Lipoplex mediated deintercalation of doxorubicin from calf thymus DNA-doxorubicin complex

Supporting Information

Estimation of Binding constant of Doxorubicin to liposomes: We used the following methodology to obtain the concentration of liposomes aggregates.[1-2] For this purpose; we first calculated the total number of lipid molecules per vesicle using the following equation,[1-2]

$$N_{Total} = \frac{\left[\left\{4\pi(d/2)^2\right\} + \left\{4\pi(d/2-h)^2\right\}\right]}{a} \quad (4)$$

where h is lipid bilayer thickness and taken as 4.45 nm,[3] d is the diameter of small unilamellar vesicle. We already reported that in case of this kind of SUV, the diameter is around 100 nm.[4] The confocal imaging measurement (the last section of the manuscript) also indicates that the size of the liposomes lies within this range. And a, the average area per lipid molecule was taken as 0.59 nm^2.[3] Thus the number of liposome per mL (N_{lip}) for known concentrations of lipids was calculated by the following equation[1-2]

$$N_{lip} = \frac{M(lipid) \times N_A}{N_{Total} \times 1000} \quad (5)$$

The number of liposome per ml was used to calculate the concentration of the liposomes aggregates in the solution. After obtaining the liposome concentration, we estimated the binding constant using a nonlinear fitting of Benesi-Hidlebrand equation[5] (Figure 2c) as following with a correlation factor around 0.99.

$$I_f = \frac{I_f{}^0 + I_{Liposome-DOX} K_1[L]}{1 + K_1[L]} \quad (6)$$

where I_f is the fluorescence intensity of DOX at different concentration of liposomes, $I_{Liposome-DOX}$ is the fluorescence intensity at maximum concentration of liposomes, $I_f{}^0$ is the initial fluorescence intensity of DOX in buffer solution in absence of liposomes, $[L]$ is the concentration of liposomes defined by equation 4.

Reference

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Supporting Information Table 1(a): Time resolved data of DOX upon varying concentration of DPPC liposome. (Uncertainty limit ± 5 %)

[DPPC] (mM)	a_1	a_2	τ_1 (ns)	τ_2 (ns)	$\langle\tau\rangle$ (ns)	χ^2
0.00	1.00	0.00	1.10	0.00	1.10	1.13
0.10	0.97	0.03	1.10	2.61	1.15	1.11
0.20	0.85	0.15	1.10	2.79	1.35	1.08
0.30	0.72	0.28	1.10	3.10	1.66	1.12
0.40	0.66	0.34	1.10	3.23	1.82	1.10
0.50	0.56	0.44	1.10	3.40	2.11	1.05
0.70	0.42	0.58	1.10	3.65	2.58	1.15
0.90	0.33	0.67	1.10	3.75	2.97	1.14
1.00	0.24	0.76	1.10	4.10	3.38	1.04

Supporting Information Table 1(b): Time resolved data of DOX upon varying concentration of DMPC liposome. (Uncertainty limit ± 5 %)

[DMPC] (mM)	a_1	a_2	τ_1 (ns)	τ_2 (ns)	$\langle\tau\rangle$ (ns)	χ^2
0.00	1.00	0.00	1.10	0.00	1.10	1.14
0.10	0.94	0.06	1.10	1.93	1.15	1.10
0.20	0.88	0.12	1.10	2.10	1.22	1.02
0.30	0.79	0.21	1.10	2.22	1.34	1.14
0.40	0.71	0.29	1.10	2.39	1.47	1.11
0.50	0.60	0.40	1.10	2.61	1.70	1.08
0.70	0.49	0.51	1.10	2.82	1.98	1.15
0.90	0.36	0.64	1.10	3.20	2.44	1.10
1.00	0.32	0.68	1.10	3.55	2.77	1.11

Supporting Information Table 1(c): Time resolved data of DOX upon varying concentration of POPC liposome. (Uncertainty limit ± 5 %)

