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Biophysical Models of Calcium-Dependent Exocytosis

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Definition

Calcium Dependent Exocytosis is the biochemically controlled fusion of the bilipid secretory vesicle membrane with the bilipid cell membrane, triggered by the binding of several calcium ions (Ca^{2+}) to control proteins such as synaptotagmins anchored at the interface between these two membranes. Exocytosis results in the release of vesicle contents into the extracellular space, namely the release of neurotransmitter into the synaptic cleft in the case of neuronal synapses and neuromuscular junctions, or the secretion of hormone into the blood stream in the case of endocrine cells. Exocytosis also allows the transmembrane proteins contained in the vesicle membrane to be incorporated into the cell membrane, although such membrane protein trafficking is more characteristic of Ca^{2+}-independent, constitutive exocytosis.

Synonyms

Non-constitutive exocytosis, regulated exocytosis

Detailed Description

In synapses, neuromuscular junctions and endocrine cells, exocytosis of a neurotransmitter or hormone containing vesicle occurs through the interaction of the fast-binding isoforms of the Ca^{2+}-sensing protein synaptotagmin (namely Syt1, Syt2 and Syt9) with the molecules of the SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) complex spanning the cell and vesicle membranes (Jahn & Fasshauer, 2012, Moghadam & Jackson, 2013). The main elements of the secretory channel-vesicle complex are schematically illustrated in Fig. 1. Despite their highly specialized morphology, a similar exocytosis triggering mechanism is found at most high-throughput ribbon sensory synapses (Cho & von Gersdorff, 2012, Sterling & Matthews, 2005), except for the cochlear hair cells. Those auditory synapses may differ in both the molecular exocytosis machinery and their Ca^{2+} sensitivity (Nouvian et al., 2011), and may involve non-neuronal isoforms of synaptotagmin (Johnson et al., 2010) or a distinct Ca^{2+} sensor, otoferlin (Michalski et al., 2017, Pangrsic et al., 2012, Roux et al., 2006). The mechanism of exocytosis of hormone-containing large dense-core vesicles in endocrine cells is very similar to the mechanisms of neurotransmitter vesicle release (Chow et al., 1992, Heinemann et al., 1994, Voets, 2000), but typically proceeds at slower rates due to a somewhat different morphology of the release site, slower Ca^{2+} binding kinetics of the relevant synaptotagmin isoforms, and looser

coupling between voltage-dependent Ca^{2+} channels and release-ready vesicles (Kasai, 1999, Martin, 2003, Moghadam & Jackson, 2013, Verhage & Toonen, 2007, Wu et al., 2009). It should be noted however that certain classes of endocrine cells such as pancreatic beta cells exhibit a close channel-vesicle coupling similar to that of neuronal synapses (Barg et al., 2001).

The dependence of the neurotransmitter or hormone release rate on Ca^{2+} concentration is known to be steeply non-linear, as was first observed (Dodge & Rahamimoff, 1967) when examining neuromuscular junction potentials at different extracellular Ca^{2+} concentrations, and later confirmed in studies that directly varied intracellular Ca^{2+} concentration using caged-Ca^{2+} release in endocrine cells, the giant calyx of Held synapse, and other synaptic terminals (Gentile & Stanley, 2005, Neher & Sakaba, 2008, Stanley, 2016). For low, sub-saturating concentrations of Ca^{2+}, this nonlinear relationship can be summarized as

$$R([Ca]) \propto [Ca]^n \qquad (1)$$

Here [*Ca*] denotes Ca^{2+} concentration at the vesicle fusion site, *R* denotes instantaneous neurotransmitter release rate and *n* is the intrinsic (biochemical) Ca^{2+} *cooperativity* of exocytosis, which varies from 3-5 in most preparations. Note however that a significantly less cooperative, near-linear Ca^{2+} dependence has been reported in mature auditory hair cells, possibly because of differences in molecular exocytosis sensors mentioned above (Cho & von Gersdorff, 2012, Johnson et al., 2010, Nouvian et al., 2011), although it has been suggested that the linear dependence could potentially arise from the averaging across synaptic contacts (Heil & Neubauer, 2010).

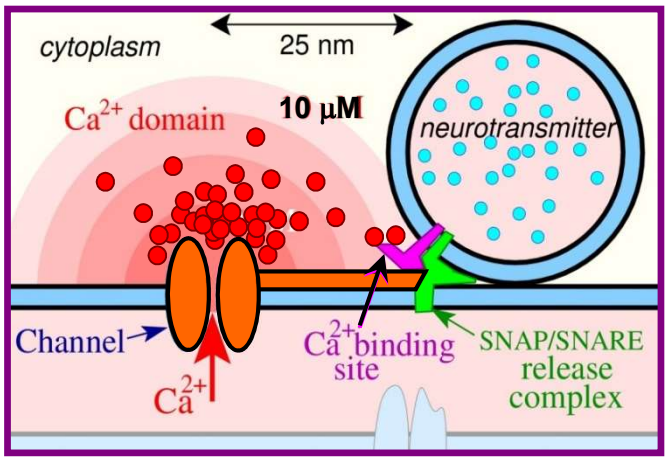


Figure 1. Secretory channel-vesicle release complex. The vesicle is shown in a fully docked state preceding fusion. The opening of a voltage-gated Ca^{2+} channel leads to the entry of Ca^{2+} ions (*red circles*) into the cell, resulting in a "domain" of elevated Ca^{2+} concentration, which can reach tens of µM close to the vesicle, some 20-30 nm away from the channel. The SNARE protein complex (*green polygon*) interacts with synaptotagmin (*magenta polygon*) that serves as the fast Ca^{2+}-sensitive trigger of the vesicle-membrane fusion. Multiple SNARE complexes around the base of the vesicle are involved in exocytosis, only one of which is shown. At many synapses, the tight co-localization of Ca^{2+} channels and SNARE proteins is promoted by direct interactions between the relevant proteins (*orange bar*).

