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# EQUILIBRIUM SUBMANIFOLD FOR A BIOLOGICAL SYSTEM

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Abstract. The complexity in a biological system may be caused by both the number of variables involved and the number of system constants that can vary. A biological system in the subcellular level often stabilizes after a certain period of time. Its asymptote can then be described as an equilibrium under certain continuity assumptions. The biological quantities at the equilibrium can be detected by experiments and they observe some mathematical equations. The purpose of this paper is to study the equilibrium submanifold of vesicle trafficking in a two-compartment system. We compute the equilibrium submanifold under some fairly general assumption on the system constants. The disconnectedness of the equilibrium submanifold may have biological implications. We show that, unlike many other systems, the equilibrium is determined largely by system constants rather than the initial state. In particular, the equilibrium submanifold is locally a real algebraic variety, with small generic dimension and large degenerate dimension. Our result suggests that some biological system may be studied by algebraic or geometric methods.

1. **Introduction.** It is perhaps safe to say that most biological system in the subcellular level will reach an equilibrium at a certain point. In many circumstances, the equilibrium will be a partial equilibrium in the sense that some parameters will stabilize while the rest will evolve after time. Very often, a biological system will involve tens or hundreds of variables. The large number of variables makes a direct analysis on the system a very difficult task. However, in a lot of biological systems, the outcome of the time evolution of a biological system is what really matters. To this end, the structure of the equilibrium submanifold, particularly the zero-th homotopy group, becomes very important.

Let $B$ be a biological system. Let $\mathbf{S} = \{s_1, s_2, \ldots, s_n\}$ be the set of variables that describe the biological quantities in this system. A point $u \in \mathbb{R}^n$ is called a state if there is a biological system such that $s_i = u_i$ for all $i \in [1, n]$. Given an initial state $\mathbf{S}(0)$, one wishes to understand all the possible state $\mathbf{S}(t)$ at time $t$. We

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call a state $\mathbf{S}(t_0)$ an equilibrium if $\mathbf{S}(t) = constant$ for $t \geq t_0$; a partial equilibrium if a subset $P(t)$ satisfies $P(t) = constant$ for $t \geq t_0$. In reality, a biological system $B$ may never reach an equilibrium in the mathematical sense. But the studies on equilibrium in abstract terms may shed lights on the structure of the complex biological system. If the set of equilibrium states has the structure of a manifold, possibly with boundary, we call it the *equilibrium submanifold*.

A biological system can often be modeled in several different ways, depending on the nature of the system. For example, when $\mathbf{S}(t)$ is multivalued, the randomness in the system may be modeled using stochastic equations. When $\mathbf{S}(t)$ is single-valued and continuous, the deterministic nature of the system may suggest a dynamical system model (see [6], [14]). In this paper, we are interested in a nonlinear dynamical system model based on the vesicle trafficking in a two-compartment system (see [4], [11], [1]). Vesicle trafficking can also be modeled by a stochastic model (see [10]). The main goal is to discuss the role of the equilibrium submanifold in analyzing the nonlinear dynamical system model and the relevant biological system.

1. First of all, most of the dynamical system model in subcelluar level can only describe a biological system *locally*. In other words, the model only makes sense when the initial state is in a certain neighborhood. Outside of this neighborhood, the model may become absurd, for example, negative quantities may appear. This neighborhood, as we shall argue, should be a neighborhood of the equilibrium submanifold. The model may still make sense outside of this neighborhood mathematically, but not biologically. Finding the equilibrium may help determine the possible initial conditions to which the dynamical system makes sense biologically. Then one can study the properties of the dynamic system near the equilibria.
2. In a dynamical system model, the dimension of the equilibrium submanifold is an important invariant. It may be interpreted biologically. For example, the equilibrium to which a system reaches, may only depend on a subset of initial state $\mathbf{S}(0)$. The dimension of the equilibrium can be used to understand how the outcome of a biological system depend on the initial state. See Theorem 5.1 and Theorem 6.2.
3. If a dynamical system is algebraic, the equilibrium submanifold will locally be a real algebraic variety. In this case, analyzing equilibrium submanifold is an interesting mathematical problem in its own right. In addition, a biological dynamical system model often contain certain system constants. How the equilibrium submanifold deforms with respect to the system constants may have remarkable biological implications. This problem is certainly of interests to algebraists.
4. In the model we are studying in this paper, the equilibrium submanifold may be disconnected. This suggests that behavior of the two-compartment system may be quite different near different equilibrium locations. Biologically, this means that the outcome of a biological system can be very different near two different branches of the equilibrium submanifold. See Theorem 5.2.
5. Equilibrium points are biologically important. Experimentally, the biological quantities are most visible at the equilibrium point. From the statistical point of view, the observability at the equilibrium can be utilized to infer the system constants, for example, the dissociation constants in our model.

6. The differential equations from a biological system may be linearly dependent, or algebraically dependent. In other words, there are relationships among the variables $\mathbf{S}(t)$. The relationships often comes from preservation of certain quantities in the biological system. See the discussions in Section 3.
7. When the number of variables is large, finding an equilibrium that makes sense biologically, can be difficult. Frequently, change of variable become necessary. For example, the absolute amount of a certain biological ingredient may vary greatly. Yet, its concentration may approach an equilibrium. So, instead of analyzing $\frac{d\mathbf{x}}{dt} = 0$, one should analyze the equation $\frac{d\mathbf{f}(\mathbf{x})}{dt} = 0$ where $\mathbf{f}(\mathbf{x})$ is a smooth function of $\mathbf{x}$ in a certain neighborhood. See Section 4.

In this paper, we did not address the problem of stability of the equilibrium. Although the stability problem can be solved theoretically ( [2], [3]), the actual computation with varying system constants can be enormous. Fortunately, system constants are often known in individual experiment. So equilibrium submanifold can be computed numerically and stability of the equilibria can be checked numerically. We intend to discuss this in a future paper. We shall finally remark that the dynamical system we discuss in this paper are all *autonomous* ([2]).

