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Deterministic and Stochastic approaches to minimal cell models: the Ribocell case study

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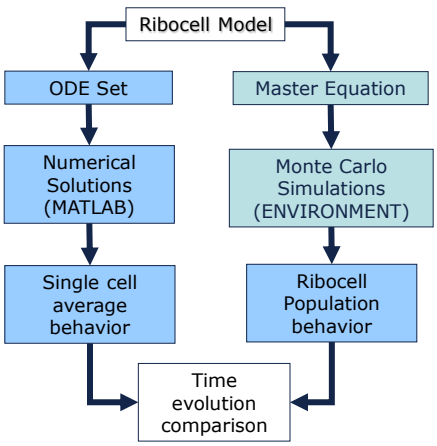
Motivation

Bridging the gap between *in silico* and *in vitro* experiments

Deterministic	Stochastic
Macroscopic	Microscopic
Positive real numbers of Molecules	Integer numbers of Molecule
Reaction Rates	Propensity Probabilities
Average Behaviour	Average + Fluctuations

Deterministic and stochastic theoretical models have been developed in order to analyse the feasibility of the *Ribocell* (ribozymes based cell):

- Emergence of synchronization between genome replication and membrane reproduction,
- Effect of intrinsic and extrinsic stochasticity in the Ribocell time behaviour.



Summary

- Ribocell hypothesis
- *in silico* Ribocell model
  - Lipid vesicle dynamics
  - The Ribocell metabolism
- Deterministic Analysis vs. Stochastic Simulations
  - kinetic parameters
- Theoretical analysis
  - Deterministic time behavior
  - Stochastic outcomes
- Conclusion

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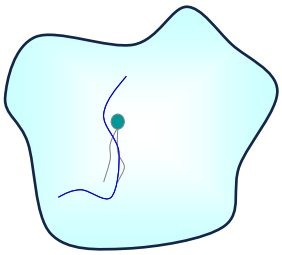
The Ribocell*

The Ribocell (ribozymes-based cell) is a theoretical cellular model that has been proposed some years ago as a possible minimal cell prototype It consists in a self-replicating minimum RNA genome (2 rybozimes, $\mathbf{T_{lip}}$ $\mathbf{R_{pol}}$) coupled with a self-reproducing lipid vesicle compartment:



$\mathbf{T_{lip}}$ is able to catalyze the conversion of molecular precursors into lipids

$\mathbf{R_{pol}}$ is able to replicate RNA strands.

In an environment rich both of lipid precursors and activated nucleotides, the Ribocell can self-reproduce if the genome self-replication and the compartment self-reproduction mechanisms are somehow synchronized.

*** Szostak, Bartel, Luisi (2001) NATURE 409, 387-390**

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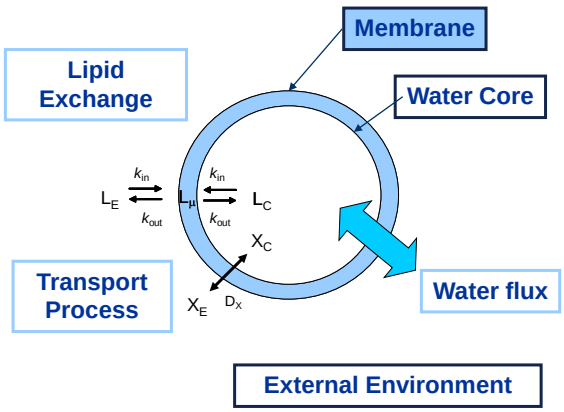
In silico Vesicles (*)

Vesicles are described as compartmentalized reacting systems (CSR) made of two different homogeneous domains:

- the membrane
- the water core

Lipids and molecules can be exchanged between the membrane and water core, between the membrane and the external environment.

Transport processes can also occur, exchanging molecules directly from the external environment to the internal water pool.