[POPC] (mM)	a_1	a_2	τ_1 (ns)	τ_2 (ns)	$\langle\tau\rangle$ (ns)	χ^2
0.00	1.00	0.00	1.10	0.00	1.10	1.11
0.10	0.95	0.06	1.10	1.71	1.15	1.10
0.20	0.86	0.15	1.10	2.06	1.26	1.05
0.30	0.76	0.24	1.10	2.11	1.34	1.03
0.40	0.62	0.39	1.10	2.31	1.58	1.05
0.50	0.52	0.47	1.10	2.41	1.70	1.02
0.70	0.45	0.56	1.10	2.57	1.93	1.11
0.90	0.35	0.64	1.10	2.85	2.21	1.05
1.00	0.34	0.66	1.10	3.00	2.35	1.12

Supporting Information Table 2(a): Time resolved data for the DOX-DNA-DPPC complex (DPPC lipoplex) upon increasing concentration of liposome. (Uncertainty limit ± 5 %)

[DPPC] (mM)	a_1	a_2	τ_1 (ns)	τ_2 (ns)	$\langle\tau\rangle$ (ns)	χ^2
0.00	0.48	0.52	1.10	2.45	1.80	1.12
0.10	0.47	0.53	1.10	2.64	1.92	1.17
0.20	0.45	0.55	1.10	2.83	2.05	1.11
0.30	0.42	0.57	1.10	3.05	2.20	1.19
0.40	0.40	0.60	1.10	3.25	2.39	1.14
0.50	0.37	0.62	1.10	3.44	2.54	1.15
0.70	0.35	0.65	1.10	3.85	2.89	1.10
0.90	0.32	0.67	1.10	4.10	3.10	1.19
1.00	0.31	0.69	1.10	4.21	3.25	1.14
0.00	0.30	0.70	1.10	4.32	3.35	1.18

Supporting Information Table 2(b): Time resolved data for the DOX-DNA-DMPC complex (DMPC lipoplex) upon increasing concentration of liposome. (Uncertainty limit ± 5 %)

[DMPC] (mM)	a_1	a_2	τ_1 (ns)	τ_2 (ns)	$\langle\tau\rangle$ (ns)	χ^2
0.00	0.48	0.52	1.10	2.45	1.80	1.05
0.10	0.46	0.53	1.10	2.58	1.87	1.11
0.20	0.44	0.55	1.10	2.74	1.99	1.13
0.30	0.43	0.57	1.10	2.95	2.15	1.14
0.40	0.42	0.59	1.10	3.10	2.29	1.12
0.50	0.38	0.61	1.10	3.24	2.39	1.11
0.70	0.35	0.65	1.10	3.47	2.64	1.09
0.90	0.32	0.67	1.10	3.55	2.73	1.11
1.00	0.31	0.68	1.10	3.63	2.81	1.12
0.00	0.29	0.69	1.10	3.66	2.84	1.14

Supporting Information Table 2(c): *Time resolved data for the DOX-DNA-POPC complex (POPC lipoplex) upon increasing concentration of liposome. (Uncertainty limit ± 5 %)*

[POPC] (mM)	a_1	a_2	τ_1 (ns)	τ_2 (ns)	$\langle\tau\rangle$ (ns)	χ^2
0.00	0.48	0.52	1.10	2.45	1.80	1.10
0.10	0.47	0.53	1.10	2.51	1.85	1.10
0.20	0.45	0.55	1.10	2.59	1.92	1.13
0.30	0.43	0.57	1.10	2.68	2.00	1.14
0.40	0.42	0.58	1.10	2.81	2.09	1.10
0.50	0.38	0.62	1.10	3.00	2.28	1.09
0.70	0.37	0.63	1.10	3.12	2.37	1.10
0.90	0.35	0.65	1.10	3.14	2.43	1.05
1.00	0.34	0.66	1.10	3.15	2.45	1.15
0.00	0.32	0.68	1.10	3.17	2.51	1.12

Supporting information Figure 1: Zeta titration curves for different lipoplexes upon increasing concentration of Ca^{2+}