2. **Vesicle Trafficking and Pollen Growth.** The biological system we are interested in is the vesicle trafficking in yeast, animal, and plant cells (eukaryotic cells). These cells are enclosed by the phospholipid bilayer (plasma membrane), which allows the cells to maintain a fluid inside while they exchange gases and small ions with the exterior. In yeast and plants, the plasma membrane is further covered by cell wall (polysaccharides) that makes the cell structure more rigid. Eukaryotic cells also have interior compartments that are enclosed again by the phospholipid bilayer. Within these compartments, several molecules that need to be physically separated from the cellular fluid (cytosol), such as phospholipids and polysaccharides, are synthesized, stored, or degraded. Depending on conditions, the cells quickly transport these molecules (cargoes) from one compartment to others that include the plasma membrane. In order to transport these cargoes, extremely small compartments (vesicles) containing the cargoes bud out from a compartment membrane and travel around in the cytosol. When a vesicle arrives at the destination compartment, the membrane of the vesicle fuses to the membrane of the destination compartment in order to release the cargoes into or on the compartment. This transporting system is called vesicle trafficking (or vesicular traffic), which is commonly evolved among eukaryotes ( [1]).

We are interested in vesicle trafficking in the pollen tube growth. Pollens are sperm cells (male gamete) of seed plants and can be found in anthers in the flower. During pollination (sexual reproduction), pollens are transferred to a stigma of flowers through the air. After that, pollens, which are actually single cells enclosed by the plasma membrane and cell wall, grow a tube into the base tower of the stigma (style) until they reach egg cells (ovules) in the bottom of the style. The length of the tubes in some plants are about 10 mm ( [15]) though the sizes of pollens on the stigma are typically 10 to 100 micron. It is known that the growth of the tube (polarized growth) relies on vesicle trafficking that delivers newly synthesized materials such as phospholipids and polysaccharides to the tip of the tube ( [13]). The cargoes that are transported to the plasma membrane at the tip of the tube are originally produced in the compartment known as the Golgi apparatus. They,

however, can be transported to other compartments within the pollen tube such as the early endosome, late endosome, and recycling endosome, depending on the developmental signal. The goal of our research is to elucidate the mechanism of vesicle trafficking that leads to the pollen tube growth at a systems level.

Because of advantages in genetic and molecular analysis, the detailed molecular mechanisms of the vesicle budding and fusion have been revealed in the past years. Biologists look at vesicle budding or fusion *in vivo* or *in vitro* in their assays. If vesicle budding or fusion is halted when they remove a molecule(s) of interest from their assays, biologists can conclude that the molecule(s) play an important role in vesicle trafficking. Through these analysis, we know now that budding and fusion of vesicles are mainly controlled by two different types of proteins; guanine triphosphatases (GTPases) and soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptors (SNAREs) respectively ( [5]). During vesicle budding, GTPase on a compartment membrane recruits machinery proteins from the cytosol, and the machinery proteins generate vesicles using the compartment membrane. Meantime the cargoes in the compartment are recruited by GTPase to the vesicles. GTPase also recruits SNAREs that localize in the compartment membrane into the vesicle membrane. After a vesicle travels around within the cytosol, GTPase and the machinery proteins that are used to generate the vesicle are released from the vesicle to the cytosol but SNAREs and the cargoes remain on or within the vesicle. When the vesicle is at the destination compartment, SNAREs on the vesicle interact with SNAREs on the destination compartment and allow the vesicle fuse using an energy generated by the SNARE-SNARE interactions. Therefore, these molecular mechanisms explain how SNAREs and a membrane in an original compartment are exported to the destination compartment together with the cargoes.

Biologists, however, observe that a specific SNARE localizing in a specific compartment is hardly identified in other compartments during the cargo transportations. For instant, SNARE localizing in the Golgi apparatus of a pollen tube is hardly identified in other compartments while the cargoes (i.e., polysaccharides) are simultaneously transported from the Golgi apparatus to other compartments (i.e., the plasma membrane) so that the pollen tube can continuously grow. This observation suggests that SNARE must be transported back to the original compartment soon after the vesicle fuses to another compartment in vesicle trafficking. It is also true that the delivery of materials to construct the plasma membrane simply relies on the fusion and budding of vesicles because both vesicles and the plasma membranes are composed of the same materials (i.e., phospholipids). However, the delivery of materials to construct the cell wall depend on the amount of the cargoes (i.e., polysaccharides) that the vesicle carries as well as the rates of vesicle fusion and budding. Hence, the pollens are thought to develop a system that balances out the rates of the vesicle flows and the amounts of the cargoes that the vesicles carry. How do the pollens control the delivery of the materials to the tube tip? How does a compartment maintain its size while the membrane is spontaneously exported during the pollen tube growth? How does a compartment maintain a specific SNARE while it is spontaneously exported? How can we engineer a pollen that grows faster or slower? These are questions that need to be addressed by not only looking at the molecular mechanisms of the vesicle budding and fusion but also looking at the system.

A mathematical model that describes vesicle trafficking between two compartments was constructed by Heinrich and Rapoport ( [11]). This model can be simplified and applied to the pollen tube growth. Clearly, this two compartment system model reaches an equilibrium when the pollen tube reaches its maturity. Finding the equilibrium conditions help to predict the rate of the pollen tube growth in mutant plants in which expressions of SNARE genes are suppressed in the pollens. Moreover, computing the equilibrium status with a function of time (t) allows us to predict the kinetics of compartment sizes and concentrations of SNAREs and cargoes in two compartments during the pollen tube growth.

