Electronic Supporting Information

Shape Changes and Budding of Giant Vesicles Induced by an Internal Chemical Trigger: an Interplay between Osmosis and pH Change

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1 Vesicles Preparation

An Eppendorf tube was filled with 500 μL of an aqueous phase, the so-called outer solution (O-solution) containing 200 mM of glucose and 10^{-3} mM of acetic acid, plus 300 μL of an interfacial phase containing HOA and POPC. The interface was settled for 10–15 minutes. A second Eppendorf tube containing a water-in-oil microemulsion was also prepared. 20 μL of an aqueous solution, the so-called inner solution (I-solution): [sucrose] = 200 mM, [urease] = 1.1 U/mL, $[CH_3COOH] = 1 \times 10^{-3}$ mM and [pyranine] = 50 μM solutions were mixed by pipetting up and down with 600 μL of an oil phase (amphiphiles concentrations were the same as in the interfacial phase). This microemulsion was poured over the first Eppendorf tube. The formation of vesicles was facilitated by centrifuging the tube at 6000 rpm for 10 minutes at room temperature ($\sim$ 22 °C). After the centrifugation step a white pellet was visible at the bottom of the Eppendorf tube. The oil phase and the aqueous phase were carefully removed with a micropipette. The pellet was gently washed with 100 μL of O-solution to remove free solutes. 30 μL of pellet were finally resuspended in 60 μL of O-solution.
To observe the shape transformation dynamics, 20 μL of the final diluted solution were placed into a well of a multi-well plate letting the vesicles deposit on the support for few minutes. 10 μL of a solution containing 180 mM urea ($[urea]_0$ = 60 mM), 200 mM glucose and 1×10^{-3} mM acetic acid were added to trigger the division. The number and the size of the vesicles was investigated by an epifluorescence microscope (ORMATEK TL-INV 100). Images were taken every 0.5 s by a CMOS camera (PIXELINK PL-D755CU) both in visible and in fluorescence (λ_{ex} = 450 nm and λ_{em} = 510 nm) mode. Fluorescence intensity was used to characterise the pH change inside the vesicles. Recorded images were analysed by means of ImageJ software.[1]

2 Determination of area and volume in different shapes

In a spherical vesicle, the volume V and the area A are calculated from the radius R_s

$$V = \frac{4}{3}\pi R_s^3 \tag{1}$$

$$A = 4\pi {R_s}^2 \tag{2}$$

In a prolate, there are two axes, a is the shortest axis and c the longest axis. V and A are calculated as

$$V = \frac{4}{3}\pi a^2 c \tag{3}$$

$$A = 2\pi a^2 \left(1 + \frac{c}{a\,e}\arcsin e\right) \tag{4}$$

where

$$e^2 = 1 - \frac{a^2}{c^2} \tag{5}$$

The pear shape was seen as the sum of two prolates with axes a_1, c_1, a_2 and c_2. V and A are calculated as

$$V = \frac{4}{3}\pi a_1^2 c_1 + \frac{4}{3}\pi a_2^2 c_2 \tag{6}$$

$$A = 2\pi a_1^2 \left(1 + \frac{c_1}{a_1\,e_1}\arcsin e_1\right) + 2\pi a_2^2 \left(1 + \frac{c_2}{a_2\,e_2}\arcsin e_2\right) \tag{7}$$

where

$$e_1^2 = 1 - \frac{a_1^2}{c_1^2} \tag{8}$$

$$e_2^2 = 1 - \frac{a_2^2}{c_2^2} \tag{9}$$

The budded limiting shape is constituted by two spheres with radii R_1 and R_2 connected by a narrow neck. The equations for A and V are

$$V = \frac{4}{3}\pi R_1^3 + \frac{4}{3}\pi R_2^3 \tag{10}$$

$$A = 4\pi {R_1}^2 + 4\pi {R_2}^2 \tag{11}$$

3 Numerical simulations

The ordinary differential equations that describe the evolution of the main chemical species are

$$\frac{\mathrm{d}[\mathrm{S}]}{\mathrm{d}t} = -R + k_{\mathrm{S}}\left([\mathrm{S}]_{\mathrm{out}} - [\mathrm{S}]\right) \tag{12}$$

