# Ribocell Modeling

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## Extended Abstract

A minimal living cell, or protocell, is a minimal supra molecular self-bounded structure that can exhibit self-maintenance, self-reproduction and evolvability (Luisi 2003). Some years ago, Szostak and colleagues proposed a minimal cell prototype called Ribocell: ribozymes based cell (Szostak et al. 2001) that, in principle, can exhibit all these three properties. This model cell consists in a self-replicating minimum genome coupled with a self-reproducing lipid vesicular container. The genome is composed by two hypothetical ribozymes: $R_{Lip}$ able to catalyze the conversion of molecular precursors into membrane lipids and $R_{Pol}$ able to duplicate RNA strands. Therefore, in an environment rich of both lipid precursors and activated nucleotides the Ribocell can self-reproduce if both processes: the genome self-replication and the membrane reproduction (growth and division), are somehow synchronized. In a recent work (Mavelli et al in press) we have presented and discussed a detailed and as realistic as possible kinetic mechanism for the Ribocell based on a previously published in silico model of self-replicating vesicles (Mavelli and Ruiz-Mirazo 2007):

$$\begin{aligned}
&R_{Pol} + {}_cR_{Pol} \underset{k_S}{\overset{k_{SS}}{\rightleftharpoons}} R_cR_{Pol} \\
&R_{Lip} + {}_cR_{Lip} \underset{k_S}{\overset{k_{SS}}{\rightleftharpoons}} R_cR_{Lip}
\end{aligned} \qquad (1)$$

$$\left.\begin{aligned}
R_{Pol} + S &\xrightarrow{k_{R@S}} R@S \\
R@S_cS_n + NTP_{n+1} &\xrightarrow{k_{NPT}} R@S_cS_{n+1} + W \\
R@S_cS &\xrightarrow{k_{R@SS}} R_{Pol} + S_cS
\end{aligned}\right\} \qquad (2)$$

$$P + R_{Lip} \xrightarrow{k_L} L + R_{Lip} + W \qquad (3)$$

$$\left.\begin{aligned}
NTP_{Ex} &\xrightarrow{P_{NTP}} NTP \\
P_{Ex} &\xrightarrow{P_P} P
\end{aligned}\right\} \qquad (4)$$

**Scheme 1**: The Ribocell metabolism: (1) reversible association of RNA polymerase ($R_{Pol}$) and RNA-synthase ($R_{Lip}$) strands with the respective complement cRPol and cRLip; (2) catalytic cycle of the RNA replication (S= $R_{Pol}$, ${}_cR_{Pol}$, $R_{Lip}$ and ${}_cR_{Lip}$); (3) conversion of the precursor P into the membrane lipid L catalyzed by the ribozyme RLip; (4) transport processes across the lipid membranes.

Using a deterministic approach, we showed that synchronization between genoma duplication and membrane reproduction can spontaneously emerge within the used approximations and the adopted kinetic parameters, all derived from the literature (see Table 1), only if the $k_L$ constant is increased of five orders of magnitude (Mavelli *et al* in press).

| Kinetic Patameters | Values | Process Description | References |
|---|---|---|---|
| $k_{SS}[s^{-1}M^{-1}]$ | $8.8\cdot10^{6}$ | Formation of dimers $R_cR_{Pol}$ and $R_cR_{Lip}$ | Christensen 2007 |
| $k_S[s^{-1}]$ | $2.2\cdot10^{-6}$ | Dissociation of dimers $R_cR_{Pol}$ and $R_cR_{Lip}$ | Christensen 2007 |
| $k_{R@S}[s^{-1}M^{-1}]$ | $5.32\cdot10^{5}$ | Formation of R@S | Tsoi and Yang 2002 |
| $k_{R@SS}[s^{-1}]$ | $9.9\cdot10^{-3}$ | Dissociation of Complexes $R@S_cS$ | Tsoi and Yang 2002 |
| $k_{NTP}[s^{-1}M^{-1}]$ | 0.113 | Nucleotide Polymerization in Oleic Vesicle | De Frenza 2009 |
| $k_L[s^{-1}M^{-1}]$ | 0.017 | Catalyzed Lipid Precursor Conversion | Stage-Zimmermann and Uhlenbeck 1998 |
| $k_{in}[dm^2s^{-1}]$ | $7.6\cdot10^{19}$ | Oleic acid association to the membrane | Mavelli et al.2008 |
| $k_{out}[dm^2s^{-1}]$ | $7.6\cdot10^{-2}$ | Oleic acid release from the membrane | Mavelli et al.2008 |

