

# Multivalent Calixarene-Based Liposomes as Platforms for Gene and Drug Delivery

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## 1. Deduction of Equation (5)<sup>1</sup>

The mass ratio corresponding to neutral liposomes can be written as:

$$\left(\frac{L}{D}\right)_{\phi} = \left(\frac{L^{+} + L^0}{D}\right)_{\phi} \quad (1)$$

The effective charge of a liposome is defined as:

$$Z_{\text{eff}} = \frac{n_{+}}{n_{-}} = \left(\frac{q_{L^{+}}^{+}}{q_{\text{ADN}}^{-}}\right) \times \left(\frac{\left(\frac{L^{+}}{M_L^{+}}\right)}{\left(\frac{D}{M_{\text{bp}}}\right)}\right) \quad (2)$$

When  $Z_{\text{eff}} = 1$ , the ADN mass (D) can be written:

$$D = \left(\frac{q_{L^{+}}^{+}}{q_{\text{ADN}}^{-}}\right) \times \left(\frac{\left(\frac{L^{+}}{M_L^{+}}\right)}{\left(\frac{1}{M_{\text{bp}}}\right)}\right) \quad (3)$$

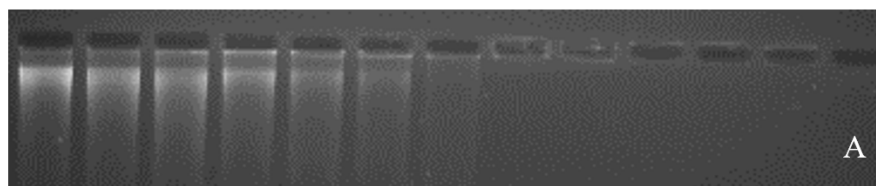
Equation (4) was obtained by considering Equations (1) and (3):

$$\left(\frac{L}{D}\right)_{\phi} = \frac{(L^{+} + L^0)}{\left(\frac{q_{L^{+}}^{+}}{q_{\text{ADN}}^{-}}\right) \times \left(\frac{\left(\frac{L^{+}}{M_L^{+}}\right)}{\left(\frac{1}{M_{\text{bp}}}\right)}\right)} = \frac{q_{\text{ADN}}^{-}}{q_{L^{+}}^{+}} \times \frac{M_L^{+}}{M_{\text{bp}}} \times \left(\frac{L^{+} + L^0}{L^{+}}\right) = \frac{q_{\text{ADN}}^{-}}{q_{L^{+}}^{+}} \times \frac{M_L^{+}}{M_{\text{bp}}} \times \frac{(n^{+}M_L^{+} + n^0M_L^0)}{n^{+}M_L^{+}} \quad (4)$$

If one multiplies by  $[(n^{+} + n^0)/(n^{+} + n^0)]$ , and introduces the molar fraction  $\alpha$ , Equation (5) is obtained. This equation relates  $(L/D)_{\phi}$  and  $\alpha$ :

$$\left(\frac{L}{D}\right)_{\phi} = \frac{q_{\text{ADN}}^{-}}{q_{L^{+}}^{+}} \times \frac{M_L^{+}}{M_{\text{bp}}} \times \frac{(n^{+}M_L^{+} + n^0M_L^0)}{(n^{+} + n^0)} \times \frac{1}{\left(\frac{n^{+}}{(n^{+} + n^0)}\right)} = \frac{q_{\text{ADN}}^{-}}{q_{L^{+}}^{+}} \times \frac{M_L^{+}}{M_{\text{bp}}} \times \frac{\alpha M_L^{+} + (1-\alpha)M_L^0}{\alpha} \quad (5)$$