3. **A Simplified Heinrich-Rapoport Model.** In this section, we review the model constructed in [11] with no cargo transportation. Denote the SNARE native to compartment 1 by $X$ and $U$. Denote the SNARE native to compartment 2 by $Y$ and $V$. There are two major processes in a vesicle trafficking system, namely the budding of vesicles from the compartments and the fusion of the vesicles with compartments. The budding vesicles are either initiated by GTPase $A$ or $B$. Suppose that vesicles budded from compartment 1 is always with GTPases $A$ and vesicles budded from compartment 2 is always with GTPases $B$. The fusion processes are mainly regulated by SNARE interactions. The interaction of SNARE X is limited to SNARE U. Similarly, the interaction of SNARE Y is limited to SNARE V. Although SNAREs X, U, Y, and V are biologically distinguishable, their functions in our model are similar; their interactions mediate the membrane fusion between the vesicles and the compartments.

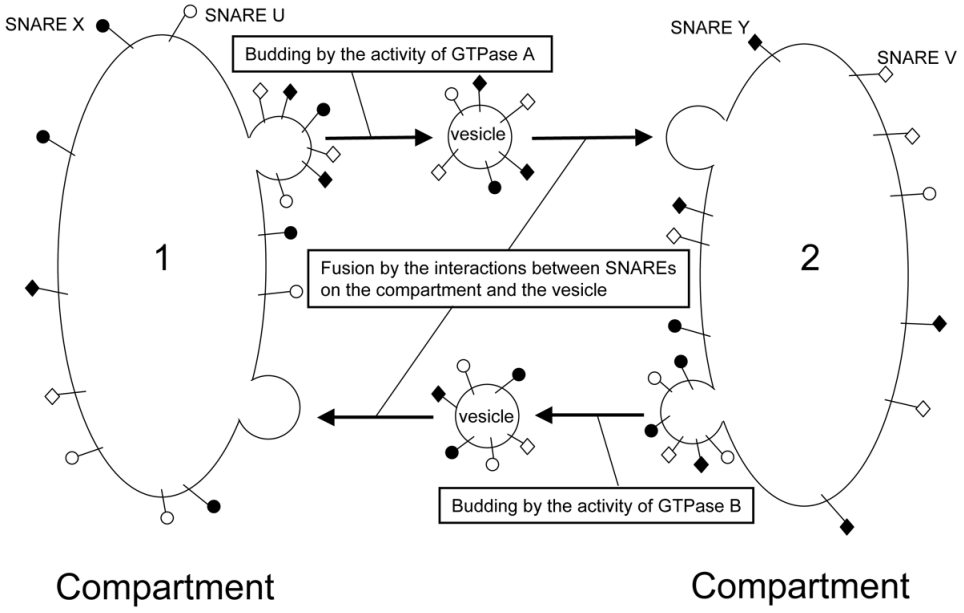


FIGURE 1. A Two Compartment Vesicle Trafficking System

Let

1. $N_i$ be the number of vesicles originated in compartment $i$;
2. $S_i$ be the size of compartment $i$;
3. $X_i$ be the amount of $X$ in compartment $i$;
4. $U_i$ be the amount of $U$ in compartment $i$;
5. $Y_i$ be the amount of $Y$ in compartment $i$;
6. $V_i$ be the amount of $V$ in compartment $i$;
7. $N_{xi}$ be the amount of SNAREs $X$ in the vesicles originated in compartment $i$;
8. $N_{ui}$ be the amount of SNAREs $U$ in the vesicles originated in compartment $i$;
9. $N_{yi}$ be the amount of SNAREs $Y$ in the vesicles originated in compartment $i$;
10. $N_{vi}$ be the amount of SNAREs $V$ in the vesicles originated in compartment $i$;

The following are dependent variables derived from above. Let

1. $x_i = X_i/S_i$ be the concentration of $X$ in compartment $i$;
2. $u_i = U_i/S_i$ be the concentration of $U$ in compartment $i$;
3. $y_i = Y_i/S_i$ be the concentration of $Y$ in compartment $i$;
4. $v_i = V_i/S_i$ be the concentration of $V$ in compartment $i$;
5. $c_{xi} = N_{xi}/N_i$ be the average concentration of $X$ in vesicles originated in compartment $i$;
6. $c_{ui} = N_{ui}/N_i$ be the average concentration of $U$ in vesicles originated in compartment $i$;
7. $c_{yi} = N_{yi}/N_i$ be the average concentration of $Y$ in vesicles originated in compartment $i$;
8. $c_{vi} = N_{vi}/N_i$ be the average concentration of $V$ in vesicles originated in compartment $i$.

Let $k_{x1}$ be the dissociation constant of SNARE $X$ with GTPase A, and $k_{x2}$ be the dissociation constant of SNARE $X$ with GTPase B. In other words, $K_{xi}$ is an equilibrium constant that measures the affinity of SNARE X to vesicles budded from compartment $i$. $k_{ui}$, $k_{yi}$ and $k_{vi}$ can be defined similarly. Define the following 8 saturation functions:

$$s_{xi} = \frac{x_i/k_{xi}}{1 + x_i/k_{xi} + u_i/k_{ui} + y_i/k_{yi} + v_i/k_{vi}},$$

$$s_{ui} = \frac{u_i/k_{ui}}{1 + x_i/k_{xi} + u_i/k_{ui} + y_i/k_{yi} + v_i/k_{vi}},$$

$$s_{yi} = \frac{y_i/k_{yi}}{1 + x_i/k_{xi} + u_i/k_{ui} + y_i/k_{yi} + v_i/k_{vi}},$$

$$s_{vi} = \frac{v_i/k_{vi}}{1 + x_i/k_{xi} + u_i/k_{ui} + y_i/k_{yi} + v_i/k_{vi}}.$$

These functions are related to the amount of cargo each vesicle can carry, which in turn, will effect the eventual size of the compartments.

We have 2 budding rates $w_i = wS_i$ where $w$ is a constant. Let $\kappa$ be the fusion rate constant.

