

## Electronic Supplementary Information (ESI)

### Microviscosity, Encapsulation, and Permeability of 2-Ketooctanoic Acid

#### Vesicle membranes

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#### First-order kinetics for $\text{OH}^-$ permeation across vesicular membranes

The acidic dissociation equilibrium of the fluorescence probe riboflavin can be represented as:



where RH and  $\text{R}^-$  represent the neutral and deprotonated riboflavin, respectively, and  $K_a$  is the acidic dissociation constant of RH. One has:

$$K_a = \frac{[\text{R}^-][\text{H}^+]}{[\text{RH}]} \quad (\text{S2a})$$

and 
$$[\text{OH}^-] = \frac{K_w[\text{R}^-]}{K_a[\text{RH}]} \quad (\text{S2b})$$

where  $[i]$  is the molar concentration of the  $i$  specie, and  $K_w$  is the dissociation constant of  $\text{H}_2\text{O}$ .

In our case, the total riboflavin molecules exist in two states: one is adsorbed on the external vesicle membrane surfaces and another is enclosed within the internal vesicle

aqueous core. Thus, before adjusting the pH of the KOCOOH solution, one has,

$$[\text{RH}]_{\text{total}} = [\text{RH}]_{\text{ex}} + [\text{RH}]_{\text{in}} \quad (\text{S3a})$$

$$[\text{R}^-]_{\text{total}} = [\text{R}^-]_{\text{ex}} + [\text{R}^-]_{\text{in}} \quad (\text{S3b})$$

where  $[\text{RH}]_{\text{total}}$  and  $[\text{R}^-]_{\text{total}}$  are the total molar concentrations of RH and  $\text{R}^-$  in the system, respectively,  $[\text{RH}]_{\text{ex}}$  and  $[\text{R}^-]_{\text{ex}}$  are the molar concentrations of RH and  $\text{R}^-$  adsorbed on the external vesicle surfaces, respectively, and  $[\text{RH}]_{\text{in}}$  and  $[\text{R}^-]_{\text{in}}$  are the molar concentrations of RH and  $\text{R}^-$  enclosed within the internal vesicle aqueous core, respectively.

From Eq. (S2b), we have,

$$[\text{OH}^-]_{\text{in}}^0 = \frac{K_w [\text{R}^-]_{\text{in}}}{K_a [\text{RH}]_{\text{in}}} \quad (\text{S4a})$$

$$[\text{OH}^-]_{\text{in}}^t = \frac{K_w ([\text{R}^-]_{\text{in}} + [\text{R}^-]_{\text{in}}^t)}{K_a ([\text{RH}]_{\text{in}} - [\text{R}^-]_{\text{in}}^t)} \quad (\text{S4b})$$

where  $[\text{OH}^-]_{\text{in}}^0$  and  $[\text{OH}^-]_{\text{in}}^t$  are the  $\text{OH}^-$  concentrations within the internal vesicles at  $t = 0$  and time  $t$ , respectively, and  $[\text{R}^-]_{\text{in}}^t$  is the molar concentrations of  $\text{R}^-$  generated after the pH adjusting in the internal vesicles at time  $t$ . Eq. (S4b) minus Eq. (S4a) gives,

$$[\text{OH}^-]_{\text{in}}^t - [\text{OH}^-]_{\text{in}}^0 = \frac{K_w [\text{R}^-]_{\text{in}}^t}{K_a ([\text{RH}]_{\text{in}} - [\text{R}^-]_{\text{in}}^t)} \left(1 - \frac{[\text{R}^-]_{\text{in}}}{[\text{RH}]_{\text{in}}}\right) \quad (\text{S5a})$$

In our case, the pH within the internal vesicles at  $t = 0$  is 6.8, and the value of  $[\text{R}^-]_{\text{in}}/[\text{RH}]_{\text{in}}$  ( $\sim 10^{-3.4}$ ) is much little than 1. Therefore, we have,

$$[\text{OH}^-]_{\text{in}}^t \approx [\text{OH}^-]_{\text{in}}^0 + \frac{K_w [\text{R}^-]_{\text{in}}^t}{K_a ([\text{RH}]_{\text{in}} - [\text{R}^-]_{\text{in}}^t)} \quad (\text{S5b})$$

The fluorescence emission intensity ( $I_{\text{em}}$ ) of riboflavin in the KOCOOH solution arises from the contribution of the two states of the dye molecules. After adjusting the pH of KOCOOH solution from 6.8 to 10.2, the ‘‘instantaneous’’ loss in the  $I_{\text{em}}$  of the RH-containing KOCOOH solution can be attributed to the deprotonation of riboflavin adsorbed on the external vesicle surfaces, and the gradual reduction in the residual  $I_{\text{em}}$  with time ( $t$ ) can be

attributed to the deprotonation of riboflavin enclosed within the internal vesicle aqueous core.