(*) **Mavelli F, Ruiz-Mirazo K, (2007) Phil. Trans. Royal Soc. London B 362, 1789.**
Mavelli F, Ruiz-Mirazo K, (2010) Phys Biol 7, 036002

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Stability of closed membrane

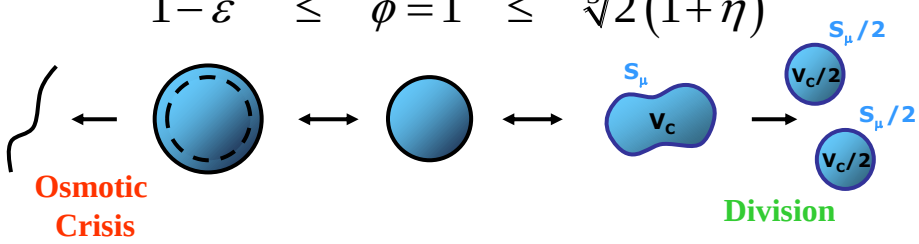
Reduced Surface

$\phi = S_\mu / \sqrt[3]{36\pi V_{core}^2}$ ratio of the actual membrane surface S_μ and the area of a sphere with the actual volume V_c of the core

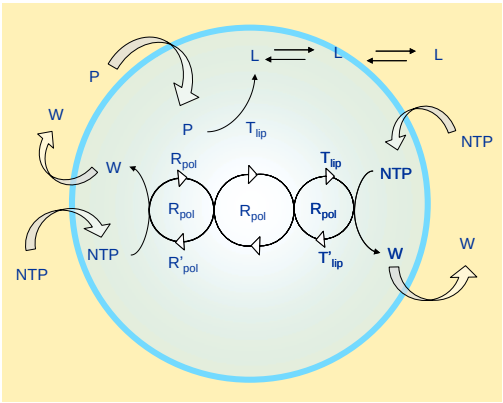
Vesicle

swollen — spherical — deflated

$$1-\varepsilon \leq \phi = 1 \leq \sqrt[3]{2}(1+\eta)$$



The Ribocell Metabolic network



$T + T' \underset{k_{ST}}{\overset{k_{DT}}{\rightleftharpoons}} TT'$

$R + T \xrightarrow{k_{RT}} R@T$

$R@TT'_n + B'_{n+1} \xrightarrow{k_{B(n+1)}} R@TT'_{n+1} + W$

$R@T_N T'_N \xrightarrow{k_{dis}} R + TT'$

$R + T' \xrightarrow{k_{RT'}} R@T'$

$R@T'_N T_n + B_{n+1} \xrightarrow{k_{B}(n+1)} R@T'_N T_{n+1} + W$

$R@T'_N T_N \xrightarrow{k_{dis}} R + TT'$

$R + R' \underset{k_{SR}}{\overset{k_{DR}}{\rightleftharpoons}} RR'$

$2R \xrightarrow{k_{RR}} R@R$

$R@R_N R'_n + B \xrightarrow{k'_{B(n+1)}} R@R_N R_{n+1} + W$

$R@R_N R'_N \xrightarrow{k'_{dis'}} RR'$

$R + R' \xrightarrow{k_{RR'}} R@R'$

$R@R'_N R_n + B \xrightarrow{k_{B(n+1)}} R@R'_N R_{n+1} + W$

$P + T \xrightarrow{k_{PT}} L + T$

$T + T' \underset{K_T^b}{\overset{K_T^f}{\rightleftharpoons}} TT'$