$$\frac{\mathrm{d}[\mathrm{NH_3}]}{\mathrm{d}t} = 2R + k_2\left[\mathrm{NH_4^+}\right] - k_{2\mathrm{r}}[\mathrm{NH_3}]\left[\mathrm{H^+}\right] + k_{\mathrm{N}}\left([\mathrm{NH_3}]_{\mathrm{out}} - [\mathrm{NH_3}]\right) \tag{13}$$

$$\frac{\mathrm{d}\left[\mathrm{NH_4^+}\right]}{\mathrm{d}t} = -k_2\left[\mathrm{NH_4^+}\right] + k_{2\mathrm{r}}[\mathrm{NH_3}]\left[\mathrm{H^+}\right] \tag{14}$$

$$\frac{\mathrm{d}[\mathrm{CO_2}]}{\mathrm{d}t} = R - k_3[\mathrm{CO_2}] + k_{3\mathrm{r}}\left[\mathrm{H^+}\right]\left[\mathrm{HCO_3^-}\right] + k_{\mathrm{C}}([\mathrm{CO_2}]_{\mathrm{out}} - [\mathrm{CO_2}]) \tag{15}$$

$$\frac{\mathrm{d}\left[\mathrm{HCO_3^-}\right]}{\mathrm{d}t} = k_3[\mathrm{CO_2}] - k_{3r}\left[\mathrm{H^+}\right]\left[\mathrm{HCO_3^-}\right] - k_4\left[\mathrm{HCO_3^-}\right] + k_{4\mathrm{r}}\left[\mathrm{CO_3^{2-}}\right]\left[\mathrm{H^+}\right] \tag{16}$$

$$\frac{\mathrm{d}\left[\mathrm{CO_3^{2-}}\right]}{\mathrm{d}t} = k_4\left[\mathrm{HCO_3^-}\right] - k_{4\mathrm{r}}\left[\mathrm{HCO_3^-}\right]\left[\mathrm{H^+}\right] \tag{17}$$

$$\begin{aligned}\frac{\mathrm{d}[\mathrm{H^+}]}{\mathrm{d}t} &= k_2\left[\mathrm{NH_4^+}\right] - k_{2\mathrm{r}}[\mathrm{NH_3}]\left[\mathrm{H^+}\right] + k_3[\mathrm{CO_2}] - k_{3\mathrm{r}}\left[\mathrm{H^+}\right]\left[\mathrm{HCO_3^-}\right] + k_4\left[\mathrm{HCO_3^-}\right] - k_{4\mathrm{r}}\left[\mathrm{CO_3^{2-}}\right]\left[\mathrm{H^+}\right] \\ &+ k_5 - k_{5\mathrm{r}}\left[\mathrm{H^+}\right]\left[\mathrm{OH^-}\right] + k_6[\mathrm{HA}] - k_{6\mathrm{r}}\left[\mathrm{A^-}\right]\left[\mathrm{H^+}\right] + k_8[\mathrm{pyrOH}] - k_{8\mathrm{r}}\left[\mathrm{pyrO^-}\right]\left[\mathrm{H^+}\right] + k_7\frac{N_{\mathrm{innerHOA}}}{N_{\mathrm{Av}}V_{\mathrm{p}}} \\ &- k_{7\mathrm{r}}\frac{N_{\mathrm{innerOA^-}}}{N_{\mathrm{Av}}V_{\mathrm{p}}}\left[\mathrm{H^+}\right] + k_7\frac{N_{\mathrm{HOA\,free\,inner}}}{N_{\mathrm{Av}}V_{\mathrm{p}}} - k_{7\mathrm{r}}\frac{N_{\mathrm{OA^-\,free\,inner}}}{N_{\mathrm{Av}}V_{\mathrm{p}}}\left[\mathrm{H^+}\right]\end{aligned} \tag{18}$$

$$\frac{\mathrm{d}[\mathrm{OH^-}]}{\mathrm{d}t} = k_5 - k_{5\mathrm{r}}\left[\mathrm{H^+}\right]\left[\mathrm{OH^-}\right] \tag{19}$$

