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Published in:
Langmuir

DOI:
[10.1021/la960322+](https://doi.org/10.1021/la960322+)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
1996

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Ravoo, B. J., Weringa, W. D., & Engberts, J. B. F. N. (1996). Design and characterization of synthetic bilayer vesicles with a polymerized inner bilayer leaflet. *Langmuir*, 12(24), 5773 - 5780.
<https://doi.org/10.1021/la960322+>

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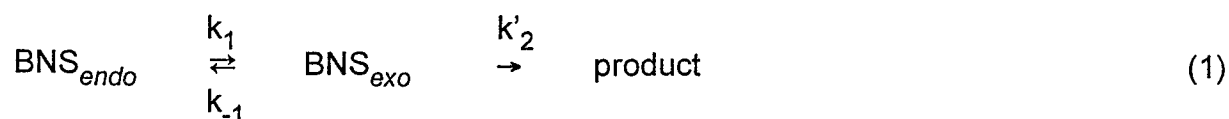
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Supplementary Material to

Design and Characterization of Synthetic Bilayer vesicles with a Polymerized Inner Bilayer Leaflet

Bart Jan Ravoo, Wilke D. Weringa and Jan B.F.N. Engberts

Kinetic data for the specific *exo*-vesicular cleavage of β -nitrostyrene (BNS) groups were analyzed in terms of a model assuming (1) partitioning of the BNS groups over the inner and outer bilayer leaflets, defining BNS_{endo} and BNS_{exo} , respectively, (2) pseudo-first-order cleavage of BNS_{exo} at the outer bilayer leaflet, characterized by k'_2 , (3) translocation of amphiphile molecules over the bilayer (*flip-flop*), resulting in exchange between BNS_{endo} and BNS_{exo} , characterized by k_1 and k_{-1} and (4) negligible permeation of hydroxide ion over the bilayer within the time scale of the cleavage experiment. Schematically:



$$d[BNS_{exo}]/dt = k_1[BNS_{endo}] - k_{-1}[BNS_{exo}] - k'_2[BNS_{exo}] \quad (2)$$

$$d[BNS_{endo}]/dt = -k_1[BNS_{endo}] + k_{-1}[BNS_{exo}] \quad (3)$$

Thus:

$$x' = k_1y - (k'_2 + k_{-1})x \quad (4)$$

$$y' = k_{-1}x - k_1y \quad (5)$$

with $x = [BNS_{exo}]$ and $y = [BNS_{endo}]$ and x' and y' representing their functions of time. Applying Laplace transformation the following subsidiary equation is obtained:

$$sX - x_0 = k_1Y - (k'_2 + k_{-1})X \quad (6)$$

$$sY - y_0 = k_{-1}X - k_1Y \quad (7)$$

in which X and Y are Laplace transforms of x and y . Expressing Y through X :

$$Y = 1/k_1(sX - x_0 + (k'_2 + k_{-1})X) \quad (8)$$

Now substitution and solution for X yields:

$$x_0k_1 - sk_1X - k'_2k_1X = s^2X - sx_0 - y_0k_1 + s(k'_2 + k_{-1})X \quad (9)$$

so:

$$X = x_0(s + k_1 + k_1y_0/x_0)/(s^2 + s(k'_2 + k_1 + k_{-1}) + k'_2k_1) \quad (10)$$

$$= x_0(s + k_1 + k_1 y_0/x_0)/((s + k_4)(s + k_5))$$

in which $k_3 = 1/2(k'_2 + k_1 + k_{-1})$, $k_4 = k_3 + (k_3^2 - k'_2 k_1)^{1/2}$ and $k_5 = k_3 - (k_3^2 - k'_2 k_1)^{1/2}$.
Now we can separate:

$$X = A_1^x/(s + k_4) + A_2^x/(s + k_5) \quad (11)$$

in which $A_1^x = (k_1(x_0 + y_0) - k_4 x_0)/2(k_3 - k_4)$ and $A_2^x = (k_1(x_0 + y_0) - k_5 x_0)/2(k_3 - k_5)$

Using reverse Laplace transformation, we obtain the solution

$$x = A_1^x \exp(-k_4 t) + A_2^x \exp(-k_5 t) \quad (12)$$

A similar solution can be obtained for y, with $A_1^y = (k_{-1}(x_0 + y_0) - k'_2 y_0 - k_4 x_0)/2(k_3 - k_4)$ and $A_2^y = (k_{-1}(x_0 + y_0) - k'_2 y_0 - k_5 x_0)/2(k_3 - k_5)$. These results imply that [BNS_{endo} + BNS_{exo}] as a function of time (which is monitored experimentally) can be expressed as:

$$x + y = (A_1^x + A_1^y) \exp(-k_4 t) + (A_2^x + A_2^y) \exp(-k_5 t) \quad (13)$$

In case of similar k_1 , k_{-1} and k'_2 it is necessary to use the full equation, but if $k_1 \approx k_{-1} \ll k'_2$ it is possible to use a simplified form. In this case we can define:

$$B = k_4 + k_5 = 2k_3 = k'_2 + k_1 + k_{-1} \approx k'_2 + 2k_1 \quad (14)$$

$$C = 1/4((k_4 + k_5)^2 - (k_4 - k_5)^2) = k_1 k'_2 = k_1(B - 2k_1) \quad (15)$$

so $k_1^2 - B/2(k_1) + C/2 = 0$ and therefore:

$$k_1 = 1/4(B - (B^2 - 8C)^{1/2}) \text{ and } k'_2 = 1/2(B + (B^2 - 8C)^{1/2}) \quad (16)$$

Since k_4 and k_5 can be determined accurately from a double exponential fit to the experimental data (in the paper, they are reported as k'_{fast} and k'_{slow}), k_1 (the rate constant for *flip-flop*) and k'_2 (the rate constant for cleavage of the BNS group in the outer bilayer leaflet) can be calculated. The results of the calculation are summarized in the following table (all values in 10^4 s^{-1}):

lipid	k_4	k_5	B	C	k_1	k'_2
6	6.25	0.095	6.35	0.610	0.100	6.15
5	5.49	0.316	5.81	1.75	0.343	5.13
5 (EtOH inj.)	7.33	0.769	8.10	5.64	0.893	6.32
4	5.11	0.584	5.69	2.97	0.680	4.32