$R + T \xrightarrow{K_{rt}} R@T$

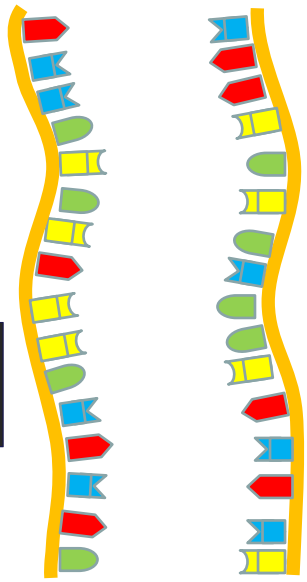
$R + T' \xrightarrow{K_{rt'}} R@T'$

$R@T_N T'_n + B'_n \xrightarrow{K_{b'(n)}} R@T_N T_{n+1}$

$R@T'_N T_n + B_n \xrightarrow{K_{b(n)}} R@T'_N T_{n+1}$

$R@T_N T'_N \xrightarrow{K_{Dis}} R + TT'$

$R@T'_N T_N \xrightarrow{K_{rd'}} R + TT'$



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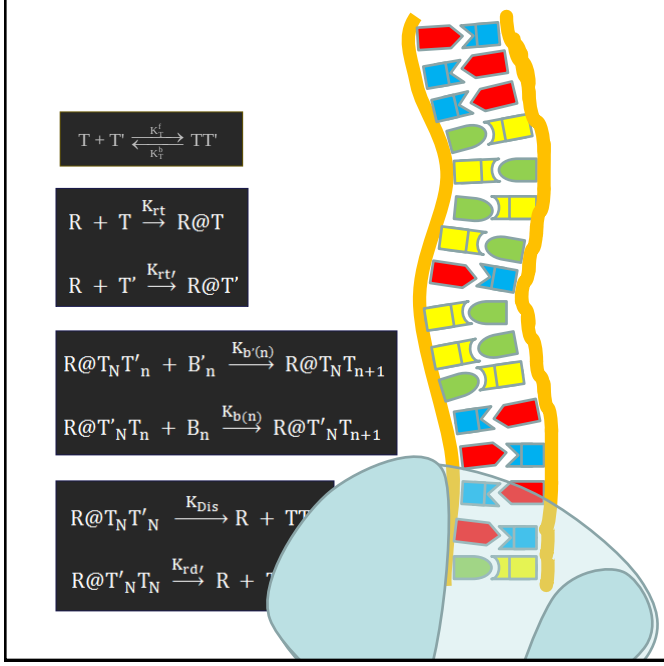

$$T + T' \underset{K_T^b}{\overset{K_T^f}{\rightleftharpoons}} TT'$$

$$R + T \xrightarrow{K_{rt}} R@T$$

$$R + T' \xrightarrow{K_{rt'}} R@T'$$

$$R@T_N T'_n + B'_n \xrightarrow{K_{b'(n)}} R@T_N T_{n+1}$$

$$R@T'_N T_n + B_n \xrightarrow{K_{b(n)}} R@T'_N T_{n+1}$$

$$R@T_N T'_N \xrightarrow{K_{Dis}} R + TT'$$

$$R@T'_N T_N \xrightarrow{K_{rd'}} R + T$$

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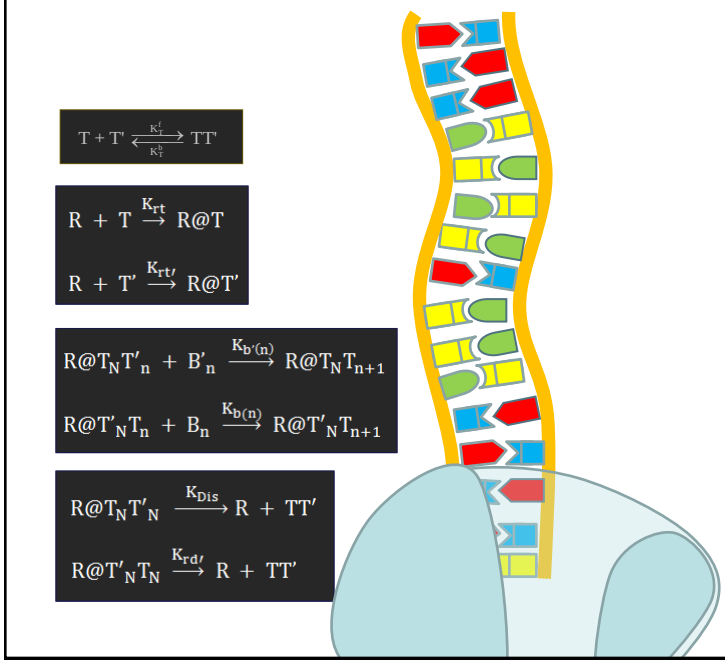

$$T + T' \underset{K_T^b}{\overset{K_T^f}{\rightleftharpoons}} TT'$$

$$R + T \xrightarrow{K_{rt}} R@T$$

$$R + T' \xrightarrow{K_{rt'}} R@T'$$

$$R@T_N T'_n + B'_n \xrightarrow{K_{b'(n)}} R@T_N T_{n+1}$$

$$R@T'_N T_n + B_n \xrightarrow{K_{b(n)}} R@T'_N T_{n+1}$$

$$R@T_N T'_N \xrightarrow{K_{Dis}} R + TT'$$

$$R@T'_N T_N \xrightarrow{K_{rd'}} R + TT'$$

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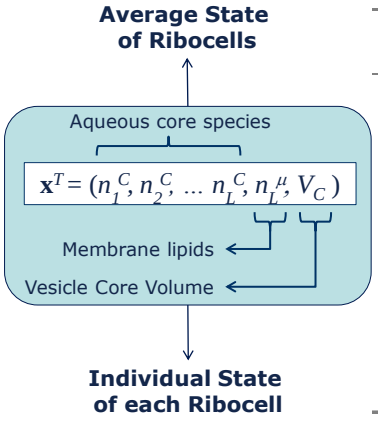
Theoretical approaches