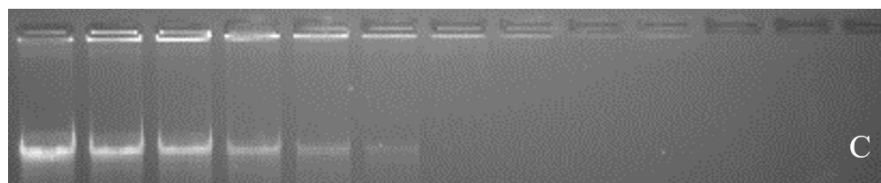
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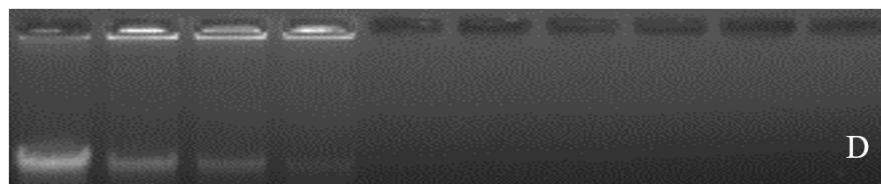
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ADN 0.5 1 1.5 2 2.5 3 4 5 6 7 8 L/D



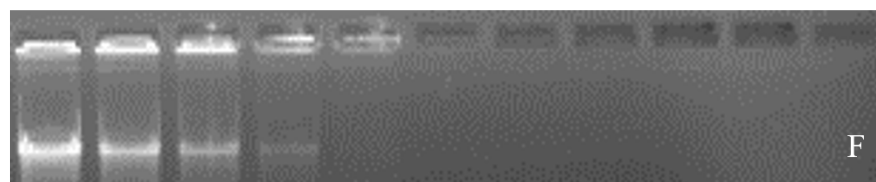
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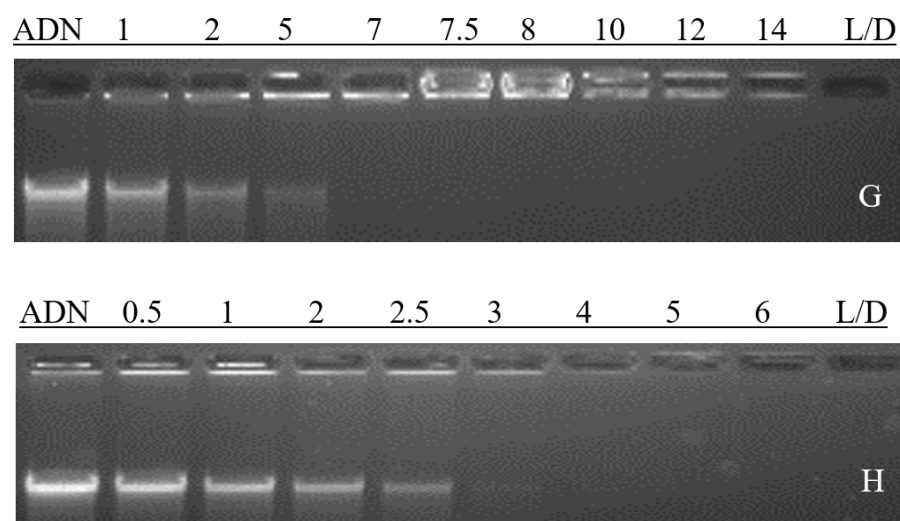