The release (exocytosis) rate *R* is usually given in units of vesicles per second, and measured as a membrane capacitance increase or by electrochemical detection of released molecules, using a carbon fiber electrode, but often assessed only indirectly by measuring postsynaptic currents

or potentials. The steep non-linear dependence given by Eq. (1) indicates that simultaneous binding of several Ca^{2+} ions to synaptotagmin Ca^{2+} sensors is needed for release. In fact, the biochemical cooperativity provides a lower bound on the number of Ca^{2+} binding events required for exocytosis. Although the C2A and C2B domains of a given synaptotagmin molecule do possess a total of five Ca^{2+} ion binding sites (Chapman, 2002), not all of these sites are necessarily involved in fast (phasic) Ca^{2+}-triggered exocytosis. Therefore, it is likely that the relevant Ca^{2+} binding sites are distributed among several synaptotagmin molecules, which dimerize and bind Ca^{2+} simultaneously to trigger vesicle fusion (Mutch et al., 2011). In fact, studies suggest that more than 10 synaptotagmin molecules typically associate with a single neurotransmitter vesicle (Takamori et al., 2006). However, in accordance with the above-mentioned Ca^{2+} cooperativity measurements, most models reviewed below assume about 5 Ca^{2+} binding sites, all of which have to be occupied for vesicle fusion to occur. An alternative detailed model that assumes an excess of Ca^{2+} binding sites and includes Monte-Carlo simulation of Ca^{2+} ion diffusion and binding have been examined (Dittrich et al., 2013; Luo et al., 2015b).

While the main characteristics of exocytosis outlined above are in general understood, and the molecular components of exocytosis have been identified, the precise sequence of molecular steps leading to exocytosis is still being debated, which explains the high degree of interest in biophysical models of this phenomenon. We also note that this process involves spatial scales of tens of nanometers, and sub-millisecond temporal resolution, well beyond the resolution of optical imaging methods, which underscores the important role of biophysical modeling in understanding exocytosis and in interpreting indirect measurements of its properties.

A complete biophysical model of secretory vesicle exocytosis requires specifying the following model components: (1) the morphology of the synaptic vesicle docking site, namely the spatial channel-vesicle arrangement; (2) the properties of endogenous Ca^{2+} buffers and other Ca^{2+} homeostasis mechanisms, (3) a method for simulating Ca^{2+} diffusion and buffering to endogenous aCa^{2+} buffers, and (4) a model of Ca^{2+} binding by the synaptotagmin release sensors. Here we focus on the latter component of the model. Examples of comprehensive models of this process are given at the end of this entry.

Sequential Ca^{2+}-binding model

Assuming for concreteness five distinct Ca^{2+} binding sites comprising the putative exocytosis gate (sensor) *X*, the most general Ca^{2+}-sensitive exocytosis process can be described by the following reaction (Heidelberger et al., 1994, Kasai, 1999):

$$X \underset{k_1^-}{\overset{[Ca]k_1^+}{\rightleftharpoons}} CaX \underset{k_2^-}{\overset{[Ca]k_2^+}{\rightleftharpoons}} Ca_2X \ldots Ca_4X \underset{k_5^-}{\overset{[Ca]k_5^+}{\rightleftharpoons}} Ca_5X \xrightarrow{\gamma} Fused \qquad (2)$$

where [*Ca*] is the Ca^{2+} concentration at the vesicle site, $k_j^{\pm}$ are the binding and unbinding rates of each binding site, and the final irreversible reaction represents the actual vesicle fusion event.

In Eq. 2 and below, Ca^{2+} binding is indicated by a product between the relevant binding rate and the Ca^{2+} concentration variable, [*Ca*]. In a deterministic simulation, this reaction is converted to a system of ordinary differential equations, using the principle of mass action:

$$\begin{cases} \dfrac{d[X]}{dt} = -k_1^+[Ca][X] + k_1^-[CaX] \\ \ldots \\ \dfrac{d[Ca_5X]}{dt} = k_5^+[Ca][Ca_4X] - \left(\gamma + k_5^-\right)[Ca_5X] \end{cases} \tag{3}$$

where [Ca_nX] represents the fraction of exocytosis gates with *n* binding sites occupied by a Ca^{2+} ion. The fusion rate is given by the product γ[Ca_5X]. Note that the Ca^{2+} concentration, [*Ca*], should be modeled independently; in the simplest case of global Ca^{2+} elevation produced by caged Ca^{2+} release, it is approximately constant. When modeling exocytosis triggered by action potentials, [*Ca*] can be represented as a brief pulse of a certain width and amplitude; more detailed models of Ca^{2+} dynamics are reviewed below.

In Eqs. 2-3, the binding and unbinding rates $k_j^{\pm}$ are in general distinct, and if the earlier unbinding rates are slow, a significant accumulation of partially bound states [Ca_nX] can result during a train of stimuli; such accumulation due to slow unbinding is the basis for the so-called bound-Ca^{2+} model of synaptic facilitation (Bertram et al., 1996; Matveev, 2006).

Parallel Ca^{2+}-binding model

It is instructive to first consider the simplest scenario where all five release sites comprising exocytosis gate *X* are identical and can bind Ca^{2+} independently, leading to the following simplified version of the reaction given by Eq. 2 (Kasai, 1999, Voets, 2000)

$$X \underset{k^-}{\overset{5[Ca]k^+}{\rightleftharpoons}} CaX \underset{2k^-}{\overset{4[Ca]k^+}{\rightleftharpoons}} Ca_2X \ldots Ca_4X \underset{5k^-}{\overset{[Ca]k^+}{\rightleftharpoons}} Ca_5X \xrightarrow{\gamma} Fused \tag{4}$$

Here the final reaction representing vesicle fusion is irreversible, and is triggered when all 5 binding sites are occupied; exocytosis proceeds at rate $r = \gamma$[Ca_5X]. Even though Eq. 4 appears to describe a series of five consecutive binding reactions, it is equivalent to five identical reactions occurring in parallel, with fusion taking place when all five sites are bound.

The following alternative version of the exocytosis model by (Bollmann & Sakmann, 2005, Bollmann et al., 2000) partially decouples the vesicle pool variable undergoing release and the Ca^{2+} sensor state variables, and also postulates the existence of an independent final release-promoting conformational transition reaction occurring after the release sensor is fully Ca^{2+} bound:

$$X \underset{k^-}{\overset{5[Ca]k^+}{\rightleftharpoons}} CaX \underset{2k^-}{\overset{4[Ca]k^+}{\rightleftharpoons}} Ca_2X \ldots Ca_4X \underset{5k^-}{\overset{[Ca]k^+}{\rightleftharpoons}} Ca_5X \underset{\delta}{\overset{\gamma}{\rightleftharpoons}} Ca_5X^*$$
$$V \overset{\rho[Ca_5X^*]}{\rightarrow} Fused \qquad (5)$$

Here V represents the vesicle pool undergoing fusion, with vesicle fusion rate given by the product $\rho V[Ca_5X^*]$, where ρ is the maximal vesicle fusion rate. The inclusion of an additional post-binding step helps to achieve more constant shape of the release time-course at different amplitudes of Ca^{2+} influx seen in experiments (Bollmann et al., 2000, Yamada & Zucker, 1992). In (Bollmann et al., 2000) the parameter values that were found to fit well the data from the calyx of Held synaptic terminal are given by ρ=40 ms^{-1}, k^+=0.3 μM^{-1}ms^{-1} , k^- =3 ms^{-1}, γ=30 ms^{-1}, δ=8 ms^{-1}.