The time behavior of the *in silico* Ribocell has been studied by means of a deterministic (DA) and stochastic approach (SA):

- DA → average time behavior of a single Ribocell
- SA → time behavior of a populations of Ribocells



Event	Deterministic Rate $(Ms)^{-1}$	Propensity Density Probability s^{-1}
Internal Chemical reactions (1) $a_{1,\rho}X_1 + \ldots a_{N,\rho}X_N \xrightarrow{r_\rho} b_{1,\rho}X_1 + \ldots b_{N,\rho}X_N$	$k_\rho \prod_j^N \left(\frac{n_j^C}{V_C N_A}\right)^{a_{j,\rho}}$	$\frac{k_\rho}{(V_C N_A)^{M_\rho - 1}} \prod_j^N \binom{n_j^C}{a_{j,\rho}}$
Solute X_n membrane transport (2)	$P_n \frac{S_\mu}{V_C}\left(C_n^E - C_n^C\right)$	$D_n S_\mu \frac{\left\|\left(C_n^E - C_n^C\right)\right\|}{\lambda_\mu}$ (3)
Membrane Lipid Release	$\frac{k_{out} n_L^\mu}{N_A V_C}$	$k_{out} n_L^\mu$
Membrane Lipid Uptake	$\left(\frac{k_\rho S_\mu [X_L^C]}{N_A V_C}\right)$	$k_\rho S_\mu [X_L^C]$
Water Flux (4)	$v_{aq} P_{aq} S_\mu \left(C_T^E - C_T^C\right)$	$V_C = \sum_{i=1}^N n_i^C \Big/ \left(N_A C_T^E\right)$

Assumptions and kinetic constants

- External concentrations of nucleotides (NTPs), lipid precursor (P), byproduct (W) and osmotic buffer (B) to be constant throughout the process time l: *continuous stirred tank reactor approximation*.
- Differences in the Nucleotides kinetic behavior are negligible
- The two ribozymes R and T are assumed both 20 nucleotides long with a random sequence of bases and with a similar kinetic behavior

- The kinetic constants of formation and dissociation of RNA dimers are experimental values measured for a 20 nucleotides sequence (Christensen 2007).
- The kinetic constants for both complex formation and complex dissociation are those for the human enzyme β-polymerase (Tsoi and Yang 2002)
- The k_L constant for the catalytic synthesis of lipids is that of the splicing reaction, catalyzed by Hammerhead ribozyme (Stage-Zimmermann and Uhlenbeck 1998).

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Kinetic Parameters at 25°C

Kinetic Patameters	Values	Process Description	Ref.
$k_{SS}[s^{-1}M^{-1}]$	$8.8 \cdot 10^{6}$	Formation of dimers RR'$_{Pol}$ and TT'$_{Lip}$	(1)
$k_{S}[s^{-1}]$	$2.2 \cdot 10^{-6}$	Dissociation of dimers RR'$_{Pol}$ and TT'$_{Lip}$	(1)
$k_{R@S}[s^{-1}M^{-1}]$	$5.32 \cdot 10^{5}$	Formation of R@S (S=R, R'T, T')	(2)
$k_{R@SS}[s^{-1}]$	$9.9 \cdot 10^{-3}$	Dissociation of Complexes R@SS'	(2)
$k_{NTP}[s^{-1}M^{-1}]$	0.113	Nucleotide Polymerization in Fatty Acid Vesicle	(3)
$k_{L}[s^{-1}M^{-1}]$	0.017	Lipid Precursor Conversion	(4)
$k_{in}[dm^{2}s^{-1}]$	$7.6 \cdot 10^{19}$	Oleic acid association to the membrane	(5)
$k_{out}[dm^{2}s^{-1}]$	$7.6 \cdot 10^{-2}$	Oleic acid release from the membrane	(5)
$P_{P}[cm \cdot s^{-1}]$	$4.2 \cdot 10^{-9}$	Membrane Permeability to Lipid Precursor	ass.(6)
$P_{NTP}[cm \cdot s^{-1}]$	$1.9 \cdot 10^{-11}$	Membrane Permeability to Nucleotides	(3)
$P_{W}=P_{S}$	0.0	Membrane Permeability to W and genetic staff	ass.
$P_{aq}[cm \cdot s^{-1}]$	$1.0 \cdot 10^{-3}$	Oleic Acid Membrane Permeability to Water	(6)

