydrophobic: for instance, alanine or leucine (Oliver and Deamer, 1994)). The process takes place similarly to the lipid uptake from an aqueous domain with the same kinetic constant $k_{P_{Core}P_\mu} = 1.0\,M^{-1}\,t^{-1}\,nm^{-2}$.

Regarding the transport across the cell membrane, we assume that a pure lipid bilayer is only permeable to a few species (in the following simulations: to the nutrient X and waste W molecules). In general, for a generic membrane penetrating substance Z, a spontaneous diffusion process can take place from one to the other aqueous solution, driven by the concentration gradient: $g_Z = ([Z]_{Env} - [Z]_{Core})/\ell_\mu$. The resulting molecular flux will be inwards or outwards depending on whether g_Z is greater or smaller than zero. In both cases, the process probability will be proportional to a diffusion constant D_Z, the absolute concentration gradient $|g_Z|$ and the surface of the membrane. If polypeptides P_n are present in the cell membrane, then selective mediated transport processes can also occur, with a probability that is calculated as before but multiplied by the number of present polypeptides and by a different (typically higher) diffusion constant D_{ZP}. This additional (passive but mediated) transport mechanism will induce faster in–out exchanges of a substance, or make the membrane permeable to other (initially impermeable) substances in the model.[3]

Finally, it is important to recall that all the simulations reported in this paper start from pure lipid spherical vesicles, with a 50 nm radius (that is, with a membrane made of 125,600 molecules). The membrane lipid uptake and release processes are at equilibrium since the lipid aqueous concentration is set equal to $[L]_{Env} = [L]_{Core} = 0.004\,M$ in the core and in the external environment. Outside and inside the cell an osmotic buffer is also present at high concentration ($[B]_{Env} = [B]_{Core} = 0.2\,M$), to reduce osmotic shocks and the possibility that osmotic crises occur due to random fluctuations in the lipid uptake and release processes (Mavelli and Ruiz-Mirazo, 2007). The external con-

[3] We should remark here that in our previous work (Mavelli and Ruiz-Mirazo, 2007) the reported values of the membrane transport diffusion constants implicitly included the factor S_μ/ℓ_μ; therefore, they should be multiplied for the factor $4\pi R^2/\ell_\mu = 7.85 \times 10^3$ to be comparable with $R = 50$ nm spherical vesicles.

centrations of X and W are supposed to be constant $[X]_{Env} = 0.001$ M and $[W]_{Env} = 0.0$ M, assuming that the environment behaves as a source of nutrients and a sink of waste, respectively. In the cell core, only the metabolite A_1 is present at the beginning, with a concentration set equal to $[A_1]_{Core} = 0.001$ M, so as to balance the osmotic pressure. In addition, the values of the all kinetic and diffusion parameters kept constant during the simulations performed are shown on the table reported in Fig. 2. It is noteworthy that these values are of the same order of magnitude than standard real data.

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