We note that most of the Ca^{2+} binding rates quoted in the literature are inferred from the studies on the calyx of Held synapse and other synapses that primarily involve the faster Syt2 isoform of synaptotagmin in its natural physiological environment. Other isoforms of synaptotagmin may have significantly different values of the Ca^{2+} binding and unbinding rates (Bornschein & Schmidt, 2018, Moghadam & Jackson, 2013). For example, the following model by (Voets, 2000) accurately predict exocytosis of the readily-releasable pool of vesicles in chromaffin cells caused by the Ca^{2+} binding to synaptotagmin isoform Syt1:

$$X \underset{k^-}{\overset{3[Ca]k^+}{\rightleftharpoons}} CaX \underset{2k^-}{\overset{2[Ca]k^+}{\rightleftharpoons}} Ca_2X \underset{3k^-}{\overset{[Ca]k^+}{\rightleftharpoons}} Ca_3X \overset{\gamma}{\rightarrow} Fused \qquad (6)$$

where k^+=4.4·10^{-3} μM^{-1}ms^{-1}, k^- =0.056 ms^{-1}, γ=1.45 ms^{-1}.

Cooperative Ca^{2+}-binding model

Some studies suggest that the Ca^{2+}-sensitive exocytosis triggers exhibit strong cooperativity, whereby the target protein undergoes a conformational change with each successive Ca^{2+} ion binding, which in turn leads to an increase in the Ca^{2+} affinity of the remaining (yet unoccupied) Ca^{2+} binding sites. The following widely-used modification of reaction given by Eq. 2 has been proposed to implement this possibility (Beutner et al., 2001, Felmy et al., 2003, Heidelberger et al., 1994, Kochubey et al., 2009, Sakaba, 2008, Schneggenburger & Neher, 2000):

$$X \underset{k^-}{\overset{5[Ca]k^+}{\rightleftharpoons}} CaX \underset{2bk^-}{\overset{4[Ca]k^+}{\rightleftharpoons}} Ca_2X \underset{3b^2k^-}{\overset{3[Ca]k^+}{\rightleftharpoons}} Ca_3X \underset{4b^3k^-}{\overset{2[Ca]k^+}{\rightleftharpoons}} Ca_4X \underset{5b^4k^-}{\overset{[Ca]k^+}{\rightleftharpoons}} Ca_5X \overset{\gamma}{\rightarrow} Fused \qquad (7)$$

Note that cooperative binding can be represented as either a progressive increase in forward binding rates or decrease of backward rates; Eq 7 corresponds to the latter possibility. The cooperativity parameter should satisfy b < 1, with a typical value used in the literature of 0.25, indicating that the final Ca^{2+}-binding reactions are only slowly reversible. In (Schneggenburger & Neher, 2000, Wolfel & Schneggenburger, 2003) quantifying the Ca^{2+}-dependence of vesicle

release at the calyx of Held synaptic terminal, the following parameter values were obtained: k^+=0.09 μM^{-1}ms^{-1}, k^-=9.5 ms^{-1}, γ=6 ms^{-1}, and the cooperativity parameter b=0.25.

We note that the use of the term "cooperativity" is ambiguous in the context of exocytosis mechanisms. Cooperativity may refer to the high number of Ca^{2+} binding sites (as inferred from the log-log slope of the Ca^{2+} concentration-response curve given by Eq. 1), or may indicate that the Ca^{2+} binding affinities of these sites are not equal but increase as the first sites become Ca^{2+} bound.

Models with independent sets of Ca^{2+}-binding sites

Several recent biophysically detailed models of exocytosis take into account the possibility that Ca^{2+} may bind to several different proteins or to different domains of the same protein, such as the C2A and C2B domains of synaptotagmin, each characterized by a distinct set of rates and cooperativity values. Examples of such models are given further below in the context of more comprehensive models of exocytosis – see model schemes 16 and 20.

Exocytosis rate at a steady Ca^{2+} concentration

To understand the exocytosis rate during prolonged Ca^{2+} elevation, for instance to reproduce caged-Ca^{2+} release experiments, it is of interest to consider the equilibrium point of the Ca^{2+}-binding reactions summarized above. Since all release models contain an irreversible transition to the fusion state, in the absence of endocytosis or vesicle refill from a reserve pool, the true equilibrium is achieved only when all vesicles are fused and release rate equals zero. Therefore, below we will assume a model with decoupled sensor binding state and vesicle pool state variables, as in Eq. 5, or consider a quasi-equilibrium in the case where resource depletion is small on the time scale of Ca^{2+} binding.

The equilibrium occupancy of a single first-order Ca^{2+}-binding reaction $Ca + X \leftrightharpoons CaX$ with forward rate k^+ and backward rate k^- is found by setting the rate of the first reaction in Eqs. 3 to zero, which yields

$$[CaX] = \frac{k^+[Ca]}{k^+[Ca] + k^-} = \frac{[Ca]}{[Ca] + K} \tag{8}$$

where the ratio of the backward and backward rates $K = k^-/k^+$ is referred to as the Ca^{2+} affinity or dissociation constant, and is an important Ca^{2+}-sensitivity parameter: the lower the K, the higher is the affinity (sensitivity) of the release sensor. It follows from Eq. 8 that K represents the Ca^{2+} concentration at which half the sensors become bound. If the final fusion reaction requires the binding of all five sensors, in the absence of vesicle depletion the release rate at equilibrium will be proportional to the 5th power of the steady-state binding occupancy given by Eq. 8:

$$R([Ca]) = \gamma \left(\frac{[Ca]}{[Ca] + K} \right)^5 \tag{9}$$

At low Ca^{2+} concentration, this agrees with the cooperativity condition given by Eq 1. Note that the release rate equals $1/2^5$ of its maximal value when $[Ca^{2+}]=K$.