1) Christensen 2007 Biosci Rep 27:327–333;
2) Tsoi and Yang 2002, Biochem. J 361:317-325 ;
3) Mansy et al 2008, Nature 454:122-126 ;
4) Stage-Zimmermann and Uhlenbeck 1998, RNA 4:875-889 ;
5) Mavelli et al. 2008, In: Arabnia HR et al. (ed) BIO-COMP'08 Proceedings;
6) Sacerdote and Szostak 2005, PNAS 102:6004-6008.

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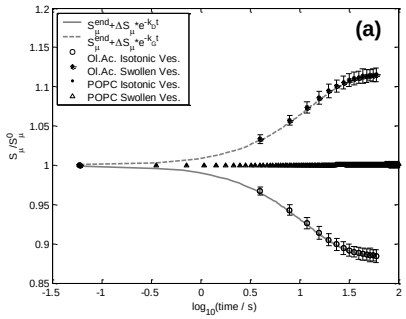
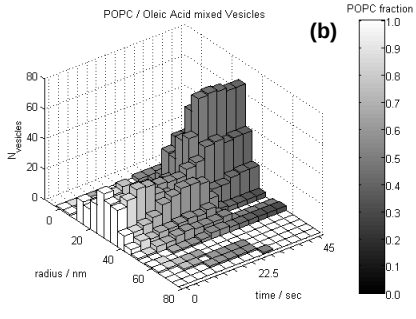
Vesicles Competition Simulations

In previous work (*) we tested the equilibrium and dynamic behavior of lipid vesicles in terms of:

- deterministic rates and propensity probabilities of lipid uptake/release processes,
- the condition of the vesicle division.





(*) Mavelli F, Ruiz-Mirazo K, (2010) Physical Biology 7, 036002

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NATURE| Vol 454| 3 July 2008

Template-directed synthesis of a genetic polymer in a model protocell

Sheref S. Mansy[1], Jason P. Schrum[1], Mathangi Krishnamurthy[1], Sylvia Tobé[1], Douglas A. Treco[1] & Jack W. Szostak[1]

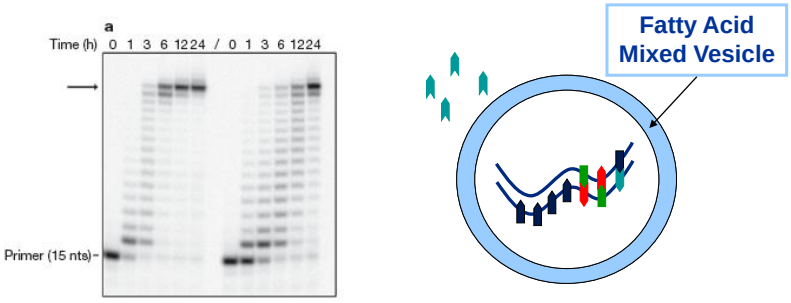

Figure 4 | Template-copying chemistry inside vesicles. Vesicles contained encapsulated primer-template complexes, and template-copying was initiated by the addition of activated monomer to the external solution. nts, nucleotides. **a**, Non-enzymatic dC_{15}-template copying in solution (lanes 1–6) and inside 2:1 MA:GMM vesicles (lanes 8–13) at 4 °C. **b**, Template-

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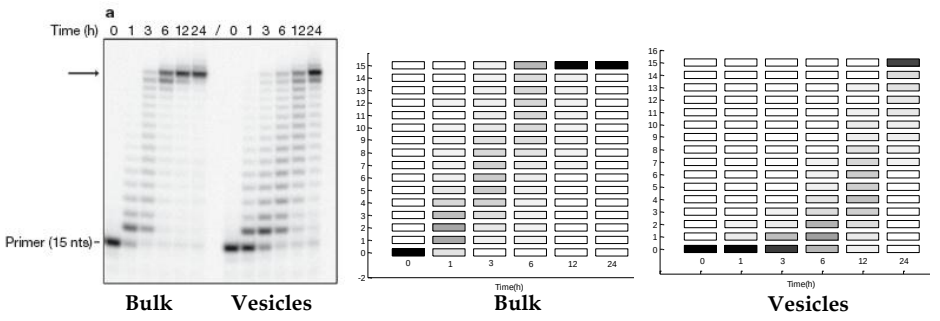
DNA Synthesis

