

## **SUPPORTING INFORMATION**

**Article accepted as a talk for ECAL 2009**

(to be published as part of the Proceedings of the Conference, by Springer – LNCS Series)

### **On the transition from prebiotic to proto-biological membranes: from ‘self-assembly’ to ‘self-production’**

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## Set of parameters used in the simulations

Almost all the variables, apart from permeability to the waste ( $P_w$ ) and the rate of decay of L into l ( $k_d$ ), were kept the same in all reported simulation runs, as described below.

We always began simulations with a single spherical vesicle of 50 nm radius, made exclusively of lipid l, with its monomer present in both the surrounding aqueous solutions, at the following equilibrium concentration:  $[l_{core}] = [l_{env}] = 0.004$  M.

Regarding the kinetic constants for self-assembly into --and release from-- the vesicle, they have been adjusted in such a way that the equilibrium concentrations of monomers l and L were 4 mM and 66.7  $\mu$ M, respectively (which are realistic values for a standard fatty acid, as compared to phospholipids, whose cac is typically much lower). So,  $k_{lm} = k_{lm} = 7.6 \times 10^{19} \text{ M}^{-1} \text{ dm}^{-2}$ ;  $k_l = 4.56 \text{ s}^{-1}$ ;  $k_L = 7.6 \times 10^{-2} \text{ s}^{-1}$ . As for the internal protometabolic reactions,  $k_f = 10$  (forward cycle reactions) and  $k_r = 0.1$  (backward cycle reactions), which amounts to say that the cycle is somewhat forced to run ‘clockwise’ (see Fig. 1 in the paper) -- or that X and Y, the nutrients, are high free energy compounds.

The initial concentrations set in the environment (env) and in the internal core (core) are specified in Table S1.  $[X]_{env}$  and  $[Y]_{env}$  are kept constant, in order to guarantee a continuous external supply of material. Metabolites  $A_i$  are assumed to be present only inside the vesicles, starting with just one of them ( $A_1$ ), and are not allowed to cross the membrane.

**Table S1:** Set of initial concentrations used in the simulation runs

Molecule	[ ] <sub>env</sub> (M)	[ ] <sub>core</sub> (M)
l	0.004	0.004
L	0.000	0.000
X	0.001*	0.000
Y	0.001*	0.000
$A_1$	0.000	0.002
$A_i$ ( $i = 2,3..6$ )	0.000	0.000
W	0.000	0.000
B	0.200	0.200

\* These concentrations are maintained constant

## Permeability calculations with ENVIRONMENT

In principle, the propensity density function related to solute exchange across a closed lipid bilayer, in our platform ENVIRONMENT, is defined as follows:

$$p_X^{Tr} = D_X S_\mu \frac{\left| (C_X^{Env} - C_X^{Core}) \right|}{\lambda}$$

where  $D_X$  is the diffusion coefficient [ $\text{dm}^2\text{s}^{-1}\text{mole}^{-1}$ ],  $S_\mu$  is the membrane surface [ $\text{dm}^2$ ],  $\lambda$  is the membrane thickness [ $\text{dm}$ ] and  $(C_X^{Env} - C_X^{Core})$  is the difference between the external and the internal concentration [ $\text{mole}/\text{dm}^3$ ] of the solute X.  $p_X^{Tr} dt$  gives the probability that one molecule of X crosses the membrane in the time interval  $[t, t+dt]$  according to the concentration gradient. The deterministic flux of solute molecules, in turn, can be described by the differential equation:

$$\frac{1}{S_\mu} \frac{dn_X}{dt} = P_X (C_X^{Env} - C_X^{Core})$$

where  $n_X$  is the mole number of X in the vesicle aqueous core, while  $P_X$  is the solute macroscopic permeability usually expressed in  $\text{cm}/\text{s}$ . By comparing the equations and , it easy to obtain the relationship:

$$D_X = P_X \lambda N_A$$

that gives the molecular diffusion coefficient in terms of the macroscopic permeability,  $N_A$  being the Avogadro's number.