For the case of cooperative binding, Eq. 7, the steady-state fusion rate R in the absence of resource depletion ($\gamma << 1$) is proportional to the equilibrium value of fully bound state Ca_5X, which yields (Weiss, 1997)

$$R([Ca]) = \frac{\gamma [Ca]^5}{[Ca]^5 + 5[Ca]^4 b^4 K + 10[Ca]^3 b^7 K^2 + 10[Ca]^2 b^9 K^3 + 5[Ca] b^{10} K^4 + b^{10} K^5} \tag{10}$$

where [Ca] is the Ca^{2+} concentration at the release site. In the limit of very small values of b (strong cooperativity limit), the above expression approaches the well-known and widely used Hill function:

$$R([Ca]) \approx \gamma \frac{[Ca]^n}{[Ca]^n + K_D^n} \tag{11}$$

where n=5 and $K_D \approx b^2 K$ ($b << 1$). The dissociation constant K_D quantifies the Ca^{2+} sensitivity of the entire 5-step process, as opposed to the sensitivity of any individual Ca^{2+}-binding site: when $[Ca]= K_D = b^2 K$, the exocytosis rate reaches its half-maximal value. Note that this is the most constrained and most important model parameter, since extensive experimental data on Ca^{2+} sensitivity has been collected at various types of synaptic terminals. In general, physiological rates of release are found when $[Ca^{2+}]$ at the vesicle location reaches the range of 10-50 μM (Neher & Sakaba, 2008), although there are reports that highly-sensitive vesicle pools exist at some endocrine cells, possibly controlled by distinct isoforms of the synaptotagmin sensor with Ca^{2+} affinity of several μM (Pedersen & Sherman, 2009).

Note that the Hill functional form given by Eq. 11 should only be viewed as a crude qualitative approximation of the true Ca^{2+}-dependence of exocytosis rate, even at equilibrium, and tends to be somewhat overused (Weiss, 1997). This is because the agreement between Eqs. 10 and 11 becomes sufficiently accurate only for values of cooperativity parameter satisfying b<0.1; in the absence of firm experimental evidence for such a strong cooperativity, the Hill function should be avoided. Assuming a more moderate but still strong cooperativity corresponding to the typically used value b=0.25, fitting the data to a Hill function would lead to a gross underestimate of the true number of Ca^{2+} binding sites, as illustrated in Fig. 2. This underestimate is mostly due to the poor match of the saturating part of the Hill curve, and can be greatly improved by omitting the data from saturating levels of $[Ca^{2+}]$. Therefore, the most model-independent way of estimating the cooperativity factor n is to fit only the log-log slope of the experimentally obtained Ca^{2+} sensitivity curve below the saturation inflection, which is often done in practice.

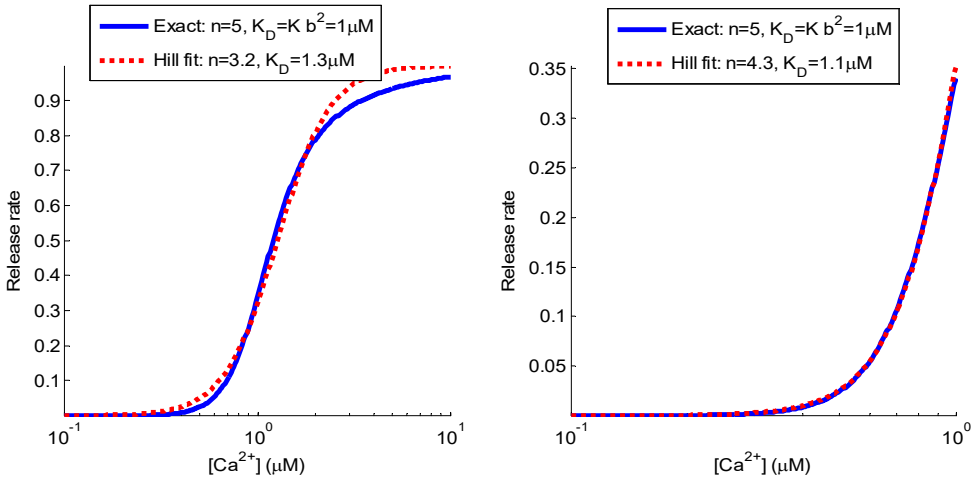


Figure 2. Hill-function fit underestimates true biochemical cooperativity and affinity of a 5th-order Ca^{2+}-binding reaction (Eqs. 7,10), even in the case of moderately strong cooperativity parameter (b=0.25). Note that the low quality of the fit is mostly a result of poor matching of the saturating part of the curve by the Hill function: the cooperativity is more accurately predicted if only the non-saturating part of the data is considered (*right panel*)

Cumulative release during prolonged Ca^{2+} elevation

The Ca^{2+}-dependence of release rate can be used to quantify the total cumulative release with prolonged Ca^{2+} elevation, as measured by the total membrane capacitance increase or the total amount of released neurotransmitter. In the case of prolonged and approximately steady Ca^{2+} elevation of duration Δt, the amount of fused vesicles is given by (Kasai, 1999, Quastel et al., 1992):

$$F([Ca]) = F_{\max}\left[1-\exp\left(-R_{eq}([Ca])\ \Delta t\right)\right] \tag{12}$$

where $F_{\max}$ represents the total amount of available exocytosis resources (say, vesicles), and $R_{eq}([Ca])$ represents the equilibrium reaction rate at Ca^{2+} concentration $[Ca]$ attained at the vesicle location. We note once again that this analysis assumes that the vesicle depletion is decoupled from the sensor binding state, as in the model given by Eq. 5. The exponential term can be interpreted as the probability of release failure. For pulses raising $[Ca^{2+}]$ to sub-saturating levels, given Eq. (1) we have $R \approx k[Ca]^n$, therefore (Quastel et al., 1992)

$$F([Ca]) = F_{\max}\left[1-\exp\left(-k\,[Ca]^n\,\Delta t\right)\right] \tag{13}$$

However, if $[Ca^{2+}]$ is high enough to saturate the release sensor during the long depolarizing pulse, the total amount of released neurotransmitter will become limited by the duration of stimulation only:

$$F = F_{\max}\left[1-\exp\left(-R_{\max}\Delta t\right)\right] \tag{14}$$

As was explained above, Eqs. (12-13) only apply to the situation where $[Ca^{2+}]$ is elevated for a long time relative to the kinetics of the Ca^{2+}-binding sites, as for instance during whole-cell $[Ca^{2+}]$ elevation produced by Ca^{2+} uncaging experiments. If on the other hand $[Ca^{2+}]$ is understood as the *peak* concentration achieved in the vicinity of the vesicle during a brief action potential, the above steady-state results would over-estimate the true release, and could only serve as an upper bound on release rate (Shahrezaei & Delaney, 2005). The "true" neurotransmitter release rate would be given by a solution to the differential equation (5), with $[Ca^{2+}]$ representing the time-dependent Ca^{2+} concentration at the vesicle release site, which has to be modeled independently, using simulations of Ca^{2+} entry through Ca^{2+} channels, diffusion and binding to intracellular Ca^{2+} buffers.

