

SUPPORTING INFORMATION

Vesicular Release Dynamics are Altered by Interaction between Chemical Cargo and Vesicle Membrane Lipids

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EXPERIMENTAL DETAILS

Chemicals

1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC, >99%), 1,2-dioleoyl-*sn*-glycero-3-phosphethanolamine (DOPE, >99%) and cholesterol (ovine wool, >98%) were purchased from Avanti Polar Lipids, USA. Serotonin hydrochloride ($\geq 98.0\%$), dopamine hydrochloride (98.0%), chloroform ($\geq 99.9\%$), methanol ($\geq 99.9\%$) were obtained from Sigma-Aldrich. All aqueous solutions were prepared using $18 \text{ M}\Omega \cdot \text{cm}^{-1}$ water from Purelab Classic purification system (ELGA, Sweden).

Solutions

Lipid film hydration buffer for preparing liposomes:

The lipid film hydration was performed in a solution of 150 mM dopamine (DA)/serotonin (5-HT) hydrochloride in 10 mM HEPES at pH 7.4.

Pre-equilibration buffers for the Sephadex G-25 column:

The Sephadex G-25 column for removing free DA/5-HT outside liposomes was pre-equilibrated with a buffer containing 142 mM NaCl in 10 mM HEPES with pH 7.4.

Liposome isotonic buffer:

The storage, amperometric measurements and nanoparticle tracking analysis (NTA) of liposome samples were performed in buffer with 10 mM HEPES, 142 mM NaCl, pH 7.

All buffers were purged with argon for 30 min before adding oxygen sensitive DA/5-HT hydrochloride.

Liposome preparation

Neurotransmitter-loaded liposomes were prepared passively by thin lipid film hydration. A solution of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-dioleoyl-*sn*-glycero-3-phosphethanolamine (DOPE) and cholesterol (60:20:20 mole ratio) in chloroform was dried in a round-bottom flask by rotary evaporation until a lipid film was obtained (~3 h). The dried lipid cake was re-hydrated to obtain a liposome suspension by gently mixing with 1.5 mL of the hydration buffer and then left to stand for 30 min under argon gas at room temperature. The liposome suspension was freeze-thawed in liquid nitrogen 3 to 5 times to form multilamellar vesicles, and then extruded 11 times through double polycarbonate membranes of $0.4 \mu\text{m}$ pore size with an Avanti Mini-Extruder (Avanti Polar Lipids, Inc., USA). This procedure yielded liposomes with a mean diameter of 376 nm, as measured by dynamic light scattering (DLS) on a Malvern Zetasizer Nano ZS (Malvern Inc., Malvern, UK). Removal of free DA or 5-HT outside liposomes was achieved by gel filtration on a Sephadex G-25 column which was pre-equilibrated in advance.

VIEC experiments

Monitoring the cargo released was accomplished by vesicle impact electrochemical cytometry to detect DA or 5-HT stored in liposomes. A 33- μm carbon fiber electrode was placed in a liposome suspension as shown in Figure 1 of the main text. Liposomes adsorbed on the microelectrode and ruptured via electroporation, the proposed mechanism for vesicle opening in the VIEC. The electrochemical recording of release of individual liposome content was performed by applying a constant positive potential (700 mV vs. Ag/AgCl) to the carbon fiber microelectrode in the form of spikes (a plot of current versus time). By analysis of each spike, important kinetic information about the release event can be obtained. Several parameters used for spike analysis in this work are depicted in the schematic in Figure 1 of the main text including the width of spike at half maximum (t_{half}) as an indicator of duration of release events; the rise-time (t_{rise}), the time from 10% to 90% of amplitude on the rising part of each spike, which reflects fusion pore opening; the fall-time (t_{fall}), the time from 90% to 10% of amplitude on the falling part of each spike that represents the time needed for chemical contents to exit the liposome, and the amplitude of spike (I_{max}) representing the maximum flux of molecules through the open pore. Additionally, the amount of monoamines (N) released from a single liposome was evaluated with Faraday's law: $N=Q/z_eF$, where Q is the charge calculated by integrating current of each amperometric spike, z_e is the number of electrons transferred during the redox reaction (2 for DA or 5-HT) and F is Faraday's constant ($96\,485\text{ C}\cdot\text{mol}^{-1}$).