$$\frac{\mathrm{d}[\mathrm{HA}]}{\mathrm{d}t} = -k_6[\mathrm{HA}] + k_{6\mathrm{r}}\left[\mathrm{A^-}\right]\left[\mathrm{H^+}\right] + k_{\mathrm{HA}}\left([\mathrm{HA}]_{\mathrm{out}} - [\mathrm{HA}]\right) \tag{20}$$

$$\frac{\mathrm{d}[\mathrm{A^-}]}{\mathrm{d}t} = k_6[\mathrm{HA}] - k_{6\mathrm{r}}\left[\mathrm{A^-}\right]\left[\mathrm{H^+}\right] \tag{21}$$

$$\frac{\mathrm{d}[\mathrm{pyrOH}]}{\mathrm{d}t} = -k_8[\mathrm{pyrOH}] + k_{8\mathrm{r}}\left[\mathrm{pyrO^-}\right]\left[\mathrm{H^+}\right] \tag{22}$$

$$\frac{\mathrm{d}[\mathrm{pyrO^-}]}{\mathrm{d}t} = k_8[\mathrm{pyrOH}] - k_{8\mathrm{r}}\left[\mathrm{pyrO^-}\right]\left[\mathrm{H^+}\right] \tag{23}$$

R is a modified Michaelis-Menten rate law, which accounts for the pH dependence, the substrate and the product inhibition of the enzyme

$$R = \frac{v_{\max}\,[\mathrm{S}]}{\left(K_{\mathrm{M}} + [\mathrm{S}]\left(1 + \frac{[\mathrm{S}]}{K_{\mathrm{S}}}\right)\left(1 + \frac{[\mathrm{P}]}{K_{\mathrm{P}}}\right)\left(1 + \frac{K_{\mathrm{es2}}}{[\mathrm{H^+}]} + \frac{[\mathrm{H^+}]}{K_{\mathrm{es1}}}\right)\right)} \tag{24}$$

$v_{\max} = k_1[\mathrm{E}]$, being [E] expressed as enzyme activity (units/mL), N_{Av} is the Avogadro's number and V_{p} is an average volume calculated from the prolate and the pear shapes expressed in dm^3 (for simplicity, the volume is kept constant during the transformation from the prolate to the budded limiting shape). The equations contain concentrations (indicated in square brackets) and numbers of molecules (N_{innerHOA}, $N_{\mathrm{innerOA^-}}$, $N_{\mathrm{OA^-\,free\,inner}}$ and $N_{\mathrm{HOA\,free\,inner}}$ in equation 18). Concentrations and numbers of molecules are correlated through the Avogadro's number and the volume of the vesicles. The initial concentrations and parameters used for the simulations are reported in Table 1. The kinetic constants used in the model are listed in Table 2.

Table 1 Initial concentrations and parameters used for the kinetic simulations. $[CO_2]$ and $[CO_2]_{out}$ are calculated by considering the solution at the equilibrium with the atmosphere at 25 °C. The transfer rates k_X (s^{-1}) were calculated from the permeabilities P_X as $k_X = 3P_X/R$ where R (dm) is the vesicle radius.

[X] (M)		Parameters	
$[S]$	0	[E] (U/mL)	1.10
$[NH_3]$	0	$[S]_{out}$ (M)	6.00×10^{-2}
$[NH_4^+]$	0	$[NH_3]_{out}$ (M)	0
$[CO_2]$	1.20×10^{-5}	$[H^+]_{out}$ (M)	1.00×10^{-6}
$[HCO_3^-]$	5.62×10^{-6}	$[OH^-]_{out}$ (M)	1.00×10^{-8}
$[CO_3^{2-}]$	3.15×10^{-10}	$[HA]_{out}$ (M)	5.45×10^{-8}
$[H^+]$	1.00×10^{-6}	$[CO_2]_{out}$ (M)	1.20×10^{-5}
$[OH^-]$	1.00×10^{-8}	P_S (dm/s) [2]	4.00×10^{-7}
$[HA]$	5.45×10^{-8}	P_N (dm/s) [3]	1.00×10^{-3}
$[A^-]$	9.45×10^{-7}	P_{HA} (dm/s) [4]	6.50×10^{-4}
[pyrOH]	4.81×10^{-5}	P_C (dm/s) [5]	1.20
$[pyrO^-]$	1.92×10^{-6}	V_P (dm^3)	6.82×10^{-13}

Table 2 Kinetic constants used in the model. Enzymatic constants were taken from refs. [6–8]. Equilibrium rate constants were derived from the pK_a according to refs. [8–10].