Asynchronous synaptic transmission

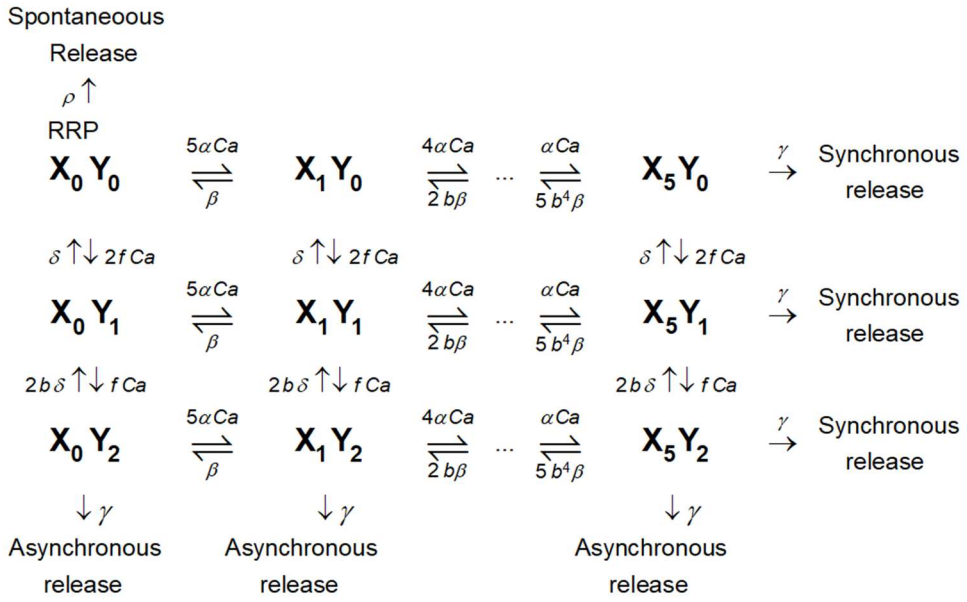
Many synapses display a delayed phase of neurotransmitter release that persists after the stimulating presynaptic depolarization has ceased. This delayed component of exocytosis is termed asynchronous or delayed exocytosis, and at some synapses it makes a significant contribution to the over-all neurotransmitter release under physiological conditions (Rozov et al., 2019). There is continued debate whether the asynchronous component of synaptic transmission is a result of synaptic Ca^{2+} accumulation, or whether it is caused by a distinct vesicle pool released via special mechanisms independent of the synchronous release component (Chung & Raingo, 2013, Smith et al., 2012). The connection between delayed release and the so-called spontaneous release seen in the absence of stimulation is also not fully understood. Biophysical modeling is used extensively in testing different hypotheses about the mechanisms of asynchronous and spontaneous neurotransmitter release.

An intriguing feature of asynchronous release is that it seems to exhibit a lower, almost linear Ca^{2+} dependence (Kochubey & Schneggenburger, 2011, Lou et al., 2005, Sun et al., 2007). The so-called allosteric model proposed by (Lou et al., 2005) explains the observed decrease in apparent cooperativity of Ca^{2+} action at low Ca^{2+} concentration by the presence of a slow conformational change of the release proteins, leading to a second route of vesicle fusion, which becomes progressively less likely at higher Ca^{2+} concentration. This is implemented by the following scheme with a reverse-cooperativity parameter $f < 1$:

$$\begin{array}{ccccccccc} \text{Reserve pool} & \rightleftharpoons & X & \underset{b^0k^-}{\overset{5[Ca]k^+}{\rightleftharpoons}} & CaX & \cdots & Ca_4X & \underset{5b^4k^-}{\overset{[Ca]k^+}{\rightleftharpoons}} & Ca_5X \\ & & \downarrow \alpha & & \downarrow \alpha f & & \downarrow \alpha f^4 & & \downarrow \alpha f^5 \\ & & \text{Fused} & & \text{Fused} & & \text{Fused} & & \text{Fused} \end{array} \qquad (15)$$

A more detailed model proposed by (Sun et al., 2007) and referred to as the *dual sensor* model provides an improved quantitative description of release at low Ca^{2+} concentrations, since it reproduced more accurately the latency of synaptic response at very low levels of Ca^{2+}. This

model assumes two independent Ca^{2+} sensors acting in parallel and each triggering a distinct mode of neurotransmitter release:



(16)

Here the synchronous release involves a sensor "X" with Ca^{2+}-cooperativity of five, while a different and independent sensor "Y" is responsible for asynchronous release, and possesses a cooperativity of two. Note that in this model implementation both sensors bind Ca^{2+} cooperatively, with equal cooperativity parameter *b*. The existence of two distinct sensors is supported by recent evidence that in some synapses asynchronous release is controlled by distinct Ca^{2+} binding proteins. Among the candidates for such a specialized asynchronous release regulator are the non-synaptotagmin sensor Doc2 (Yao et al., 2011), a distinct isoform of vesicle SNARE protein VAMP4 (Raingo et al., 2012), and the Syt7 isoform of synaptotagmin characterized by slow but high-affinity Ca^{2+} binding (Luo et al., 2015a, Turecek & Regehr, 2018, Weber et al., 2014). An alternative possibility is that asynchronous release becomes prominent when the faster synaptotagmin isoforms like Syt1 are depleted, which removes their release-clamping influence (Turecek & Regehr, 2019). However, as mentioned above, the mechanisms of asynchronous as well as spontaneous release are still under debate and may well vary across synaptic types (Chung & Raingo, 2013, Kaeser & Regehr, 2014, Smith et al., 2012).

