Dynamics of pore synthesis and degradation in protocells

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Abstract. Liposomes have found countless applications as microreactors or for studying the evolution of protocells. However, to keep reactions ongoing, exchange with the environment is required. Based on experiments with nanopores expressed by an enclosed gene expression system, we developed a model describing the observed growth dynamics quantitatively. The model depends on one parameter only and allowed estimations of hitherto unknown parameters: the diffusion coefficient of amino acids through a single pore and the initial amino acid concentration. The long-term consequences of different degradation mechanisms are also discussed: we found a surprisingly sharp threshold deciding on the question of survival of the protocell.

Contents

1. Introduction 2
2. Setup of the basic model 3
3. Discussion 4
4. Protein degradation 6
5. Summary 9
Acknowledgments 9
References 9

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1. Introduction

A large number of bio-molecules can be catalysed from inorganic components in an aqueous medium (pond or ocean) [1–4]. The simultaneous presence of functional combinations of molecules such as ribozymes [5] or nucleotides together with proteins and enzymes is a prerequisite during the genesis of the cell as their function relies on cooperativity. Consequently, the absence of even one partner would prohibit the function and thus be negative for selection and survival. Hence, we assume that the first protocell emerged as an entire functional unit, which is subsequently selected as a whole.

Once polynucleotides and enzymes are present at sufficient concentration, some of those molecules may accidentally get enclosed in a spontaneously forming liposome defining such functional units [6]. Liposomes have been explored previously as model ‘protocells’ [6–13]. It is conceivable that a very large number of such protocells were created with various combinations of molecules. If only one of those protocells is ‘accidently’ able to self-reproduce, its copies may improve and dominate the medium on a long-term scale. On the other hand, however, a price has to be paid: cell membranes are highly impermeable to most bio-molecules and exchange with the environment is largely blocked. As a consequence, the protocell is condemned to equilibrium death. This paper focuses on one possible way out of this dilemma, although important questions are not addressed: where do the presupposed DNA and the enzymes come from, how does a protocell divide, and how do collective phenomena improve the survival of each protocell?

We set up a small theoretical model to address the general problem of feeding the protocell with nutrients by assuming the presence of a complete gene expression system and DNA or RNA encoding nanopores within the vesicle.

The model (figure 1) was inspired by the experiments of Noireaux, Libchaber, Monnard, Luptak and Deamer [6, 14, 15] and is quantitative, as it fits well the experimental data. It is based on liposomes in crystal phase which were filled with, among others, cell extract, plasmid DNA encoding alpha-haemolysin pores (diameter 1.4 nm, molecular mass cut-off 2–3 kDa and monomer sequence length 294 amino acids) and the fluorescent molecule GFP (on the same plasmid). As the pores and GFP were co-expressed, the fluorescent signal allowed measurement of the pore quantity; the system produced pores until the nutrients were depleted. The synthesized α-haemolysin monomers get inserted into the membrane and are self-assembled spontaneously to form pores, creating selective permeability across the membrane. The liposome is then ‘fed’ with new nutrients and the protein synthesis reaction increases again, which is described later.

Changes in the volume of the liposome (diameter approximately 10 µm) are not considered in [14] and in the presented model. Furthermore, due to the size of the liposome and the high diffusion speed of amino acids, fluctuations are also neglected in our basic model. Extension of the proposed model to accommodate this aspect would be straightforward, however. Fluctuations and noise dominate cellular dynamics at the molecular level. Furthermore, the cell extract used in [14] accounts for a large number of molecules and enzymes. Hence, the noise due to gene expression and translation, diffusion, as well as pore assembly and disassembly is averaged as the time scale of the described dynamics is much larger.

The model is quantitative, robust and therefore an appropriate base for further quantitative extensions towards an artificial cell: it would allow studying the influence of controlled cell membrane permeability on the regulatory feedback loops yielding oscillatory behaviour as
Figure 1. Sketch of a protocell model containing an $\alpha$-haemolysin pore coding plasmid $\Pi$, mRNAs, ribosomes (R), $\alpha$-haemolysin pores ($\alpha$H) and cell extract.

described, e.g., by the ‘repressilator’ model [16]. New unexpected features emerge from multicellular systems as synchronization of cellular oscillations [17].