Put $\mathbf{SN}_i = (X_i, U_i, Y_i, V_i)$ and $\mathbf{sn}_i = (x_i, u_i, y_i, v_i)$. Let $\mathbf{c}_i = (c_{xi}, c_{ui}, c_{yi}, c_{vi})$. Define an inner product on $\mathbb{R}^4$ by

$$\langle \mathbf{p}, \mathbf{q} \rangle = p_1q_2 + p_2q_1 + p_3q_4 + p_4q_3. \tag{1}$$

We have 2 forward fusion rates of vesicles:

1. $f_1 = \kappa(c_{x1}u_2 + c_{u1}x_2 + c_{y1}v_2 + c_{v1}y_2) = \kappa\langle \mathbf{c}_1, \mathbf{sn}_2\rangle$. This is the fusion rate of vesicles originated in compartment 1 that fusion with compartment 2;
2. $f_2 = \kappa(c_{x2}u_1 + c_{u2}x_1 + c_{y2}v_1 + c_{v2}y_1) = \kappa\langle \mathbf{c}_2, \mathbf{sn}_1\rangle$. This is the fusion rate of vesicles originated in compartment 2 that fusion with compartment 1.

We have 2 backward fusion rates of vesicles:

1. $r_1 = \kappa\langle \mathbf{c}_1, \mathbf{sn}_1\rangle$. This is the fusion rate of vesicles originated in compartment 1 that fusion with compartment 1;
2. $r_2 = \kappa\langle \mathbf{c}_2, \mathbf{sn}_2\rangle$. This is the fusion rate of vesicles originated in compartment 2 that fusion with compartment 2.

We can now write down the system of differential equations according to [11] (Eq. (1)(2)):

$$\frac{dS_1}{dt} = -wS_1 + r_1S_1N_1 + f_2S_1N_2, \qquad \frac{dS_2}{dt} = -wS_2 + r_2S_2N_2 + f_1S_2N_1$$
$$\frac{dN_1}{dt} = wS_1 - r_1S_1N_1 - f_1S_2N_1, \qquad \frac{dN_2}{dt} = wS_2 - r_2S_2N_2 - f_2S_1N_2.$$

Theoretically, the changes of the sizes of the compartments are determined by the outgoing flux of vesicles budded from the compartments and the incoming flux of vesicles from back fusion and forward fusion. The changes of the number of vesicles are determined similarly. We normalize the size of vesicles. The sizes of the compartments $S_1$ and $S_2$ will now be measured in terms of the number of vesicles. It follows that the total size of the compartments and vesicles is preserved in vesicle trafficking. We have

$$S_1 + S_2 + N_1 + N_2 = constant.$$

Biologically, the size of vesicles may vary slightly. There are typically 2000 vesicles in the pollen tube and the average size of a vesicle can be determined. Now the back and forward fusions are dictated by the amount of SNAREs in the compartments and in the vesicles. There are 8 differential equations concerning $X_i, U_i, Y_i, V_i$, the SNARE amounts in the respective compartment [11] (Page 5, Supplement 1):

$$\frac{dX_1}{dt} = -wS_1s_{x1} + r_1S_1N_{x1} + f_2S_1N_{x2}, \qquad \frac{dX_2}{dt} = -wS_2s_{x2} + r_2S_2N_{x2} + f_1S_2N_{x1}; \tag{2}$$

$$\frac{dU_1}{dt} = -wS_1s_{u1} + r_1S_1N_{u1} + f_2S_1N_{u2}, \qquad \frac{dU_2}{dt} = -wS_2s_{u2} + r_2S_2N_{u2} + f_1S_2N_{u1}; \tag{3}$$

$$\frac{dY_1}{dt} = -wS_1s_{y1} + r_1S_1N_{y1} + f_2S_1N_{y2}, \qquad \frac{dY_2}{dt} = -wS_2s_{y2} + r_2S_2N_{y2} + f_1S_2N_{y1}; \tag{4}$$

$$\frac{dV_1}{dt} = -wS_1s_{v1} + r_1S_1N_{v1} + f_2S_1N_{v2}, \qquad \frac{dV_2}{dt} = -wS_2s_{v2} + r_2S_2N_{v2} + f_1S_2N_{v1}. \tag{5}$$

There are also 8 differential equations concerning $N_{xi}, N_{ui}, N_{yi}, N_{vi}$, the amount of SNAREs in the vesicles outside of the compartments, [11] (Page 6, Supplement 1):

$$\frac{dN_{x1}}{dt} = wS_1s_{x1} - r_1S_1N_{x1} - f_1S_2N_{x1}, \qquad \frac{dN_{x2}}{dt} = wS_2s_{x2} - r_2S_2N_{x2} - f_2S_1N_{x2}.$$

The other equations are similar. Among these 16 equations, we have the following relations:

$$X_1 + X_2 + N_{x1} + N_{x2} = constant, \qquad U_1 + U_2 + N_{u1} + N_{u2} = constant,$$

$$Y_1 + Y_2 + N_{y1} + N_{y2} = constant, \qquad V_1 + V_2 + N_{v1} + N_{v2} = constant.$$

So only 12 of them are linearly independent. Among the total of 20 equations, only 15 are linearly independent. We shall remark that, in a more complex biological system, sorting out the linear dependence among the equations can be a tedious task.

4. **Equations on Concentrations.** Let $\frac{d\mathbf{x}}{dt} = \mathbf{F}(\mathbf{x})$ be a system of autonomous differential equations. We call the system algebraic if $\mathbf{F}(\mathbf{x})$ is algebraic. Clearly, if a system is algebraic, the equilibrium submanifold will be a real algebraic variety.