Assuming that the  $I_{em}$  of riboflavin is proportional to  $[RH]$ , i.e.,

$$I_{em} = \varepsilon[RH] \quad (S6)$$

in which  $\varepsilon$  is a coefficient, we have,

$$I_0^0 = \varepsilon([RH]_{ex} + [RH]_{in}) \quad (S7a)$$

$$I_0 = \varepsilon([RH]_{ex}^0 + [RH]_{in}^0) \quad (S7b)$$

and

$$I_0^0 - I_0 = \varepsilon[R^-]_{ex}^0 \quad (S8a)$$

$$I_0 - I_\infty = \varepsilon[R^-]_{in}^\infty \quad (S8b)$$

where  $I_0^0$  and  $I_0$  are the  $I_{em}$  of the RH-containing solution before and just after ( $t = 0$ ) adjusting its pH, respectively,  $I_\infty$  is the  $I_{em}$  of the solution at  $t \rightarrow \infty$  (the permeation equilibrium state),  $[RH]_{ex}^0$  and  $[RH]_{in}^0$  are the molar concentrations of RH adsorbed on the external vesicle surfaces and enclosed within the internal vesicle aqueous core, respectively,  $[R^-]_{ex}^0$  and  $[R^-]_{in}^\infty$  are the molar concentrations of  $R^-$  generated after the pH adjusting in the external vesicles at  $t = 0$  and in the internal vesicles at  $t \rightarrow \infty$ , respectively. Note that  $[RH]_{in} = [RH]_{in}^0$  owing to no  $OH^-$  permeation occurring at  $t = 0$ , and that  $[RH]_{ex}^0 = [RH]_{ex}^\infty$  and  $[R^-]_{ex}^0 = [R^-]_{ex}^\infty$  owing to the deprotonation of RH adsorbed on the external vesicles is instantaneously completed after the pH adjusting.

Based on Eqs. (S8a) and (S8b), we have,

$$\frac{I_0^0 - I_0}{I_0 - I_\infty} = \frac{[R^-]_{ex}^0}{[R^-]_{in}^\infty} \equiv \frac{[R^-]_{ex}^\infty}{[R^-]_{in}^\infty} \quad (S9)$$

Assuming that when the  $OH^-$  permeation equilibrium is achieved (i.e., at  $t \rightarrow \infty$ ), the  $OH^-$  concentration of the internal vesicles,  $[OH^-]_{in}^\infty$ , is equal to that of the external vesicles (bulk

solution),  $[\text{OH}^-]_{\text{ex}}^0$  (where  $[\text{OH}^-]_{\text{ex}}^0 \equiv [\text{OH}^-]_{\text{ex}}^\infty$ ). From Eq. (S2b), we have,

$$[\text{OH}^-]_{\text{ex}}^\infty = \frac{K_w([\text{R}^-]_{\text{ex}} + [\text{R}^-]_{\text{ex}}^\infty)}{K_a([\text{RH}]_{\text{ex}} - [\text{R}^-]_{\text{ex}}^\infty)} \quad (\text{S10a})$$

$$[\text{OH}^-]_{\text{in}}^\infty = \frac{K_w([\text{R}^-]_{\text{in}} + [\text{R}^-]_{\text{in}}^\infty)}{K_a([\text{RH}]_{\text{in}} - [\text{R}^-]_{\text{in}}^\infty)} \quad (\text{S10b})$$

Because in our case,  $[\text{R}^-]_{\text{ex}}^\infty \gg [\text{R}^-]_{\text{ex}}$  and  $[\text{R}^-]_{\text{in}}^\infty \gg [\text{R}^-]_{\text{in}}$ , from Eqs. (S10a) and (10b), we can obtain,

$$\frac{[\text{R}^-]_{\text{ex}}^\infty}{[\text{R}^-]_{\text{in}}^\infty} \approx \frac{[\text{RH}]_{\text{ex}}}{[\text{RH}]_{\text{in}}} \quad (\text{S11})$$