Numerous new features can be obtained by including a pore insertion probability smaller than one. In this case, the probability becomes susceptible to the electrostatic potential differences across the membrane [18]. Degradation and insertion delay can lead to oscillatory behaviour. By integrating calcium-sensitive actin cortex, the membrane tension and consequently mechano-sensitive pores may get modulated as well. Modulated calcium and nutrient concentrations lead to modulation of gene expression [19]. Transporters of amino acids can actively move amino acids in a specific direction across the membrane, using the cross-membrane ion gradient, e.g. sodium [20], as the energy source. Together with passive ion channels, the protocell becomes a highly controllable microreactor for molecular essays down to the single molecule level. Further possibilities consist in the expression of glycine-controlled chloride channels. These channels can be open or closed in milliseconds by the external application of glycine. The on–off switch of ion transportation in combination with sodium chloride symporter allows modulating rapidly the electro-chemical potential across the membrane controlling transporters. This may be used to externally control the gene expression inside the protocell. Therefore, protocells with switchable pores, e.g. pH- or mechano-sensitive, can feature simple feedback loops and rudimentary perception of the environment. This would allow us to couple gene expression to mechanical cues in artificial bottom-up systems on the way towards the artificial cell and tissues.

2. Setup of the basic model

An adequate supply of nutrients is achieved by the creation and insertion of pore proteins in the protocells’ membrane. The resulting change in concentration of the amino acids, $\dot{c}_{k, in}(t) = \dot{N}_k(t)/V$, with the number of amino acids $N_k$ of type $k$ (in units of mole/l), of a protocell is expressed as

$$\dot{c}_{k, in}(t) = -\frac{1}{V} \left[ b_k \dot{P}(t) + J_k(t)ANP(t) \right], \tag{1}$$
where $N_A$ is the Avogadro constant and $b_k$ is the number of amino acids of type $k$ required to build the pore proteins $P$ (in units of mol). The supply of amino acids is served by all inserted pore proteins, with the cross-sectional area $A$ of the pore channel, which enables a flux $J_k$ of new amino acids entering from the surrounding solution into the protocell. Assuming a linear concentration gradient leading to diffusive transport of amino acids inside the pore channel, the flux can be determined from

$$J_k(t) = -D_{k,\text{eff}} \hat{n} \cdot \nabla c_k(t) = -\frac{D_{k,\text{eff}}}{L} \left[ c_{k,\text{out}} - c_{k,\text{in}}(t) \right]$$

with the length $L$ of the pore channel, the concentrations $c_{k,\text{in}}$ and $c_{k,\text{out}}$ of amino acids inside and outside the protocell, the trans-membrane effective diffusion coefficient $D_{k,\text{eff}}$ of the amino acids and the outside normal vector of the membrane $\hat{n}$.

In the scenario mentioned above, pore proteins are only expressed as long as amino acids are abundant. The expression process slows down if the concentration of even one type of amino acid runs low and eventually stops once the concentration of the amino acid reaches zero. The kinetics of each specific amino acid in equation (1) cannot be considered, as the precise concentrations of the feeding medium are unknown from the experiments of Noireaux and Libchaber (amino acid concentrations approximately $10–100 \mu \text{m}$ [14]). However, it turned out that the sum of the concentration of amino acids is sufficient: $c_{\text{in}}(t) = \sum_k c_{k,\text{in}}(t)$. For an extension, the buffer composition can be refined based on equations (1) and (2).

In our protocell messenger RNAs (mRNAs) are permanently produced, degraded (ribonucleases) and recycled. The mRNA concentration has been shown to stabilize rapidly [21], so we can assume a constant mRNA concentration for our model. We suppose that there is a sufficiently high concentration of the ribonucleotide (building blocks of mRNA) mixture, and assume saturated enzymes with an mRNA synthesis rate of approximately 10 base pairs per second [22]. $\alpha$-Haemolysin is a pore-forming protein, which consists of self-assembling monomers forming a heptamer [23]. The synthesis of $\alpha$-haemolysin monomers happens with a turnover rate of one to two amino acids per second, which corresponds to an assembling time of 5–10 min per $\alpha$-haemolysin heptamer. The enzymatic reaction links a reversible binding and an irreversible synthesis step. Hence, the reaction follows the Michaelis–Menten kinetics:

$$\dot{P}(t) = \frac{v_{\text{max}}}{N_A} \frac{c_{\text{in}}(t)}{K_M + c_{\text{in}}(t)} = r \frac{c_{\text{in}}(t)}{1 + c_{\text{in}}(t)/K_M}$$

with the amount of pore proteins $P$, the maximum reaction rate $v_{\text{max}}$, the Michaelis constant $K_M$ and the reaction activity defined by $r = v_{\text{max}}/(N_A K_M)$ of the protocell. Combining equations (1)–(3) in the low-concentration approximation, $c_{\text{in}}(t)/K_M \ll 1$, therefore $\dot{P}(t) = r c_{\text{in}}(t)$, one obtains for the time-dependent amount of pore proteins:

$$LV \ddot{P}(t) + Lbr \dot{P}(t) + (\dot{P}(t) - rc_{\text{out}}) D_{\text{eff}} A N_A P(t) = 0.$$  (4)