As we shall discuss in the next section, sometimes it is more desirable to work with differential equations on concentration, rather than the absolute quantity. Recall that

$$\begin{aligned}
\frac{dX_1}{dt} &= -wS_1s_{x1} + \kappa\langle \mathbf{c}_1, \mathbf{sn}_1\rangle S_1N_{x1} + \kappa\langle \mathbf{c}_2, \mathbf{sn}_1\rangle S_1N_{x2},\\
\frac{dX_2}{dt} &= -wS_2s_{x2} + \kappa\langle \mathbf{c}_2, \mathbf{sn}_2\rangle S_2N_{x2} + \kappa\langle \mathbf{c}_1, \mathbf{sn}_2\rangle S_2N_{x1},\\
\frac{dN_{x1}}{dt} &= wS_1s_{x1} - \kappa\langle \mathbf{c}_1, \mathbf{sn}_1\rangle S_1N_{x1} - \kappa\langle \mathbf{c}_1, \mathbf{sn}_2\rangle S_2N_{x1},\\
\frac{dN_{x2}}{dt} &= wS_2s_{x2} - \kappa\langle \mathbf{c}_2, \mathbf{sn}_2\rangle S_2N_{x2} - \kappa\langle \mathbf{c}_2, \mathbf{sn}_1\rangle S_1N_{x2}.
\end{aligned}$$

Obviously, $\frac{dX_1}{dt} + \frac{dX_2}{dt} + \frac{dN_{x1}}{dt} + \frac{dN_{x2}}{dt} = 0$. This is basically saying that the total number of SNARE $X$ is preserved. So only three equations are linearly independent. The equations for SNAREs U, Y, V can be written similarly. Now we want to make a simplification by considering the concentration $x_i$ and $c_{xi}$:

$$\begin{aligned}
\frac{dc_{x1}}{dt} &= \frac{d\frac{N_{x1}}{N_1}}{dt} = \frac{1}{N_1}\frac{dN_{x1}}{dt} - \frac{N_{x1}}{N_1^2}\frac{dN_1}{dt}\\
=&\frac{1}{N_1}[wS_1s_{x1} - \kappa\langle \mathbf{c}_1, \mathbf{sn}_1\rangle S_1N_{x1} - \kappa\langle \mathbf{c}_1, \mathbf{sn}_2\rangle S_2N_{x1}]\\
&- \frac{N_{x1}}{N_1^2}[wS_1 - \kappa\langle \mathbf{c}_1, \mathbf{sn}_1\rangle S_1N_1 - \kappa\langle \mathbf{c}_1, \mathbf{sn}_2\rangle S_2N_1]\\
=&\frac{wS_1s_{x1}}{N_1} - \frac{c_{x1}wS_1}{N_1} = \frac{wS_1}{N_1}(s_{x1} - c_{x1}).
\end{aligned} \tag{6}$$

We see here that the change of $c_{x1}$ is related to $c_{x1}$ "negatively". This often occurs in a biological system in terms of concentration, but not always. Similarly, we compute

$$\begin{aligned}
\frac{dx_1}{dt} &= \frac{d(\frac{X_1}{S_1})}{dt} = \frac{1}{S_1}\frac{dX_1}{dt} - \frac{X_1}{S_1^2}\frac{dS_1}{dt}\\
=&\frac{1}{S_1}[-wS_1s_{x1} + \kappa\langle \mathbf{c}_1, \mathbf{sn}_1\rangle S_1N_{x1} + \kappa\langle \mathbf{c}_2, \mathbf{sn}_1\rangle S_1N_{x2}]\\
&- \frac{X_1}{S_1^2}[-wS_1 + \kappa\langle \mathbf{c}_1, \mathbf{sn}_1\rangle S_1N_1 + \kappa\langle \mathbf{c}_2, \mathbf{sn}_1\rangle S_1N_2]\\
=&- ws_{x1} + \kappa\langle \mathbf{c}_1, \mathbf{sn}_1\rangle N_{x1} + \kappa\langle \mathbf{c}_2, \mathbf{sn}_1\rangle N_{x2}\\
&+ wx_1 - x_1\kappa\langle \mathbf{c}_1, \mathbf{sn}_1\rangle N_1 - x_1\kappa\langle \mathbf{c}_2, \mathbf{sn}_1\rangle N_2\\
=&w(-s_{x1} + x_1) + \kappa\langle \mathbf{c}_1, \mathbf{sn}_1\rangle (c_{x1} - x_1)N_1 + \kappa\langle \mathbf{c}_2, \mathbf{sn}_1\rangle (c_{x2} - x_1)N_2
\end{aligned} \tag{7}$$

**Theorem 4.1.** *We have*

$$\frac{dc_{xi}}{dt} = \frac{wS_i}{N_i}(s_{xi} - c_{xi}),$$

$$\frac{dx_1}{dt} = w(-s_{x1} + x_1) + \kappa\langle \mathbf{c}_1, \mathbf{sn}_1\rangle(c_{x1} - x_1)N_1 + \kappa\langle \mathbf{c}_2, \mathbf{sn}_1\rangle(c_{x2} - x_1)N_2,$$

$$\frac{dx_2}{dt} = w(-s_{x2} + x_2) + \kappa\langle \mathbf{c}_2, \mathbf{sn}_2\rangle(c_{x2} - x_2)N_2 + \kappa\langle \mathbf{c}_1, \mathbf{sn}_2\rangle(c_{x1} - x_2)N_1$$

*If $s_{x1} = c_{x1} = x_1 = x_2 = c_{x2} = s_{x2}$, then the system reaches a partial equilibrium on $c_{xi}$ and $x_i$.*

The equations we have now give us better ideas about how the rate of concentration evolves.

5. **Equilibrium: A symmetric model.** Unless otherwise stated, we suppose that $X$ and $U$ SNAREs are symmetric, because a molecular model suggests that a molar ratio of SNARE X and U are 1 to 1 when they interact ([12]). We suppose SNAREs Y and V are also symmetric. In other words,

$$x_i = u_i, k_{xi} = k_{ui}; \qquad y_i = v_i, k_{yi} = k_{vi}.$$

We also assume that

$$s_{xi} = x_i/k_{xi}; \qquad s_{yi} = y_i/k_{yi}.$$

Automatically, we have $s_{xi} = s_{ui}$ and $s_{yi} = s_{vi}$. Finally, we assume that all the quantities we have are nonzero and

$$k_{x1} \ll k_{x2}; \qquad k_{y1} \gg k_{y2}. \tag{8}$$

These assumptions are natural and reflect what is going on in a real vesicle trafficking system.