Now, eq. implies that a variation in the membrane composition can affect the propensity probability, since both the bilayer thickness  $\lambda$  and the solute diffusion coefficient  $D_X$  depend on the type of lipidic or amphiphilic molecule involved. In order to account for this, ENVIRONMENT estimates  $\lambda$  by the ratio between the actual surface  $S_\mu$  and volume  $V_\mu$  of the membrane:

$$\lambda = \frac{V_\mu}{S_\mu} = \frac{\sum_j N_j v_j}{\frac{1}{2} \sum_j N_j a_j}$$

where  $a_j$  and  $v_j$  are the surface head area and the molecular volume of the  $j$ -th species and  $N_j$  are the respective number of amphiphilic molecules present in the bilayer. Furthermore, for a binary mixture of two amphiphiles, in this first approximation to the problem, we assume that the solute diffusion coefficient of the mixed membrane  $D_X^{Mix}$  will depend linearly on the membrane composition, according to the formula:

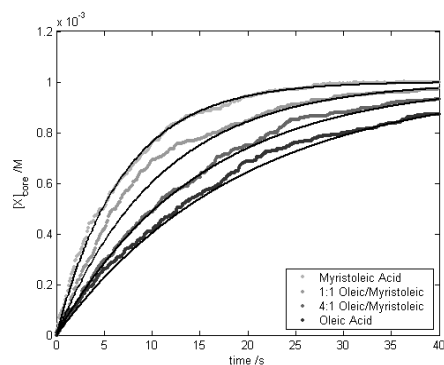
$$D_X^{Mix} = D_X^1 - (D_X^2 - D_X^1) \chi_2^S$$

where  $D_X^j$  ( $j=1,2$ ) are the diffusion coefficients of solute X across the pure membrane of amphiphiles 1 and 2, respectively, and  $\chi_2^S = a_2 N_2 / (2 S_\mu)$  is the surface contribution of the second amphiphile.

In Table S2, the permeability and diffusion coefficients of ribose, calculated by means of eqs.- for mixed membranes are shown (and compared with the values for the pure vesicles, composed in this example by either myristoleic or oleic acid, as reported in [Mansy et al. 2008]). Those values have been then used to check (see Fig. S1) the simulated ribose transport process by means of ENVIRONMENT with the deterministic curve obtained by solving eq.:

$$C_X^{Core} = C_X^{Env} \left( 1 - \exp \left( -3 \frac{P_X}{R_{Ves}} t \right) \right)$$

under the assumption that the volume and the surface of the vesicle do not change during the transport process:  $S_{\mu}/V_{Core} = 3/R_{Ves}$ .



**Fig. S1:** Comparison between stochastic simulations and deterministic solutions for the transport process of a solute X across 50-nm radius membranes with different amphiphile composition. See **Table** for further details concerning the transport parameters and membrane properties. The external concentration of X was set  $[X_{Env}] = 1.0 \text{ mM}$ . The continuous black curves were obtained by solving eq. assuming that the volume and the surface of vesicle do not change during the transport process.

**Table S2:** Experimental and calculated values for Myristoleic/Oleic acid 50nm-Radius mixed vesicles. \*Permeability values are relative to ribose [Mansy et al. 2008].

Parameters	Myristoleic Acid	1:1	4:1	Oleic Acid
Permeability ( $\text{cm s}^{-1}$ ) $\times 10^{-8}$	24.0*	15.4	11.2	8.6*
Molecular Diffusion Coefficient ( $\text{dm}^2 \text{mole}^{-1} \text{s}^{-1}$ ) $\times 10^8$	4.62	3.35	2.58	2.07
Membrane Thickness (nm)	3.2	3.6	3.84	4.0
Surface head area ( $\text{nm}^2$ )	0.3	--	--	0.3
Molecular volume ( $\text{nm}^3$ )	0.48	--	--	0.6

**Ref.:**

Mansy S, Schrum JP, Krishnamurthy M, Tobé S, Treco DA, Szostak JW (2008) Template directed synthesis of a genetic polymer in a model protocell. *Nature* 454, 122-126. And supplementary material.