Enzymatic		pH equilibria		
k_1 (U^{-1} mL M s^{-1})	3.7×10^{-6}		forward (s^{-1})	reverse (M^{-1} s^{-1})
K_m (M)	3.0×10^{-3}	k_2	24	4.3×10^{10}
K_{es1} (M)	5.0×10^{-6}	k_3	3.7×10^{-2}	7.9×10^{4}
K_{es2} (M)	2.0×10^{-9}	k_4	2.8	5×10^{10}
K_S (M)	3.0	k_5	1×10^{-3} (M^{-1} s^{-1})	1×10^{11}
K_P (M)	2.0×10^{-3}	k_6	7.8×10^{5}	4.5×10^{10}
		k_7	3.2×10^{2}	1×10^{10}
		k_8	1	2.5×10^{7}

The variation of the number of molecules is described by the following differential equations

$$\frac{\mathrm{d}N_{\mathrm{outerPOPC}}}{\mathrm{d}t} = \frac{\mathrm{d}N_{\mathrm{innerPOPC}}}{\mathrm{d}t} = 0 \tag{25}$$

$$\frac{\mathrm{d}N_{\mathrm{outerHOA}}}{\mathrm{d}t} = -k_7 N_{\mathrm{outerHOA}} + k_{7\mathrm{r}} N_{\mathrm{outerOA^-}} \left[\mathrm{H^+_{out}}\right] - k_{\mathrm{f}} \left(N_{\mathrm{outer}} - N_{\mathrm{inner}} \frac{R_{\mathrm{s}}^2}{(R_{\mathrm{s}}-h)^2} \right) \tag{26}$$

$$\frac{\mathrm{d}N_{\mathrm{outerOA^-}}}{\mathrm{d}t} = k_7 N_{\mathrm{outerHOA}} - k_{7\mathrm{r}} N_{\mathrm{outerOA^-}} \left[\mathrm{H^+_{out}}\right] \tag{27}$$

$$\frac{\mathrm{d}N_{\mathrm{innerHOA}}}{\mathrm{d}t} = -k_7 N_{\mathrm{innerHOA}} + k_{7\mathrm{r}} N_{\mathrm{innerOA^-}} \left[\mathrm{H^+}\right] + k_{\mathrm{f}} \left(N_{\mathrm{outer}} - N_{\mathrm{inner}} \frac{R_{\mathrm{s}}^2}{(R_{\mathrm{s}}-h)^2} \right) \tag{28}$$

$$\frac{\mathrm{d}N_{\mathrm{innerOA^-}}}{\mathrm{d}t} = k_7 N_{\mathrm{innerHOA}} - k_{7\mathrm{r}} N_{\mathrm{innerOA^-}} \left[\mathrm{H^+}\right] - N_{\mathrm{innerOA^-}} \frac{k_{\mathrm{off}}}{1+e^{(-k_{\mathrm{t}}(\mathrm{pH}-\mathrm{pH_{thres}}))}} \tag{29}$$

$$\frac{\mathrm{d}N_{\mathrm{OAfree\,inner}}}{\mathrm{d}t} = N_{\mathrm{innerOA^-}} \frac{k_{\mathrm{off}}}{1+e^{(-k_{\mathrm{t}}(\mathrm{pH}-\mathrm{pH_{thres}}))}} + k_7 N_{\mathrm{HOA\,free\,inner}} - k_{7\mathrm{r}} N_{\mathrm{OA^-\,free\,inner}} \left[\mathrm{H^+}\right] \tag{30}$$