To compute the equilibrium, set

$$\frac{dS_i}{dt} = 0; \frac{dN_i}{dt} = 0; \frac{dc_{xi}}{dt} = 0; \frac{dc_{yi}}{dt} = 0; \frac{dX_i}{dt} = 0;; \frac{dY_i}{dt} = 0.$$

Notice here that it's more convenient for us to use $X_i$ and $Y_i$ instead of $x_i$ and $y_i$ for computational purposes. We have

$$r_1N_1 + f_2N_2 = w; \qquad r_2N_2 + f_1N_1 = w. \tag{9}$$

$$wS_1 = r_1S_1N_1 + f_1S_2N_1; \qquad wS_2 = r_2S_2N_2 + f_2S_1N_2. \tag{10}$$

$$c_{xi} = s_{xi} = x_i/k_{xi}; \qquad c_{yi} = s_{yi} = y_i/k_{yi}. \tag{11}$$

$$ws_{x1} = r_1c_{x1}N_1 + f_2c_{x2}N_2; \qquad ws_{x2} = r_2c_{x2}N_2 + f_1c_{x1}N_1. \tag{12}$$

$$ws_{y1} = r_1c_{y1}N_1 + f_2c_{y2}N_2; \qquad ws_{y2} = r_2c_{y2}N_2 + f_1c_{y1}N_1. \tag{13}$$

Now substituting Equations (11) into Equations (12), (13), we obtain

$$w\frac{x_1}{k_{x1}} = r_1N_1\frac{x_1}{k_{x1}} + f_2N_2\frac{x_2}{k_{x2}}; \qquad w\frac{x_2}{k_{x2}} = r_2N_2\frac{x_2}{k_{x2}} + f_1N_1\frac{x_1}{k_{x1}} \tag{14}$$

$$w\frac{y_1}{k_{y1}} = r_1N_1\frac{y_1}{k_{y1}} + f_2N_2\frac{y_2}{k_{y2}}; \qquad w\frac{y_2}{k_{y2}} = r_2N_2\frac{y_2}{k_{y2}} + f_1N_1\frac{y_1}{k_{y1}} \tag{15}$$

**Lemma 5.1.** *The system reaches an equilibrium when Equations (9), (10), (11), (14), (15) hold.*

Now there are eight variables $N_i$, $S_i$, $x_i$, $y_i$ and eight equations. These equations are linearly dependent with the constraint:

$$N_1 + N_2 + S_2 + S_2 = Q \tag{16}$$

$$x_1S_1 + x_2S_2 + \frac{x_1}{k_{x1}}N_1 + \frac{x_2}{k_{x2}}N_2 = T_x; \qquad y_1S_1 + y_2S_2 + \frac{y_1}{k_{y1}}N_1 + \frac{y_2}{k_{y2}}N_2 = T_y \tag{17}$$

Here $Q$ is the total size, $T_x$ and $T_y$ are the total amount of $X$ SNAREs and $Y$ SNAREs.

Combining Equation (9) with Equation (14) (15), we obtain

$$\frac{x_1}{k_{x1}} = \frac{x_2}{k_{x2}}; \qquad \frac{y_1}{k_{y1}} = \frac{y_2}{k_{y2}}. \tag{18}$$

So $\mathbf{c}_1 = \mathbf{c}_2$. We can now eliminate Equations (14 15). Equation ( 18) immediately implies that

$$r_1 = f_2 = \frac{2\kappa x_1^2}{k_{x1}} + \frac{2\kappa y_1^2}{k_{y1}}, \qquad r_2 = f_1 = \frac{2\kappa k_{x2}x_1^2}{k_{x1}^2} + \frac{2\kappa k_{y2}y_1^2}{k_{y1}^2}.$$

By Equation (9), we must have $r_1 = f_2 = r_2 = f_1$ and $N_1 + N_2 = \frac{w}{r_1}$. Put $r = r_1$. Equation (10) is then equivalent to $S_1N_2 = N_1S_2$.

**Lemma 5.2.** *At the equilibrium, we have*

$$c_{xi} = c_{ui} = \frac{x_1}{k_{x1}} = \frac{x_2}{k_{x2}}, \qquad c_{yi} = c_{vi} = \frac{y_1}{k_{y1}} = \frac{y_2}{k_{y2}}, \tag{19}$$

$$r_i = f_i = r = \frac{2\kappa x_1^2}{k_{x1}} + \frac{2\kappa y_1^2}{k_{y1}} = \frac{2\kappa x_2^2}{k_{x2}} + \frac{2\kappa y_2^2}{k_{y2}}, \tag{20}$$

$$N_1 + N_2 = \frac{w}{r}, \qquad S_1 + S_2 = Q - \frac{w}{r}, \qquad \frac{S_1}{N_1} = \frac{S_2}{N_2}, \tag{21}$$

$$x_1S_1 + x_2S_2 + \frac{x_1}{k_{x1}}N_1 + \frac{x_2}{k_{x2}}N_2 = T_x, \qquad y_1S_1 + y_2S_2 + \frac{y_1}{k_{y1}}N_1 + \frac{y_2}{k_{y2}}N_2 = T_y. \tag{22}$$

Now put $c_x = c_{xi}$ and $c_y = c_{yi}$. Then $x_i = k_{xi}c_x$ and $c_{yi} = k_{yi}c_y$. By Equation (20), we have

$$r = 2\kappa k_{x1}c_x^2 + 2\kappa k_{y1}c_y^2 \qquad r = 2\kappa k_{x2}c_x^2 + 2\kappa k_{y2}c_y^2 \tag{23}$$