By reproducing the DNA synthesis time course both in bulk solution and in vesicles we determine:

- the kinetic constant for the NTP-DNA binding — $k = 0.1125\ M^{-1}s^{-1}$
- the NTP transport diffusion coefficient — $D = 4.5 \times 10^{4}\ dm^2/s$



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Deterministic Analysis

In all the studied cases, we start from a single 100nm-radius spherical vesicle containing only two pairs of ribozymes in form of duplex changing the value of the lipid formation constant k_L.

Three different long time regimes are in principle expected:
(1) the stationary grow-division regime (GD), i.e. a stable steady state where the protocell grows in size and divides keeping the same radius after any division;
(2) the Ribocell burst due to an osmotic crisis (OC) when the core volume increases more rapidly than the membrane surface;
(3) the death for dilution regime (DD) when the membrane surface increases rapidly and the protocell divides before the genome duplication is completed.

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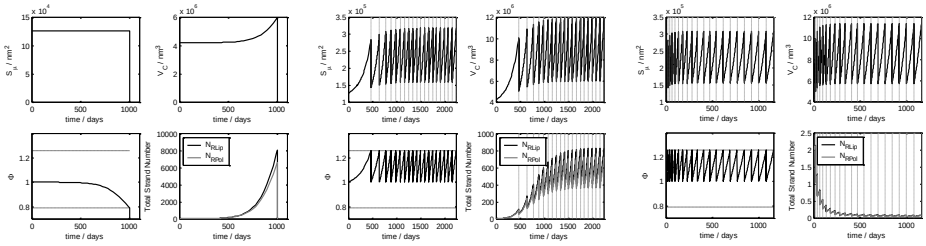
Ribocell Deterministic Analysis

	k_L ($s^{-1}M^{-1}$)		Δt_{div} (days)	Radius (nm)	Total Number or RNA strands	T_{Lip} (%)	T'_{Lip} (%)	R_{Pol} (%)	R'_{Pol} (%)
→	**1.7×10^{-2}**	**OC**							
	$1.7\times10^{+2}$	OC							
	$1.7\times10^{+3}$	GD	127.8	157.3	41384	28.43	28.43	26.13	17.01
→	**$1.7\times10^{+4}$**	**GD**	**81.1**	**112.4**	**790**	**26.08**	**26.08**	**25.82**	**22.03**
	$1.7\times10^{+5}$	GD	80.3	110.7	8	25.00	25.00	25.00	25.00
→	**$1.7\times10^{+6}$**	**DD**	**79.8**	**110.7**	**0**				



Osmotic Crisis
k_L=1.7e-2

Growth and Division
k_L=1.7e+4

Death for dilution
k_L=1.7e+6

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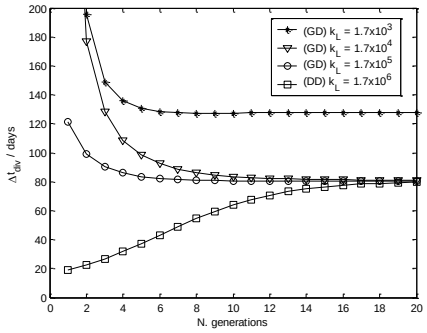
Ribocell time life



The Ribocell time life Δt_{div}, i.e. the interval of time between two subsequent divisions, are reported for different k_L values against the number of generations. This plot shows that in all the studied cases after 20 generations a stationary state is attained.

(*) Mavelli F, Theoretical approaches to the Ribocell modeling, in Luisi PL & Stano P (Eds*) Minimal Cell*, Springer (forthcoming)

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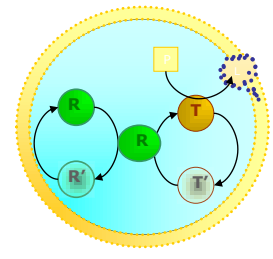
Random solute distribution

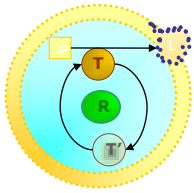
The Ribocell can self-replicate only if it contains at least 3 ribozymes:

- R
- R or R'
- T or T'

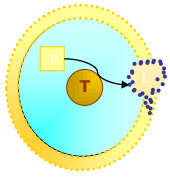
During the Ribocell life time vesicle divisions can separate the ribozymes producing different types of self-reproducing and inert vesicles.