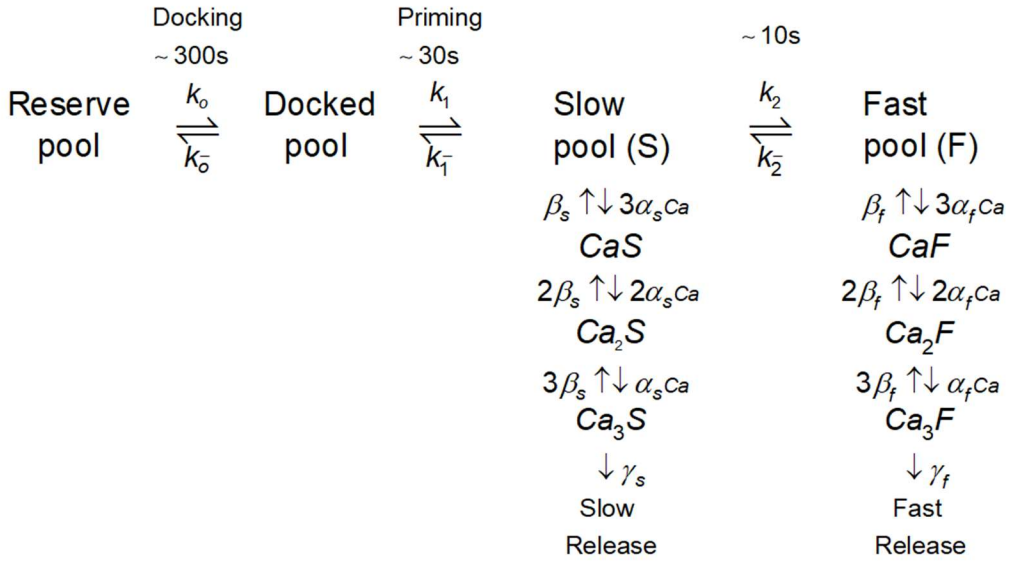
Multiple vesicle pool models

Apart from the intrinsic heterogeneity in Ca^{2+} affinity and speed of exocytosis due to the existence of distinct release pathways guiding exocytosis of each vesicle, an additional explanation for the observed heterogeneous components of vesicle release is the existence of independent pools of vesicles with variable degree of "preparedness" for exocytosis. This heterogeneity is especially pronounced in endocrine cells, which exhibit a fast-decaying initial phase of release followed by

a second, more slowly decaying component, but such behavior is also found in other high-throughput neuronal synapses such as the calyx of Held synaptic terminal and in ribbon synapses (Neher, 2012, Neher & Sakaba, 2008, Pedersen & Sherman, 2009, Verhage & Toonen, 2007, Voets, 2000). The identity of such distinct vesicle pools is currently under debate: they could be distinguished either by the variations in the distance between vesicles and corresponding voltage gated Ca^{2+} channels ("positional priming": (Wadel et al., 2007)) and/or by differences in the arrangement of the molecular machinery needed for exocytosis ("molecular priming" and "super-priming": (Chung & Raingo, 2013, Lee et al., 2013, Neher, 2017, Sorensen, 2004, Taschenberger et al., 2016, Verhage & Toonen, 2007)).

Exocytosis models comprising multiple vesicle pools allow reproducing the observed heterogeneous release components. However, simple two-pool models comprising a readily releasable pool and a reserve pool of vesicles were already proposed in the earliest modeling studies of vesicle release, in order to account for the short-term depression of synaptic response arising from vesicle depletion (Elmqvist & Quastel, 1965, Liley & North, 1953, Neher, 1998, Zucker & Regehr, 2002).

Most quantitative models of exocytosis that include multiple releasable vesicle pools are based on the two-pool model of (Heinemann et al., 1993, Voets, 2000, Voets et al., 1999) that was put forward to quantify two temporal components of secretory vesicle release in adrenal chromaffin cells. One implementation of such a two-pool model was explored by (Sorensen, 2004):

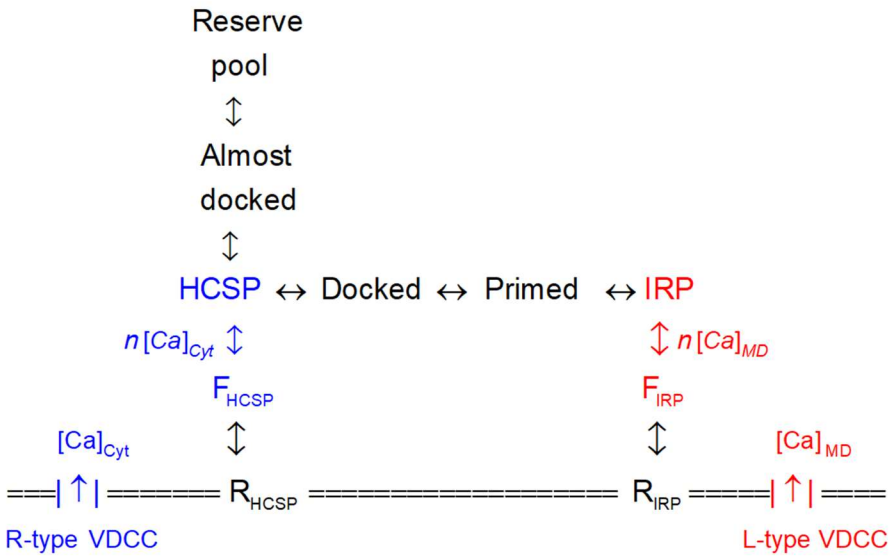


(17)

In this model exocytosis proceeds with cooperativity value of three from both the slow ("sustained") and fast synchronous pools, which are replenished via two preparatory steps, docking and priming, from the reserve pool.

There are indications that in some cells, the slowly releasing pool may in fact have a much higher sensitivity to Ca^{2+}, in the range of several µM rather than 10s of µM, which allows it to participate

in exocytosis despite a much longer separation from the Ca^{2+} channels compared to the immediately-releasable fast pool (reviewed in (Pedersen & Sherman, 2009)). The delay in the release of the highly-sensitive vesicles is explained by the longer diffusional distance and therefore longer time required for Ca^{2+} concentration to reach μM range far from the Ca^{2+} channels. Such so-called Highly Ca^{2+} Sensitive Pool (HCSP) has been described in adrenal chromaffin cells (Yang et al., 2002), rod photoreceptor ribbon synapses (Thoreson et al., 2004) and in insulin secreting beta cells (Yang & Gillis, 2004). A recent multiple-pool model that includes the HCSP pool along with the lower-affinity immediately-releasable pool (IRP), and implements a deterministic bi-domain model of $[Ca^{2+}]$ dynamics, can be found in (Pedersen & Sherman, 2009). This model is based on an earlier model of (Chen et al., 2008), and it accurately predicts the characteristic bi-phasic release of insulin from pancreatic beta cells. The first phase of secretion is mostly due to the docked vesicles that are rapidly recruited to the immediately releasable pool (IRP) and exocytosed in response to local "micro-domain" $[Ca^{2+}]$ ($[Ca]_{MD}$) entering through L-type Ca^{2+} channels. In contrast, the second, delayed phase of insulin release builds up more slowly due to the recruitment of the reserve vesicles into the HCSP, and gradual accumulation of cytosolic Ca^{2+} ($[Ca]_{Cyt}$) entering through R-type Ca^{2+} channels:



(18)

Here Ca^{2+}-dependent transitions are indicated in color, according to the primary source of Ca^{2+} for the corresponding transition. The R-type and L-type voltage-dependent Ca^{2+} channels (VDCCs) contribute respectively to the cytosolic and the microdomain pools of Ca^{2+}, $[Ca]_{Cyt}$ and $[Ca]_{MD}$ (the diffusional exchange between these two Ca^{2+} pools is not shown). The Ca^{2+}-dependent exocytosis steps lead to the two "fused" states, F_{HCSP} and F_{IRP}, which in turn feed into the final released insulin states R_{HCSP} and R_{IRP} through a final Ca^{2+}-independent pore-expansion transition.