3. Discussion

By rescaling the parameters and variables in equation (4), it becomes clear that only one parameter is relevant (time dependence omitted for clarity):

$$\frac{d^2}{dt^2} \tilde{P} + \frac{d}{dt} \left( \tilde{P} + \frac{1}{2} \dot{\tilde{P}}^2 \right) - f^2 \tilde{P} = 0$$  (5)

New Journal of Physics 14 (2012) 103008 (http://www.njp.org/)
Figure 2. The amino acid concentration inside the protocell is shown, which is directly coupled to the membrane pore proteins via $\dot{P}(t) = r c_{\text{in}}(t)$. Small values of $f$ (dashed) yield an increasingly pronounced depletion of basic components, resulting in a strongly reduced building rate of new pores. For $f = 0.1$ the short-, intermediate- and long-term domains are shown as highlighted areas.

with the coefficient $f = \sqrt{VDc_{\text{out}}}$ depending on the reduced volume $\tilde{V} = V/b$ and the reduced transport coefficient $\tilde{D} = D_{\text{eff}}AN_{A}/(Lbr)$ and we solve the equation for the $1/D$-rescaled amount of pore proteins $\tilde{P}$ with the rescaled time $t \rightarrow rt/\tilde{V}$.

Equation (5) has similarity to the harmonic oscillator, although without extensions it cannot provide oscillatory solutions because of the sign of the restoring force term, which results in a constant $\dot{\tilde{P}}$ for large times. The second term describes friction and is responsible for a permanent energy loss and damping of the dynamics.

For $f \lesssim 0.1$, which is equivalent to choosing a small protocell volume $V$, a low outer concentration $c_{\text{out}}$ and a high reaction activity $r$ (see the definition of $f$ above), the numerical solution displays, in general, characteristic behaviour on short, intermediate and long time scales. For short time scales and small initial concentrations, the continuous depletion of amino acids dominates the dynamics of the system. Assuming that the external concentration remains constant, the model only depends on the constant parameter $f$ determining the shape of the pore concentration graph over time. Therefore, for short time scales, or with negligible trans-membrane transport, equation (4) is approximated by an exponential solution, $P(t) = (c_{\text{out}}V/b)\{1 - \exp[-b r/(Vt)]\}$ (figure 2, the first highlighted area).

The intermediate time scale is dominated by the transient depletion of basic components in the protocell. In the beginning, the protocell consumes the stock of basic nutrients for the assembly of pores. Once a few pores have been formed through the membrane, new basic components are slowly provided to the protocell. Eventually, the system reaches the point of dynamic equilibrium where the loss and gain of basic components are balanced. In the case of $\dot{c}_{k,\text{in}}(t) = 0$

4 The simulation was done with Mathematica 8, Wolfram Research, Inc., USA.

New Journal of Physics 14 (2012) 103008 (http://www.njp.org/)
during the intermediate time scale, the pore protein amount increases exponentially:

\[ P(t) = \frac{c_{\text{out}} V}{b} \exp \left( -\frac{t}{\tau} \right), \quad \text{with} \quad \tau = \frac{b}{J_{\text{max}} AN_A}. \]  

(6)

Here the parameters determining the time scale \( \tau \) can be extracted well since the equation is independent of the reaction activity \( r \). \( J_{\text{max}} = D_{\text{eff}} c_{\text{out}} / L \) corresponds to the maximum possible flux, cf equation (2), through a membrane pore. The time scale \( \tau \) gives the effective diffusion coefficient \( D_{\text{eff}} \), which is the only unknown parameter. In addition, we also obtain from experimental data \( c_{\text{out}} V / b \), corresponding to the number of \( \alpha \)-haemolysin pores that can be produced by the initial stock of basic components within a permanently closed protocell volume.

With the growing number of membrane-bound pore proteins, the influx of basic components increases and eventually surmounts the loss due to pore protein synthesis and \( c_{\text{in}}(t) \) increases again. The inner concentration approaches homoeostasis with the outer concentration on large time scales. Using \( c_{\text{in}}(t) \approx c_{\text{out}} \) in equation (3) the low-concentration approximation, the increase of pore proteins for large time scales becomes simply \( P(t) = r c_{\text{out}} t + \text{const.} \)

In figure 2, the kinetics of the inner concentration, \( c_{\text{in}}(t)/c_{\text{out}} = \dot{P}(t)/(rc_{\text{out}}) \), is plotted for different values of \( f \). It shows the influence of the specific reaction activity \( r/V \). For smaller \( f \), the basic components are steadily depleted for an increasing time span, which may lead to protocell death if activity is stopped for a time period.