Carbon fiber microelectrode (CFE) fabrication

Vesicle impact electrochemical cytometry (VIEC) measurements were performed using amperometry at a carbon fiber microelectrode placed in a liposome suspension. The microelectrodes were prepared by aspirating a 33- μm carbon fiber into a glass capillary (o.d. 1.2 mm; i.d. 0.69 mm; no filament; Sutter Instrument Co., USA). A commercial micropipette puller (PE-21, Narishige, Japan) was used to heat and pull the capillary producing two carbon fiber-containing pipettes. A scalpel was used to cut the protruding carbon fiber close to the glass tip and it was then dipped into freshly made epoxy (EpoTek 301, Epoxy Technology, USA) for 3 min. The glued electrodes were cured at 100 °C overnight and subsequently cut at the glass junction. The electrodes were consequently polished at a 45° angle on a commercial micro grinder (EG-400, Narishige, Japan) and backfilled with 3 M KCl. Each electrode was then tested in a 0.1 mM solution of dopamine in phosphate-buffered saline (PBS; pH 7.4) by performing cyclic voltammetry. Only electrodes with proper I - E curves were used for experiments.

Amperometric measurements

The electrode was kept at 700 mV vs. a Ag/AgCl reference/counter electrode (World Precision Instruments, Inc., USA) using an amplifier instrument (Axopatch 200B, Axon Instruments, USA). The signal was digitized at 10 kHz and filtered with an internal low pass Bessel filter at 2 kHz. The signal was displayed in real time (AxoScope 8.1, Axon Instruments, USA) and stored digitally.

Data Acquisition and Statistical analysis

The amperometric traces were processed by a macro of Igor Pro 6 (Version 6.3.7.2; Page 16 of 24 WaveMetrics, Lake Oswego, OR) designed for analysis of quantal release by the group of David Sulzer at Columbia University. Peaks were detected if they exceeded a threshold of 3 times the RMS noise. Statistical analysis for amperometric measurements data were performed in GraphPad Prism 7 (GraphPad Software, Inc., San Diego, CA). The average of all parameters are calculated as the mean of medians of all recorded traces.

NUMERICAL SIMULATION ANALYSIS

The building of the finite elemental model

The finite elemental model for liposomal release was based on the model of a disk electrode detecting exocytosis in our previous work¹, with a new module for calculating the adsorption-desorption dynamics was added (scheme in Figure S1).

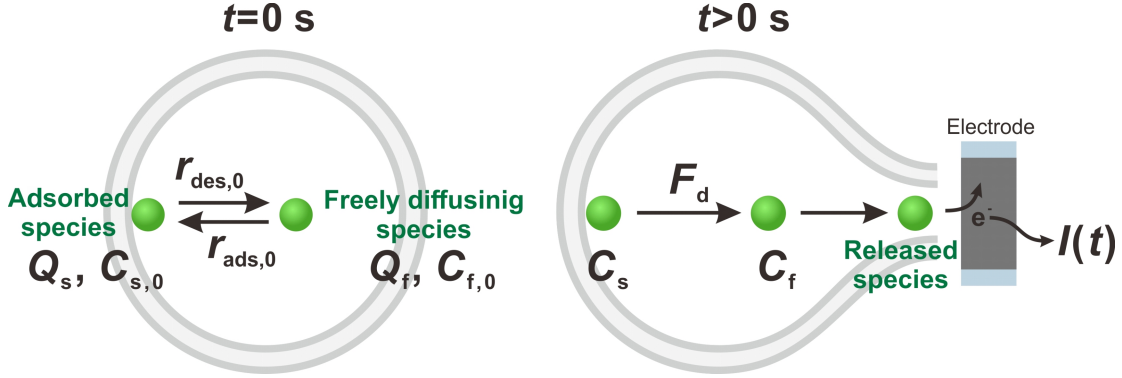


Figure S1. Schematic of the model configuration at initial time ($t=0$ s) and after instantaneous electroporation ($t>0$ s).

Schematically, a circle (the radius, $R_{lip}=188$ nm) and a pore on the membrane with variable size (R_p) was used to simulate the electroporated liposome membrane where a total quantity of cargo ($Q=48.4$ fC, the average experimental charge for spikes from DA/5-HT groups) was initially set inside the vesicles, but separated into adsorbed species (Q_s , s indicates the membrane inner surface) and free species (Q_f , f indicates freely moving species). The values for Q_s and Q_f were calculated *a priori* based on the Langmuir adsorption model.