Based on Eqs. (S7a), (S9), and (S11), we can obtain,

$$[\text{RH}]_{\text{in}} = \frac{I_0^0(I_0 - I_\infty)}{\varepsilon(I_0^0 - I_\infty)} \quad (\text{S12})$$

At time  $t$ , we have,

$$[\text{R}^-]_{\text{in}}^t = \frac{I_0 - I_t}{\varepsilon} \quad (\text{S13a})$$

$$[\text{RH}]_{\text{in}}^t = [\text{RH}]_{\text{in}} - [\text{R}^-]_{\text{in}}^t \quad (\text{S13b})$$

Based on Eqs. (S5b), (S12) and (S13), we have,

$$[\text{OH}^-]_{\text{in}}^t = [\text{OH}^-]_{\text{in}}^0 + \frac{K_w}{K_a} \left[ \frac{(I_0 - I_t)(I_0^0 - I_\infty)}{I_0^0(I_0 - I_\infty) - (I_0 - I_t)(I_0^0 - I_\infty)} \right] \quad (\text{S14})$$

The first-order kinetics for the permeation of  $\text{OH}^-$  across vesicular membranes can be derived as:

$$\ln\left(\frac{[\text{OH}^-]_{\text{ex}}^0 - [\text{OH}^-]_{\text{in}}^t}{[\text{OH}^-]_{\text{ex}}^0 - [\text{OH}^-]_{\text{in}}^0}\right) = -k_1 t \quad (\text{S15})$$

In our case,  $[\text{OH}^-]_{\text{ex}}^0 \gg [\text{OH}^-]_{\text{in}}^0$ . From Eqs. (S14) and (S15), we have,

$$\ln\left(1 - \frac{K_w}{K_a[\text{OH}^-]_{\text{ex}}^0} \left[ \frac{(I_0 - I_t)(I_0^0 - I_\infty)}{I_0^0(I_0 - I_\infty) - (I_0 - I_t)(I_0^0 - I_\infty)} \right]\right) = -k_1 t \quad (\text{S16a})$$

$$\ln\left(1 - \frac{[\text{H}^+]_{\text{ex}}^0}{K_a} \left[ \frac{(I_0 - I_t)(I_0^0 - I_\infty)}{I_0^0(I_0 - I_\infty) - (I_0 - I_t)(I_0^0 - I_\infty)} \right] \right) = -k_1 t$$

$$\ln\left(1 - \frac{[\text{RH}]_{\text{ex}}^0}{[\text{R}^-]_{\text{ex}}^0} \left[ \frac{(I_0 - I_t)(I_0^0 - I_\infty)}{I_0^0(I_0 - I_\infty) - (I_0 - I_t)(I_0^0 - I_\infty)} \right] \right) = -k_1 t \quad (\text{S16b})$$

From Eqs. (S7b), (S12), and (S13), we have,

$$[\text{RH}]_{\text{ex}}^0 = \frac{I_0}{\varepsilon} - [\text{RH}]_{\text{in}}^0 = \frac{I_0}{\varepsilon} - ([\text{RH}]_{\text{in}} - [\text{R}^-]_{\text{in}}^0) \quad (\text{S17a})$$

$$[\text{RH}]_{\text{ex}}^0 = \frac{I_0}{\varepsilon} - \frac{I_0^0(I_0 - I_\infty)}{\varepsilon(I_0^0 - I_\infty)} \quad (\text{S17b})$$

Based on Eqs. (S8a), (S16b), and (S17b), we can obtain,

$$\ln \left[ \frac{I_0^0(I_t - I_\infty)}{I_0^0(I_0 - I_\infty) - (I_0 - I_t)(I_0^0 - I_\infty)} \right] = -k_1 t \quad (\text{S18})$$

In the early stage of permeation,  $I_0^0(I_0 - I_\infty) \gg (I_0 - I_t)(I_0^0 - I_\infty)$ . The relative error for this

approximation was estimated to be lower than 2% for our case. Therefore, the first-order

kinetics for the permeation of  $\text{OH}^-$  across vesicular membranes can be written as:

$$\ln\left(\frac{I_t - I_\infty}{I_0 - I_\infty}\right) = -k_1 t \quad (\text{S19})$$