| | | | |
|---|---|---|---|
| $P_P$ [$cm \cdot s^{-1}$] | $4.2 \cdot 10^{-9}$ | Membrane Permeability to Lipid Precursor | Sacerdote and Szostak 2005 |
| $P_{NTP}$[$cm \cdot s^{-1}$] | $1.9 \cdot 10^{-11}$ | Membrane Permeability to Nucleotides | De Frenza 2009 |
| $P_W=P_S$ | 0.0 | Membrane Permeability to W and genetic staff | |
| $P_{aq}$[$cm \cdot s^{-1}$] | $1.0 \cdot 10^{-3}$ | Oleic Acid Membrane Permeability to Water | Sacerdote and Szostak 2005 |

**Table 1**: Kinetic Constants and Permeability of the Ribocell *in silico* model at room temperature (S= $R_{pol}$, $_cR_{pol}$, $R_{lip}$ and $_cRLip$).

In this contribution we will focus the attention on the role of random fluctuations on the Ribocell time behaviour by using a Monte Carlo program developed in recent years for simulating chemically reacting compartmentalized systems (Mavelli *et al* 2008). The random nature of reacting events (*intrinsic stochasticity*) can highly differentiated the time course of each single protocell in the population, since the effect of fluctuations is enlarged by the autocatalytic character of genome replication. Moreover, another source of time course dispersion is the random distribution of the cell internal content after each division (*extrinsic stochasticity*). Also in this case, displacement from the deterministic equality of the genetic staff amount in both the daughter cells is amplified by the nature of the internal metabolism. However, while intrinsic stochasticity can determine equivalent behaviours with different time scales (Fig.1A), the extrinsic randomness can produce completely different outcomes bringing to the death for dilution of the Ribocell if a complete segregation of ribozymes in diverse protocells takes place (Fig 1B,C).

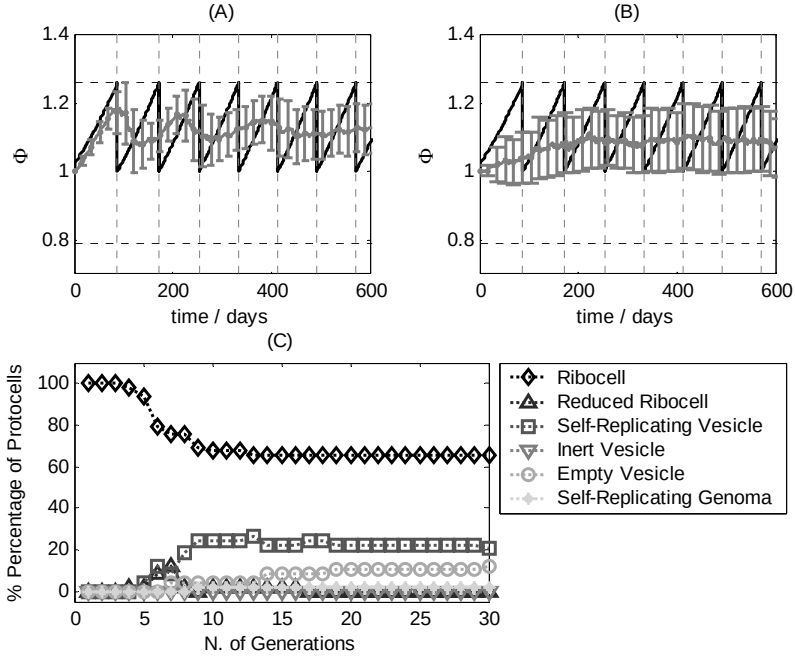


**Figure 1**: Comparison between deterministic curves (black lines) and stochastic simulation data (gray lines with error bars) of the Ribocell reduced surface Φ obtained setting (A) $k_L=1.7x10^4$ $s^{-1}M^{-1}$ and (B) $k_L=1.7x10^5$ $s^{-1}M^{-1}$ (Vertical dashed lines are the deterministic division times). (C) Composition of the Ribocells population against the generation number ($k_L=1.7x10^5$ $s^{-1}M^{-1}$).

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