ADN 1 4 6 6.5 7 8 9 10 11 12 14 L/D

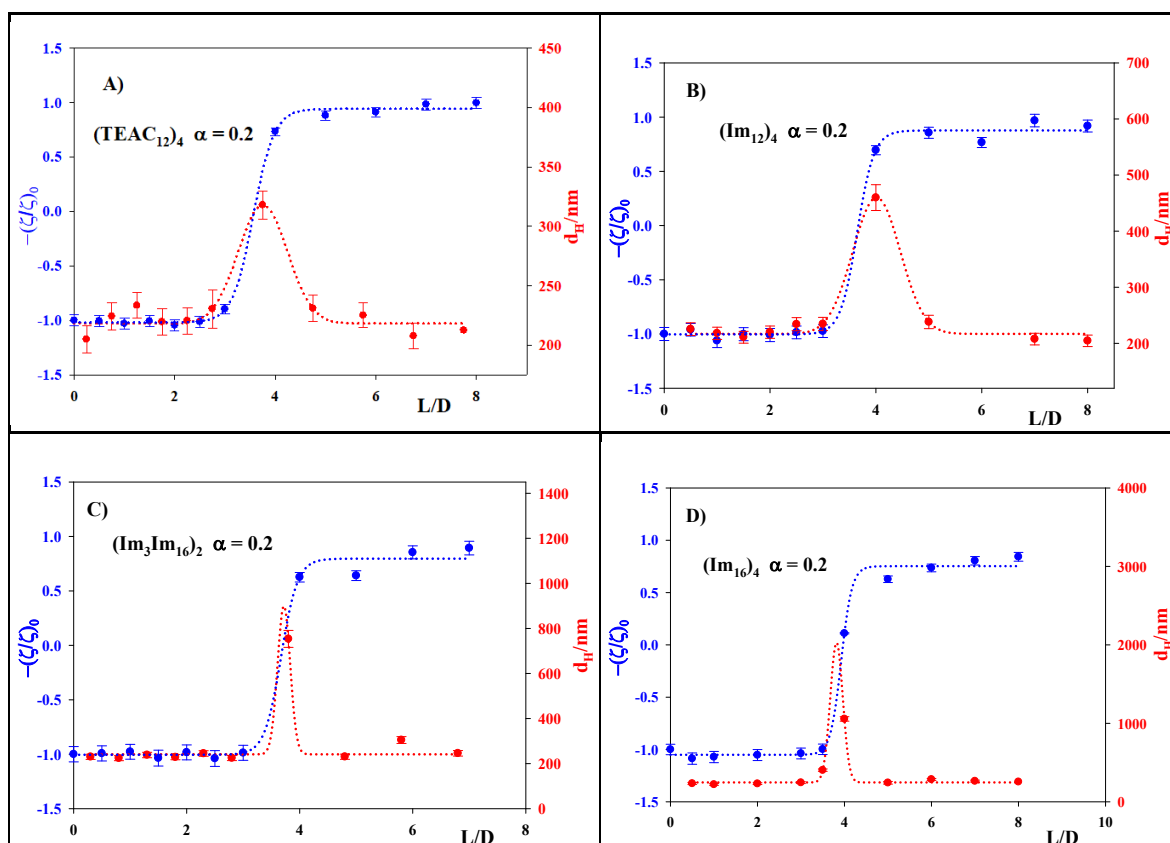


ADN 0.25 0.5 1 1.5 2 2.5 3 4 5 L/D





**Figure S1.** Electrophoretic mobility shift assay on an agarose gel (1%) for CAL/DOPE/DNA lipoplexes at different  $\alpha$  values, varying the mass ratio L/D. Experiments were run in TAE buffer (40 mM tris-acetate, 1 mM EDTA) and stained with Red Safe. Electrophoresis was performed at 90 V until completion. (TEAC<sub>12</sub>)<sub>4</sub>: (A)  $\alpha = 0.2$  y (B)  $\alpha = 0.5$ ; (Im<sub>12</sub>)<sub>4</sub>: (C)  $\alpha = 0.2$  y (D)  $\alpha = 0.5$ ; (Im<sub>16</sub>Im<sub>3</sub>)<sub>2</sub>: (E)  $\alpha = 0.1$  y (F)  $\alpha = 0.5$ , and (Im<sub>16</sub>)<sub>4</sub>: (G)  $\alpha = 0.1$  y (H)  $\alpha = 0.3$ .



**Figure S2.** Dependence of the relative zeta potential,  $-(\zeta/\zeta_0)$ , and of the hydrodynamic diameter,  $d_H$ , of CAL/DOPE/DNA lipoplexes on L/D for  $\alpha = 0.2$ . T = 303.0.1  $\pm$  0.1 K. (A) (TEAC<sub>12</sub>)<sub>4</sub>; (B) (Im<sub>12</sub>)<sub>4</sub>; (C) (Im<sub>3</sub>Im<sub>16</sub>)<sub>2</sub>; (D) (Im<sub>16</sub>)<sub>4</sub>.

#### Reference:

1. M. Muñoz-Úbeda, S.K. Misra, A.L. Barrán-Berdon, C. Aicart-Ramos, M.B. Sierra, J. Biswas, P. Kondaiah, E. Junquera, S. Bhattacharya, E. Aicart, *J. Am. Chem. Soc.* **2011**, *133*, 18014–18017. <https://doi.org/10.1021/ja204693f>.