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An "off-on"-type Electrochemiluminescent Immunosensor based on Resonance Energy Transfer and Liposome-assisted Strategy for Signal Amplification

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The calculation for amount of GSH per liposome

The strong encapsulation ability of liposomes to achieve a large amount of GSH encapsulation, and finally play an important role in improving the ECL signal of the system. The TEM image of GSHLL (seen as Fig. 2A in the manuscript body) shows that the liposome has a uniform closed vesicle structure with a diameter of approximately 180 nm. Assuming the liposomes were homogeneous and unilamellar spheres, the characterizations of liposomes could be calculated by means of the following equations:

(1) The volume of liposome:

$$V = (4/3)\,\pi R^3 = (4/3)\,\pi\,(90 \times 10^{-7})^3 \times 10^3\ \mu L = 3.1 \times 10^{-12}\ \mu L$$

(2) The encapsulated volume of a single liposome:

$$V = (4/3)\,\pi\,(R - T)^3 = (4/3)\,\pi\,[(90 - 4) \times 10^{-7}]^3 \times 10^3\ \mu L = 2.7 \times 10^{-12}\ \mu L$$

(3) The average head group surface area per lipid molecule:

$A = A_1P_1 + A_2P_2 = 0.71 \times (1/2) + 0.19 \times (1/2) = 0.45\ nm^2$

(4) The number of lipids per liposome:

$N_{tot} = 4\pi [R^2 + (R - T)^2]/A = 4\pi [90^2 + (90 - 4)^2]/0.45 = 4.3 \times 10^5$

(5) The number of liposomes per L:

$N_{lip} = (M_{lipid} \times N_A)/N_{tot} = (40 \times 10^{-6} \times 6.02 \times 10^{23})/4.3 \times 10^5 = 5.6 \times 10^{13}$

(6) Assuming that the GSH concentration inside the liposomes (M_{lipid}) is identical to the original solution used , the amount of GSH per liposome was calculated as follows:

$N_{GSH} = (M_{lipid} \times N_A)/N_{lipld} = (0.1 \times 6.02 \times 10^{23})/5.6 \times 10^{13} = 1.1 \times 10^9$

Where R is the diameter size of GOLL from TEM measurements, A is the average head group surface area per lipid, T is the bilayer thickness (4 nm). The components in the lipid composition are taken into account to theoretically calculate the average head group surface area per lipid (A). A_1 and A_2 are 0.71 nm and 0.19 nm^2 for ePC and cholesterol, respectively. P_1 and P_2 are the mole fractions of ePC, cholesterol, respectively, from the molar ratio of 1:1. The A obtained for these liposomes is 0.45 nm^2 through using these values and weighting by the mole fraction of each component. N_{tot} is the number of lipids per liposome, and the N_{lipid} is the number of liposomes per L. M_{lipid} means the molar concentration of lipid.