Note that the existence of multiple vesicle pools can also serve as a potential explanation for asynchronous release reviewed above (Chung & Raingo, 2013, Neher, 2017). Another possibility

is that the intrinsic sources of heterogeneity in release properties captured by the allosteric and the dual-sensor models (Eqs. 15,16) are present in each vesicle (Kaeser & Regehr, 2014, Sun et al., 2007). In particular, as reviewed above, in some synapses asynchronous release may be mediated by specialized Ca^{2+} sensors. Further, both the synchronous and the delayed components are observed with global (whole-terminal) Ca^{2+} elevations that activate vesicles in all pools. Therefore, both sources of heterogeneity, the ones intrinsic to each vesicle and the extrinsic ones (existence of distinct pools), may be relevant in many synapses (Kaeser & Regehr, 2014). In order to take into account all factors of vesicle release heterogeneity and predict more accurately the exocytosis rate under low Ca^{2+} conditions, one can combine the dual-pool model with the allosteric model of (Lou et al., 2005) or the dual-sensor model of (Sun et al., 2007). This was done for instance by (Wolfel & Schneggenburger, 2003), who considered allosteric Ca^{2+} binding for both pools, as in Eq. 15 (neglecting however the exchange between the two pools due to the short time scales considered in that work). Finally, it should be noted that the separation of releasable vesicles into only two pools instead of a larger set of pools or a continuum of states is most probably a simplification, but this level of detail is sufficient to accurately quantify release under most physiological conditions (Neher, 2017). Including more than two pools should be done with great care to avoid an underdetermined model and data over-fitting.

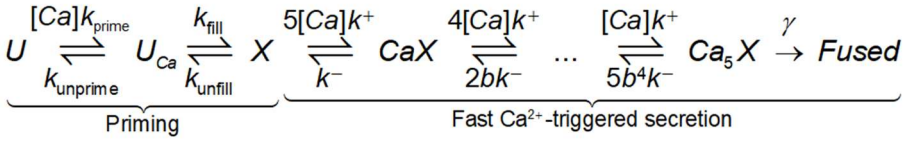
Models with Ca^{2+}-dependent vesicle priming

Vesicle docking and priming steps in reactions 17-18 most likely include multiple molecular processes (involving Munc18 and other proteins) and morphological steps (e.g. positional priming), the identity and sequence of which are still under investigation (Lee et al., 2013, Verhage & Toonen, 2007). Experimental evidence suggests that at least some of these vesicle priming steps are Ca^{2+} dependent in many synapses, albeit with a lower Ca^{2+} cooperativity compared to exocytosis itself, with a near-linear dependence on intracellular Ca^{2+} concentration (Neher & Sakaba, 2008). Depending on the balance between the different vesicle pools at resting Ca^{2+}, the Ca^{2+}-dependent priming can manifest itself in two different properties of short-term synaptic plasticity (Zucker & Regehr, 2002):

1) Under conditions of low initial release-ready vesicle pool size, Ca^{2+}-dependent priming would cause the secretion rate to increase during stimulation as a result of an increase in vesicle “mobilization” to the release-ready primed pool, which can serve as a mechanism of short-term synaptic facilitation (Dittman et al., 2000, Millar et al., 2005, Pan & Zucker, 2009, Worden et al., 1997) (see also encyclopedia entry on “Facilitation, Biophysical Models”).

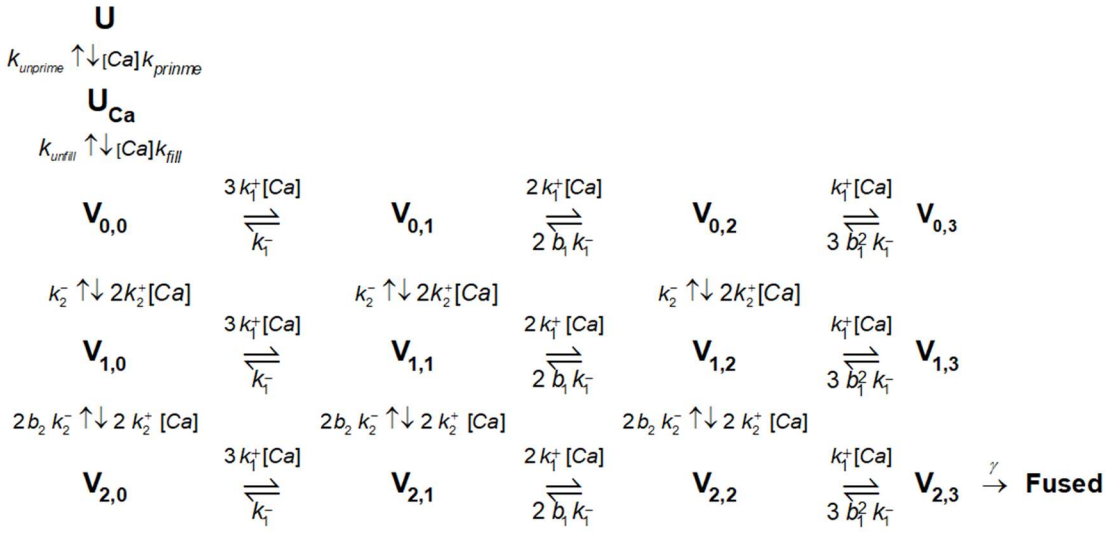
2) Under conditions of high vesicle release probability, Ca^{2+}-dependent priming would manifest itself through activity-dependent acceleration of recovery from short-term synaptic depression (Dittman & Regehr, 1998, Hosoi et al., 2007, Stevens & Wesseling, 1998, von Ruden & Neher, 1993, Wang & Kaczmarek, 1998)

The simplest model of release that includes a Ca^{2+}-dependent priming step can be obtained by modifying reaction 7: for instance, (Millar et al., 2005) considered the following scheme (see also (Bornschein et al., 2013, Brachtendorf et al., 2015, Sakaba, 2008) for a slightly modified version of this scheme):



(19)