This separation of time scales is well suited to the determination of the diffusion constant of amino acids through the pores, since the sensitivity of the model to the parameter values is dependent on \( f \) and increases for smaller values. With this goal in mind, it is advantageous to design the experiment for a small \( f \) in order to optimize the separation of the time scales and maximize the duration of the short and intermediate time scales.

In figure 3, we fitted the model curve to the experimental data by the mean-square method using the following parameters from [14]: \( L \approx 10 \text{ nm}, A \approx 1.8 \text{ nm}^2, V \approx 4.2 \text{ pl} \) and \( r \approx 7.1 \times 10^{-10} \text{ pl s}^{-1} \). We note that \( b = 3794 \) represents the number of amino acids to build one pore-heptamer including seven attached enhanced GFPs. By fitting the curves for the evolution of membrane pore proteins, our model allowed us to determine the initial inner amino acid concentration of the vesicle and the hitherto unknown effective diffusion coefficient of amino acids through a pore: \( c_{\text{out}} \approx 2.3 \text{ mM}, D_{\text{eff}} \approx 2.2 \times 10^{-15} \text{ m}^2 \text{ s}^{-1} \). Furthermore, the predicted concentration of our model is slightly higher than the indicated amino acid concentration in [14].

4. Protein degradation

The effect of protein degradation, e.g. by proteases, or more general protein activity loss of the dynamics of our system has not been thoroughly investigated experimentally so far. Therefore, assumptions have to be made here. Long-term behaviour including protein activity loss may be integrated into the model by adding hypothetical terms for zeroth-order or first-order degradation.

Zeroth-order degradation is described by a constant degradation rate of pore proteins, \( \dot{P}_{\text{deg}}(t) = -\lambda_0 \), where first-order degradation is proportional to the number of undegraded pore proteins, \( \dot{P}_{\text{deg}}(t) = -\lambda_1 P(t) \).

However, experimental studies of protein degradation, e.g. driven by AAA+ proteases ClpXP and ClpAP, is likely to follow Michaelis–Menten kinetics with small Michaelis constants.
comparison of the experimental data from Noireaux and Libchaber[15] to the presented model is shown here ( \( \tilde{\lambda}_1 = 0, \tilde{\lambda}_0 = 0, f = 0.47 \) (see footnote 4). The dotted lines represent the maximum error of the model when varying \( c_{\text{out}}, D_{\text{eff}}, r \) and \( V \) by 10%. The inset shows the evolution of pore proteins inside the protocell including protein degradation for varying \( \tilde{\lambda}_1, f = 0.3 \) and \( \tilde{d} = 0 \). The data were obtained from [14].

\( K_{\text{M,deg}} \) (approximately 1–100 nM), resulting in a pronounced zeroth-order degradation over a wide range of protein concentrations [21, 24, 25]. With increasing protein concentration, small Michaelis constants \( K_{\text{M,deg}} \) lead to faster saturation of the degradation rate. Zeroth- and first-order degradation are the limit cases for high protein concentrations, \( P(t) \gg K_{\text{M,deg}} \), and low protein concentrations, \( P(t) \ll K_{\text{M,deg}} \), respectively.

In order to implement zeroth-order degradation, a constant decay rate \( \lambda_0 \), in units of \( \text{mol s}^{-1} \), has to be subtracted from the right-hand side of equation (3). Further, \( \lambda_0 d_k \) has to be added to the brackets in equation (1), where \( d_k \) is the amount of irreversible degraded amino acids of type \( k \) per degraded pore protein. The irreversible degradation probability \( \tilde{d} = d/b \) refers to the purely reversible, \( \tilde{d} = 0 \), and purely irreversible, \( \tilde{d} = 1 \), degradation, respectively. Amino acids from reversible degradation may be reused to build new amino acids in contrast to the irreversible degradation case, e.g. where chemical modifications degrade functional ability and amino acids get irrecoverably lost. The corresponding rescaled degradation term to be added to the left-hand side of equation (5) is \( \tilde{\lambda}_0 f^2 (\tilde{P} + \tilde{d}) \) with the reduced decay rate \( \tilde{\lambda}_0 = \lambda_0/(r c_{\text{out}}) \).