$$\frac{\mathrm{d}N_{\mathrm{HOAfree\,inner}}}{\mathrm{d}t} = -k_7 N_{\mathrm{HOA\,free\,inner}} + k_{7\mathrm{r}} N_{\mathrm{OA^-\,free\,inner}} \left[\mathrm{H^+_{inner}}\right] \tag{31}$$

where N_{innerHOA} and $N_{\mathrm{innerOA^-}}$ indicate respectively the number of molecules for the unionized and deionized form in the inner leaflet, N_{outerHOA} and $N_{\mathrm{outerOA^-}}$ are the oleic acid molecules in the outer leaflet, $N_{\mathrm{OAfree\,inner}}$ and $N_{\mathrm{HOAfree\,inner}}$ are the molecules of oleate and oleic acid dissolved in the aqueous lumen of the vesicles, h is the neutral bilayer thickness and R_{s} is the radius of the initial spherical vesicle. Oleic acid molecules diffuse from the outer leaflet to the inner leaflet with a rate constant k_{f}. Oleate solubilization is expressed as a function of pH through a logistic equation with the parameters k_{off}, k_{t} (it affects the slope) and $\mathrm{pH_{thres}}$ (this is the threshold value of pH at which the solubility of oleate changes dramatically). The initial conditions and the parameters used are reported in Table 3.

Table 3 Initial conditions and parameters used for the kinetic simulations.

Initial conditions		Parameters	
N_{outerHOA}	5.795×10^8	$N_{\mathrm{outerPOPC}}$	6.476×10^8
$N_{\mathrm{outerOA^-}}$	1.832×10^7	$N_{\mathrm{innerPOPC}}$	6.473×10^8
N_{innerHOA}	5.792×10^8	R_{S} (nm)	7.12×10^3
$N_{\mathrm{innerOA^-}}$	1.831×10^7	h (nm)	2
$N_{\mathrm{HOA\,free\,inner}}$	0	k_{f} ($\mathrm{s^{-1}}$)	0.4
$N_{\mathrm{OA^-\,free\,inner}}$	0	k_{off} ($\mathrm{s^{-1}}$)	0.008
		k_{t}	100
		$\mathrm{pH_{thres}}$	6.3

The initial total number of molecules for the outer (N_{outer}) and the inner leaflet (N_{inner}) was calculated from the spherical vesicle:

$$N_{\mathrm{outer}} = \frac{4\pi R_{\mathrm{s}}^2}{<\tilde{a}>} \tag{32}$$

$$N_{\mathrm{inner}} = \frac{4\pi\,(R_{\mathrm{s}}-h)^2}{<\tilde{a}>} \tag{33}$$

where $<\tilde{a}>$ is the mean cross-sectional area expressed in $\mathrm{nm^2}$. The number of molecules of POPC and Oleic acid present

in membrane was derived from the initial composition ($[\text{Oleic}]_0 / [\text{POPC}]_0 = 2.4\text{mM}/2.6\text{mM}$)

$$N_{\text{outer}} = N_{\text{outerPOPC}} + N_{\text{outerOleic}} \tag{34}$$

$$N_{\text{outerPOPC}} = \frac{N_{\text{outer}}}{1 + \frac{[\text{Oleic}]_0}{[\text{POPC}]_0}} \tag{35}$$

$$N_{\text{outerOleic}} = N_{\text{outer}} - N_{\text{outerPOPC}} \tag{36}$$

The number of oleic acid and oleate molecules was calculated from the initial pH of the solution (at t=0 s $\text{pH}_{\text{outer}} \simeq \text{pH}_{\text{inner}} = 6$) and from the p$K_a$ (the pK_a of oleic acid incorporated in lipid membranes is 7.5) [11].

$$N_{\text{outerOleic}} = N_{\text{outerHOA}} + N_{\text{outerOA}^-} \tag{37}$$

$$N_{\text{outerHOA}} = \frac{N_{\text{outerOleic}}}{1 + 10^{\text{pH}-\text{p}K_a}} \tag{38}$$

$$N_{\text{outerOA}^-} = N_{\text{outerOleic}} - N_{\text{outerHOA}} \tag{39}$$

Equations 34 - 39 hold also for the inner leaflet.

Notes and references

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