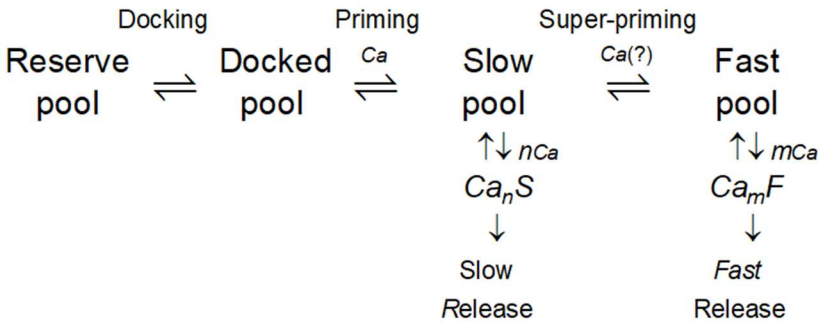
The study of (Millar et al., 2005) also considered a more detailed version of this model that takes into account the independence of Ca^{2+} binding to C2A and C2B domains of synaptotagmin, leading to the following modification of above (see also (Pan & Zucker, 2009)):



(20)

Here the horizontal state transitions represent the binding of the three C2A sites of synaptotagmin, while the vertical transitions correspond to the binding of the C2B sites, which is assumed to be independent of the Ca^{2+} binding of the C2A domain. Both domains are assumed to bind Ca^{2+} cooperatively (with cooperativity parameter $b_1=b_2=0.5$). These models were successful in explaining the difference between release properties of the facilitating “tonic” and depressing “phasic” crustacean neuromuscular junctions (Millar et al., 2005, Pan & Zucker, 2009)

Vesicle priming has also been considered in the context of more detailed models that include multiple releasable vesicle pools. In such models, the priming step is usually assumed to occur upstream of the process of exchange between the slow-releasing and the fast-releasing vesicle pools. Adding the priming step to the scheme 17 leads to the following class of models (Verhage & Toonen, 2007):



(21)

In this scheme, “super-priming” refers to the conversion of the slowly releasable pool to the fast releasable pool, the nature and the Ca^{2+}-dependence of which is currently unknown. Note that the use of the term “super-priming” is model-dependent; recently it has been used to describe a newly identified additional kinetic step in the priming of the fast pool at the calyx of Held synaptic terminal (Lee et al., 2013). The possible Ca^{2+} dependence of the conversion of the slow pool to the fast pool is unknown; it is possible that this conversion may even be retarded by Ca^{2+} at some synapses (Neher & Sakaba, 2008). At the calyx of Held, the contribution of the fast component of release is observed to increase with increasing Ca^{2+} levels, at the expense of the slow component (Wolfel et al., 2007). An explanation of this observation was suggested that the slow pool may be small at rest but increases due to activity-dependent conversion of vesicles from the fast pool, and that this conversion is less pronounced at high Ca^{2+} levels, so it is not able to reduce the fast pool at more intense stimulation levels (Neher & Sakaba, 2008).

Spatially resolved exocytosis models and the vesicle-channel coupling

Models of Ca^{2+} binding by synaptotagmin sensors controlling the release of one or more vesicle pools are often combined with simplified models of intracellular Ca^{2+} dynamics that involve only one or two well-mixed spatial Ca^{2+} compartments. This level of detail is sufficient to simulate exocytosis in response to global elevations of Ca^{2+} produced by caged-Ca^{2+} compounds. However, deep understanding of physiological synaptic or endocrine transmission requires full three-dimensional simulation of intracellular Ca^{2+} diffusion and buffering. This in turn requires taking into account the latest data on the spatial arrangement of voltage-dependent Ca^{2+} channels and membrane-docked vesicles, which varies considerably across distinct cell types and stages of development (Bornschein & Schmidt, 2018, Eggermann et al., 2012, Gentile & Stanley, 2005, Matveev et al., 2011, Moser et al., 2006, Oheim et al., 2006, Stanley, 2015, Stanley, 2016, Walter et al., 2018). In fact, the heterogeneous release probability of secretory vesicles captured by the multiple vesicle pools models reviewed above can be a result of heterogeneous distance between vesicles and the presynaptic Ca^{2+} channels.

Since the geometric arrangement of vesicles and functional Ca^{2+} channels at the release sites is hard to probe using existing experimental techniques, testing the hypotheses on channel-vesicle distance has been one of the primary aims of the more comprehensive biophysical models of

exocytosis. To simulate three-dimensional Ca^{2+} dynamics, such detailed models use either stochastic simulation (Bennett et al., 2004, Dittrich et al., 2013, Glavinovic & Rabie, 2001, Ma et al., 2015, Nadkarni et al., 2012, Scimemi & Diamond, 2012, Shahrezaei et al., 2006, Shahrezaei & Delaney, 2005), deterministic solution of reaction-diffusion equations (Bohme et al., 2018, Bucurenciu et al., 2008, Gandasi et al., 2017, Matveev et al., 2009, Matveev et al., 2011, Meinrenken et al., 2002, Meinrenken et al., 2003, Schmidt et al., 2013, Weber et al., 2010, Zucker & Fogelson, 1986), or steady-state approximations of Ca^{2+} distribution near an array of open Ca^{2+} channels (Bertram et al., 1999, Coggins & Zenisek, 2009, Matveev, 2016, Montefusco & Pedersen, 2018).

Several particularly detailed modeling studies combine together many of the mechanisms reviewed above to build a comprehensive model of secretory vesicle exocytosis:

1) The model implemented in (Dittrich et al., 2013, Luo et al., 2015b) and (Ma et al., 2015) is one of the most complete recent models of exocytosis that includes stochastic simulations of Ca^{2+} ions diffusion, buffering and binding to synaptotagmin sensors, taking into account the copy number of synaptotagmin molecules and SNARE complexes per vesicle (Chapman, 2002, Han et al., 2004, Mutch et al., 2011). This is the first modeling study that considered the possibility that only a subset of synaptotagmin sites have to be Ca^{2+}-bound to trigger exocytosis, and (Dittrich et al., 2013) examines the effective Ca^{2+} cooperativity of exocytosis as an emergent characteristic of such partial binding of a subset of Ca^{2+} sensors.

2) The study of (Pan & Zucker, 2009) builds a comprehensive model of release and short-term plasticity at tonic and phasic crustacean neuromuscular junctions, based on Eq. 20 above, but adds positional priming, leading to a transition scheme describing vesicle exchange between the following distinct vesicle pools: reserve, docked, primed channel-detached and primed channel-attached. One of the main aims of this study was to build a model that most fully accounts for the properties of short-term synaptic facilitation observed at crustacean tonic neuromuscular junctions, as well as the properties of short-term depression and asynchronous release at phasic neuromuscular junctions.

These models are just two representatives of a class of comprehensive models of Ca^{2+}-dependent exocytosis that go beyond the simulation of vesicle release in response to a single presynaptic depolarization, aiming to reproduce short-term changes in synaptic transmission strength in response to prolonged or repeated stimulation. Such modeling work is reviewed in more detail in the entry "Facilitation, Biophysical Models".

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