The first-order degradation terms are \( -\lambda_1 P(t) \) to be added to equation (3), with \( \lambda_1 \) in units of \( s^{-1} \), \( \lambda_1 d_k P(t) \) to equation (1) and \( \tilde{\lambda}_1 (\tilde{P} + \tilde{d} \tilde{P} + \tilde{P}^2) \) to equation (5), with \( \tilde{\lambda}_1 = \lambda_1 \tilde{V}/r \).

The value for \( \tilde{d} \) is arbitrarily chosen and serves as an exemplar of irreversible degradation (\( \tilde{d} > 0 \)). We further discuss \( \tilde{d} = 0.3 \) to show the difference of reversible, figure 3 inset, and partially irreversible degradation, figure 4.

Figure 4 shows the qualitative difference of irreversible zeroth- and first-order degradation. In the case of zeroth order, no saturation in the number of pore proteins is found. A small enough degradation rate (short-dashed line, \( \tilde{\lambda}_0 = 0.1 \), left graph) decelerates the dynamics of the system.
Figure 4. Evolution of pore proteins for zeroth-order (left) and first-order (right) protein degradation ($f = 0.3$). We have chosen $\lambda = 0.3$ for convenience as an exemplar of irreversible degradation. The dynamics of the pore production slows down with increasing degradation rate. First-order degradation reveals saturation in the number of pore proteins for long-term scales. In both cases, a critical degradation rate can be seen ($\tilde{\lambda}_{0,\text{crit}} \approx 0.182$ and $\tilde{\lambda}_{1,\text{crit}} = 0.3$). Above these rates, the irreversible degradation of pore proteins, and therefore amino acids, becomes larger than the incoming flux of new amino acids. Eventually, this results in complete degradation of pore proteins and complete depletion of amino acids inside the protocell (dashed and dash-dot-dotted lines).

In contrast, above a critical degradation rate, the production grows on long-term scales. Below the threshold, we observed infinite growth in zeroth-order and stationary in first-order dynamics. The irreversible degradation rate exceeds the production of pore proteins. This diminishes the incoming flux of new amino acids and eventually results in complete depletion of the protocell and degradation of all pore proteins (dashed line, left graph). The protocell dies. For $f \lesssim 0.1$, the degradation rates for closing protocells has an upper bound $\tilde{\lambda}_{0,c} > f^2/(\tilde{d} + f^2)$.

First-order degradation dynamics offers saturation of the number of pore proteins (short-dashed line, $\tilde{\lambda}_{1} = 0.1$, right graph) up to a critical degradation rate (solid line, right graph). The system approaches steady state on the long-term scale. The inner concentration and the number of pore proteins for steady state can be calculated as $c_{s.s.}/c_{\text{out}} = 1 - \tilde{d}\tilde{\lambda}_{1}/f^2$ and $\tilde{P}_{s.s.} = f^2 - \tilde{d}\tilde{\lambda}_{1}$. Above a critical rate, $\tilde{\lambda}_{1,c} = f^2/\tilde{d}$, the degradation of pore proteins becomes larger than the production, decreasing the inflow of new amino acids. The number of pore proteins and the concentration of amino acids approach zero asymptotically. In this case, first-order degradation shares the same fate as zeroth-order on the long-term scale.

In conclusion, we found complex dynamics due to the two discussed degradation scenarios. If degradation surpasses a sharp threshold, all pores degrade, which amplifies this dynamics even more. In the case of zeroth order, this dynamics is strongly pronounced. In first-order case, below this threshold the system ends up in a stationary situation, which is not the case for zeroth-order degradation. The protocell is therefore required to sustain a production rate over this threshold to survive.
5. Summary

The discussed theoretical model describes the experimentally measured turnover dynamics of a simple protocell consisting of a liposome enclosing gene expression machinery. The protocell produces α-haemolysin pore proteins encoded in the included minimal genome. The impermeability of the membrane leads to basic component depletion, which shuts down the system turnover. Once the pores are inserted spontaneously into the membrane, the basic nutrients enter and the turnover increases again. Our one-parameter model describes quantitatively the transient dynamics. The model fits very well the corresponding experimental curve in [14], which allows us to predict numerical values for the amino acid diffusion through a single pore as well as the initial amino acid concentration of liposomes. We also considered the possible consequences of long-term degradation, although measurements are not yet available. The extended model describes zeroth- and first-order irreversible degradation of proteins and the qualitative differences on a long-term scale. Contrary to zeroth order, the number of pore proteins for first-order degradation will saturate on the long-term scale. Increasing the degradation rate up to the critical, well-defined threshold value slows down the pore production dynamics. For higher degradation rates, the protocells end up in equilibrium death.

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