At time= 0 s, the initial surface concentration ($C_{s,0}$) and initial concentration of intraliposomal freely moving molecules ($C_{f,0}$) was assumed to be at equilibrium, so the initial adsorption rate ($r_{ads,0}$) was equal to the desorption rate ($r_{des,0}$).

$$r_{des,0} = k_{des}C_{s,0} = r_{ads,0} = k_{ads}C_{f,0}(\Gamma_s - C_{s,0})$$

Where k_{ads} is the adsorption rate constant, k_{des} is the desorption rate constant, Γ_s is the surface concentration of adsorbed molecules in saturated state on the membrane. Hence, the initial surface concentration ($C_{s,0}$) can be calculated by

$$C_{s,0} = \frac{k_{ads}C_{f,0}\Gamma_s}{k_{des}+k_{ads}C_{f,0}}$$

Meanwhile, the total spike charge $Q=Q_s+Q_f$, and

$$Q_s = 2F(4\pi R_{lip}^2)C_{s,0} ; Q_f = 2F(4\pi R_{lip}^3/3)C_{f,0}$$

where F is the Faraday constant. Then $C_{f,0}$ was solved by equation 1,

$$\frac{1}{3}R_{lip}C_{f,0}^2 + (\Gamma_s + \frac{1}{3}R_{lip}K_1 - Q/8F\pi R_{lip}^2)C_{f,0} - QK_1/8F\pi R_{lip}^2 = 0 \quad (\text{Eq.1})$$

where K_1 is the Langmuir constant, which equals k_{des}/k_{ads} .

After the initial state was set, a flux inward, from the circle, simulating the desorption, was determined by F_d ,

$$F_d = k_{ads}C_f(I_s - C_s) - k_{des}C_s$$

Notably, the value of $k_{ads}C_f(I_s - C_s)$ is usually much less than $k_{des}C_s$, so $F_d \approx -k_{des}C_s$, which results in the influence of k_{ads} and I_s is small and, hence, their estimation is quite unreliable.

When $t > 0$ s, the real time concentrations of surface adsorption molecules (C_s) and freely moving molecules (C_f) were solved by use of Fick's diffusion equation with a flux inward.

$$\frac{\partial C_s}{\partial t} + \nabla \cdot (-D_s \nabla C_s) = -F_d$$

$$\frac{\partial C_f}{\partial t} + \nabla \cdot (-D_f \nabla C_f) = F_d$$

Here, t is the time, D_s and D_f is the diffusion coefficient of surface molecules and freely moving molecules. The input parameters are listed in **Table S1**.

Finally, a current reflecting the release dynamics was solved by use of Faraday's law and surface integration of the flux density of molecules across the pore,

$$I(t) = \iint_{S_{pore}} z_e F J(t) dS$$

where $J(t)$ is the flux density of molecules across the liposome pore, z_e is the charge transfer number of the electrochemical reaction which is 2, and F is the Faraday constant.

Table S1. Model input parameters

Parameters	Description	Value	Unit
D_f	diffusion coefficient of chemicals	6.0×10^{-10} (Ref. 2)	$\text{m}^2 \cdot \text{s}^{-1}$
D_s	surface diffusion coefficient of chemicals	0	$\text{m}^2 \cdot \text{s}^{-1}$
$C_{f,0}$	initial concentration of free chemical inside liposome	solution of Eq.1	mol/m^3
z_e	number of electrons transferred in the electrochemical reaction	2	
R_{lip}	the radius of liposome	188	nm
Q	the total charge according to chemical in a liposome	4.84×10^{-14}	C

Inverse estimation of desorption parameters based on the Monte Carlo method

The inverse estimation was based on a Monte Carlo least-squares optimization method. The model parameters include k_{des} , k_{ads} , I_s , R_p . The experimental spike signal was set as the target, and the least sum of the squared difference between the target and the corresponding value calculated by the above finite elemental model was used to search

the best-fitting combination of desired parameters. As discussed above, the estimation of k_{ads} and Γ_s is unreliable, so only the estimated k_{des} and R_p of the typical spikes are listed in **Table 1**.

References

- (1) Gu, C.; Zhang, X.; Ewing, A. G. Comparison of Disk and Nanotip Electrodes for Measurement of Single-Cell Amperometry during Exocytotic Release. *Anal. Chem.* **2020**, *92*, 10268-10273.
- (2) Trouillon, R.; Lin, Y.; Mellander, L. J.; Keighron, J. D.; Ewing, A. G. Evaluating the Diffusion Coefficient of Dopamine at the Cell Surface During Amperometric Detection: Disk Vs Ring Microelectrodes. *Anal. Chem.* **2013**, *85*, 6421-6428.