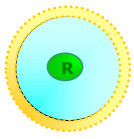
Self-replicating Ribocell



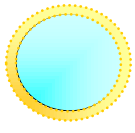
Reduced Ribocell



Enzymatic Vesicle



Inert Vesicle



Empty Vesicle

Stochastic analisys

Stochastic simulation are carried out for the two cases $k_L=1.7\times10^{+4}$ and $k_L=1.7\times10^{+5}$ starting from 50 spherical vesicles of 100nm radius with a genetic staff composition near to the steady state regime.

As for the deterministic calculations, after any division only one of the two daughters is kept, in order to perform simulations at a constant number of protocells and to avoid a huge increase of the running time.

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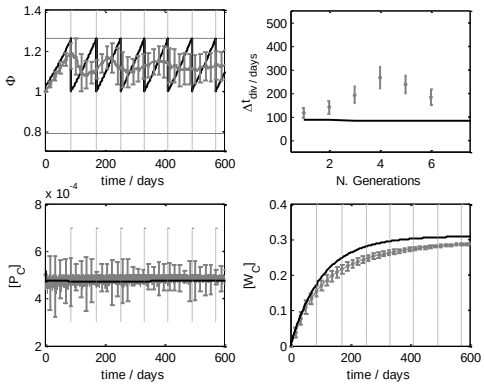
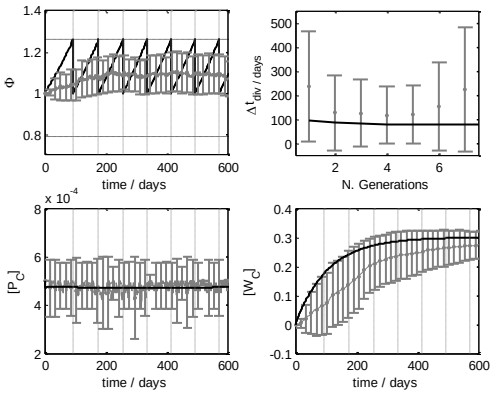
Stochastic Simulations: k_L=1.7e4



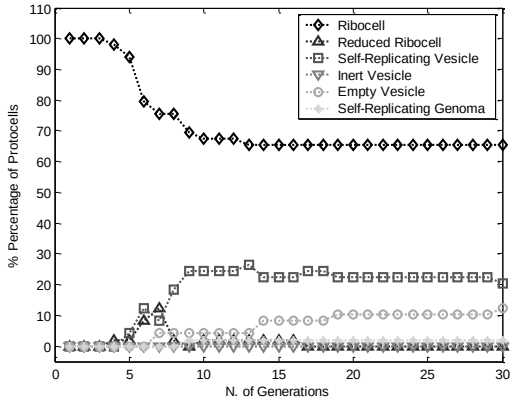
Comparison between deterministic curves (black lines) and stochastic simulation data (gray lines with error bars) of the Ribocell time behaviour obtained setting $k_L=1.7\text{x}10^4$ Vertical dashed lines are the deterministic division times.

Stochastic Simulations: k_L=1.7e5



Comparison between deterministic curves (black lines) and stochastic simulation data (grey lines with error bars) of the Ribocell time behaviour obtained setting k_L=1.7x10^5 Vertical dashed lines are the deterministic division times.

Protocell population: k_L=1.7e5



Different composition of the Protocell population against the generation number: (k_L=1.7x10^5)

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Conclusions

The Ribocell time behavior needs a more deep theoretical analysis and some improvements of the *in silico* model are also necessary (i.e. high temperature kinetic parameters), neverthless some conclusions can be drawn from these preliminary results:

Deterministic analysis:

- The emergence of a spontaneous synchronization between the genome self-replication and the lipid membrane.
- The high values of division times can be due to the low value of the dissociation constant of RNA dimers at room temperature.

Stochastic analysis

- The random nature of reacting events (intrinsic stochasticity) can highly differentiated the time course of each single since its effect is enlarged by the autocatalytic character of genome replication.
- The random distribution of the cell internal content after each division (extrinsic stochasticity) can produce completely different outcomes bringing to the death for dilution.

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- Marco Lerario
- Adrea De Frenza

students

*"All models are wrong,
but some are useful"*

George Box