Viewing $r$ as the only variable, we obtain

$$c_x = \sqrt{\frac{k_{y1} - k_{y2}}{\Delta}\frac{r}{2\kappa}}, \qquad c_y = \sqrt{\frac{k_{x2} - k_{x1}}{\Delta}\frac{r}{2\kappa}}. \tag{24}$$

with $\Delta = k_{y1}k_{x2} - k_{x1}k_{y2}$. Combining with Equation (21), we obtain

$$S_1 = N_1(\frac{rQ}{w} - 1), \qquad S_2 = N_2(\frac{rQ}{w} - 1), \qquad N_1 + N_2 = \frac{w}{r}. \tag{25}$$

From Equation (22), we obtain

$$c_xN_1(\frac{k_{x1}rQ}{w} - k_{x1} + 1) + c_xN_2(\frac{k_{x2}rQ}{w} - k_{x2} + 1) = T_x \tag{26}$$

$$c_yN_1(\frac{k_{y1}rQ}{w} - k_{y1} + 1) + c_yN_2(\frac{k_{y2}rQ}{w} - k_{y2} + 1) = T_y. \tag{27}$$

$$rN_1 + rN_2 = w. \tag{28}$$

**Theorem 5.3.** *At the equilibrium, $r$ satisfies*

$$\det\begin{pmatrix} r & r & w \\ (\frac{k_{x1}rQ}{w}-k_{x1}+1)\sqrt{\frac{k_{y1}-k_{y2}}{\Delta}\frac{r}{2\kappa}} & (\frac{k_{x2}rQ}{w}-k_{x2}+1)\sqrt{\frac{k_{y1}-k_{y2}}{\Delta}\frac{r}{2\kappa}} & T_x \\ (\frac{k_{y1}rQ}{w}-k_{y1}+1)\sqrt{\frac{k_{x2}-k_{x1}}{\Delta}\frac{r}{2\kappa}} & (\frac{k_{y2}rQ}{w}-k_{y2}+1)\sqrt{\frac{k_{x2}-k_{x1}}{\Delta}\frac{r}{2\kappa}} & T_y \end{pmatrix}=0 \tag{29}$$

*with $\Delta = k_{y1}k_{x2}-k_{x1}k_{y2}$, $Q$ the total size, $T_x$ the total amount of $X$ and $T_y$ the total amount of $Y$. Suppose that Equation (29) is nontrivial and has solutions. Then $c_x$ and $c_y$ can be obtained from Equation (24); $x_i$ and $y_i$ can be obtained from Equation (19); $N_1$ and $N_2$ can be then obtained from Equation (26) (27); $S_1$ and $S_2$ can be obtained from Equation (25).*

*Proof.* Notice that Equations 26 27 28 give three linear relations for $N_1$ and $N_2$. These three relations must be linearly dependent. So we obtain Eq. 29. The other assertions follow immediately. □

It is important to notice that Equation (29) can be reduced into a quartic equation. Its solutions have a closed form. More precisely, let

$$P(r)=\det\begin{pmatrix} \sqrt{r} & \sqrt{r} & w \\ \frac{k_{x1}rQ}{w}-k_{x1}+1 & \frac{k_{x2}rQ}{w}-k_{x2}+1 & T_x\sqrt{\frac{2\kappa\Delta}{k_{y1}-k_{y2}}} \\ \frac{k_{y1}rQ}{w}-k_{y1}+1 & \frac{k_{y2}rQ}{w}-k_{y2}+1 & T_y\sqrt{\frac{2\kappa\Delta}{k_{x2}-k_{x1}}} \end{pmatrix}$$

Since $k_{x1} \ll k_{x2}, k_{y1} \gg k_{y2}$, we see that $P(+\infty) = -\infty$. By the same reason, $P(0) < 0$. We know from the biological context that $P(r) = 0$ must have a positive solution. So in a generic sense, $P(r) = 0$ must have two solutions, say $r_1$ and $r_2$. It is quite mysterious how $r_1$ and $r_2$ are related. Biologically, one rate may indicate a slower rate of growth, or no growth, the other rate may indicate a greater rate of growth.

**Theorem 5.4.** *With $T_x$, $T_y$, $Q$ all fixed, there can be at most four equilibrium. So under the symmetric condition and the condition that $k_{x1} \ll k_{x2}$ and $k_{y1} \gg k_{y2}$, the equilibrium submanifold has at most dimension 3.*

So what is remarkable here is that the equilibrium of our biological system depends largely on the dissociation constants $k_{xi}$, $k_{yi}$, fusion rate $\kappa$, budding constant $w$ and the total size $Q$, the total amount of $X$ SNAREs $T_x$ and the total amount of $Y$ SNAREs $T_y$, barely on the size of the compartments we start with. It does not depend on the initial balance of SNAREs. This seems to confirm some observations made in [11].

We shall make a remark concerning the nonsymmetric case for SNAREs. Numerical computation under the non-symmetrical situation suggests that the system reach to different equilibrium (data not shown in the manuscript). A biological example for the non-symmetric case can be seen when a person uses botulinum A (Botox), a cosmetic drug to reduce the contractions of the facial muscles that cause persistent frown lines (facial wrinkles). This drug specifically degrades a SNARE protein in neuron cells in animals, resulting the inhibition of the transport of neurotransmitter (cargo) ( [9]). An example in plants, organisms of our interest, can be seen in a mutant of a specific SNARE gene. This mutation impairs the defense mechanism against a plant pathogen, most likely due to the inhibition of the transport of anti-pathogen molecules (cargo) in plants ( [7]). Therefore, we speculate that the

mathematical proof for the non-symmetrical case would quantitatively elucidate the importance of the balance in the SNARE expressions in cells of interest in the future.

6. **The Degenerate case:** $k_{x1} = k_{x2}; k_{y1} = k_{y2}$**.** Suppose that $k_{x1} = k_{x2}$ and $k_{y1} = k_{y2}$. Then $\Delta = 0$. So Theorem 5.3 is no longer valid. We can still find equilibrium in this degenerate case. But the equilibrium will depend on the initial size of the compartments. Surprisingly, there are at most four classes of them.

Put $k_{xi} = k_x$ and $k_{yi} = k_y$. By Lemma 5.2, we have

$$x_1 = x_2 = k_x c_{x1} = k_x c_{x2}, \qquad y_1 = y_2 = k_y c_{y1} = k_y c_{y2} \tag{30}$$

Put $x = x_i$, $y = y_i$, $c_x = c_{xi}$ and $c_y = c_{yi}$. By Equation (20), we have

$$r = 2\kappa k_x c_x^2 + 2\kappa k_y c_y^2. \tag{31}$$

By Equation (22), we have

$$k_x c_x (Q - \frac{w}{r}) + c_x \frac{w}{r} = T_x \qquad k_y c_y (Q - \frac{w}{r}) + c_y \frac{w}{r} = T_y. \tag{32}$$

**Theorem 6.1.** *If $k_{x1} = k_{x2}$ and $k_{y1} = k_{y2}$, a partial equilibrium is reached when:*

$$\frac{r}{2\kappa} = k_x (\frac{rT_x}{rk_x Q - wk_x + w})^2 + k_y (\frac{rT_y}{k_y Qr - wk_y + w})^2 \tag{33}$$

$$c_{xi} = \frac{rT_x}{rk_x Q - wk_x + w}, \qquad c_{yi} = \frac{rT_y}{k_y Qr - wk_y + w}. \tag{34}$$

*and $x_i = c_{xi} k_x$, $y_i = c_{yi} k_y$. Within this partial equilibrium,*

$$S_1(t) = \frac{S_1(0)(-w + rQ)\exp(-w + rQ)t}{r(S_1(0) + S_2(0))(\exp(-w + rQ)t - 1) - w + rQ}, \tag{35}$$

$$S_2(t) = \frac{S_2(0)(-w + rQ)\exp(-w + rQ)t}{r(S_1(0) + S_2(0))(\exp(-w + rQ)t - 1) - w + rQ}. \tag{36}$$

*So the equilibrium in this situation does depend on the initial size of $S_1$ and $S_2$.*

This is to be compared with Theorem 5.4 where the equilibrium does not depend on the initial size of $S_1$ and $S_2$.

*Proof.* Equation (33, 34) follows from Equation (31, 32). Now we can go back and consider the following equations:

$$\frac{dS_1}{dt} = (-w + rN_1 + rN_2)S_1, \qquad \frac{dS_2}{dt} = (-w + rN_1 + rN_2)S_2;$$

$$\frac{dN_1}{dt} = wS_1 - rS_1N_1 - rS_2N_1; \qquad \frac{dN_2}{dt} = wS_2 - rS_2N_2 - rS_1N_2.$$

Let $Q = N_1 + N_2 + S_1 + S_2$. Then $N_1 + N_2 = Q - S_1 - S_2$. We are mainly concerned about $S_i$. Let $S = S_1 + S_2$ and $D = S_1 - S_2$. Notice that

$$\frac{dS}{dt} = \frac{d(S_1 + S_2)}{dt} = (-w + rQ - rS)S; \tag{37}$$

$$\frac{dD}{dt} = \frac{d(S_1 - S_2)}{dt} = (-w + rQ - rS)D. \tag{38}$$

We can now solve the first equation. It is a logistic differential equation.

**Lemma 6.2.** *The solution to* $\frac{dS}{dt} = (-w + rQ - rS)S$ *with initial condition* $S(0)$ *is*

$$S(t) = \frac{(-w + rQ)\exp(-w + rQ)t}{r\exp(-w + rQ)t - r + \frac{-w+rQ}{S(0)}}.$$

The proof is omitted here. Equation (38) can now be written as

$$\frac{d\ln D}{dt} = (-w + rQ) - rS. \tag{39}$$

Integrating both sides, we obtain

$$\ln D(t) = \int (-w+rQ) - rSdt = (-w+rQ)t - \ln(\exp(-w+rQ)t - 1 + \frac{-w + rQ}{rS(0)}) + C \tag{40}$$

So we obtain

$$D(t) = \frac{(\exp(-w + rQ)t)(\exp C)}{\exp(-w + Q)t - 1 + \frac{-w+rQ}{rS(0)}}.$$

Let $t = 0$. We have $D(0) = \frac{rS(0)\exp C}{-w+rQ}$. So $\exp C = \frac{D(0)(-w+rQ)}{rS(0)}$. Hence

$$D(t) = \frac{D(0)(-w + rQ)\exp(-w + rQ)t}{rS(0)\exp(-w + Q)t - rS(0) + (-w + rQ)}.$$

We obtain Equation (35, 36). In particular, as $t \to \infty$,

$$S_1(t) \to \frac{-w + rQ}{r}\frac{S_1(0)}{S_1(0) + S_2(0)}, \qquad S_2(t) \to \frac{-w + rQ}{r}\frac{S_2(0)}{S_1(0) + S_2(0)}.$$

So after the concentration rates stabilize, the compartment sizes $S_1$ and $S_2$ approach the equilibrium $\frac{-w+rQ}{r}\frac{S_1(0)}{S_1(0)+S_2(0)}$ and $\frac{-w+rQ}{r}\frac{S_2(0)}{S_1(0)+S_2(0)}$ respectively. □

Notice that Equation 33 can be reduced to a quartic equation. So $r$ can again have at most 4 different values.

**Theorem 6.3.** *Suppose that* $k_{x1} = k_{x2}$ *and* $k_{y1} = k_{y2}$. *With* $T_x$, $T_y$, $Q$ *and* $\frac{S_1(0)}{S_2(0)}$ *fixed, under the symmetric conditions, there can be at most* 4 *equilibrium points. The equilibrium submanifold is at most* 4 *dimensional.*

Notice that the equilibrium does not depend on the initial compartment size $S_i$, but only the ratio of initial $S_i$. We shall remark that the degenerate case is included here only for theoretical purposes. For biological implications of the nondegenerate case, please see [8].

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