Encyclopedia of Computational Neuroscience
DOI 10.1007/978-1-4614-7320-6_178-1


# Calcium-Dependent Exocytosis, Biophysical Models of

Victor Matveev*
Department of Mathematical Sciences, New Jersey Institute of Technology, Newark, NJ, USA

## Synonyms

Non-constitutive exocytosis; Regulated exocytosis

## 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 $Ca^{2+}$ ions 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 bloodstream 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.

## Detailed Description

In synapses, neuromuscular junctions, and endocrine cells, fast $Ca^{2+}$-triggered exocytosis of a neurotransmitter or hormone-containing vesicle occurs primarily through the interaction of the so-called SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) proteins spanning the synaptic and vesicle membranes with specific isoforms of the $Ca^{2+}$-sensitive protein synaptotagmin and with involvement of other control proteins such as complexins (Jahn and Fasshauer 2012). Despite highly specialized morphology, a similar exocytosis-triggering mechanism is found at most high-throughput ribbon sensory synapses (Cho and von Gersdorff 2012; Sterling and Matthews 2005), except for cochlear hair cells which may differ in both their molecular exocytosis machinery and $Ca^{2+}$ sensitivity (Nouvian et al. 2011) and where exocytosis may require nonneuronal isoforms of synaptotagmin (Johnson et al. 2010) or a different $Ca^{2+}$ sensor, otoferlin (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; Voets 2000), but is believed to proceed at a somewhat slower rate due to less tight morphology of the release site and more lose coupling between voltage-dependent calcium channels and release-ready vesicles (Verhage and Toonen 2007; Wu et al. 2009). However, this distinction may be specific to only certain subclasses of endocrine cells, since a close channel-vesicle coupling characteristic of that in neurons has been found in pancreatic beta cells (Barg et al. 2001).

*Email: matveev@njit.edu

Encyclopedia of Computational Neuroscience
DOI 10.1007/978-1-4614-7320-6_178-1


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

$$R([\mathrm{Ca}]) \propto [\mathrm{Ca}]^n \tag{1}$$

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 three to five 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 and 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 and Neubauer 2010).

The release (exocytosis) rate $R$ is usually estimated 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 nonlinear dependence given by Eq. 1 indicates that simultaneous binding of several $Ca^{2+}$ ions to exocytosis control proteins, most likely isoforms of synaptotagmin, is needed for release. In fact, the biochemical cooperativity can be viewed as a lower bound on the number of $Ca^{2+}$-binding events required for exocytosis (Dittrich et al. 2013). 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, and therefore it is possible that the relevant $Ca^{2+}$-sensitive sensors could be distributed among several synaptotagmin molecules, which dimerize and bind $Ca^{2+}$ simultaneously to trigger vesicle fusion (Mutch et al. 2011). However, most models (reviewed below) assume for simplicity a small number of $Ca^{2+}$-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 has recently been examined in Dittrich et al. (2013).

## 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:

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

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 a deterministic simulation, this reaction is converted to a system of ordinary differential equations, using the principle of mass action:

DOI 10.1007/978-1-4614-7320-6_178-1


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

where $[\mathrm{Ca}_n\mathrm{X}]$ represents the fraction of exocytosis gates with $n$-binding sites occupied by a $Ca^{2+}$ ion. The maximal fusion rate is given by the product $\gamma[\mathrm{Ca_5X}]$. 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, or it can be represented as a brief pulse of certain width and amplitude when modeling vesicle release produced by a train of action potentials.

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 of X 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 (see entry on "▶ Facilitation, Biophysical Models"; Bertram et al. 1996; Bornschein et al. 2013; Matveev et al. 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:

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

Here the final reaction representing vesicle fusion is irreversible and is triggered when all five binding sites are occupied; exocytosis proceeds at rate $R = \gamma\,[\mathrm{Ca_5X}]$. 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, as follows:

$$\begin{cases} \mathrm{Y} \underset{k_1^-}{\overset{[\mathrm{Ca}]\,k_1^+}{\rightleftharpoons}} \mathrm{CaY}, \quad 5\mathrm{CaY} \overset{\gamma}{\rightarrow} \mathrm{Fused} \\ \dfrac{d[\mathrm{CaY}]}{dt} = k^+[\mathrm{Ca}](1 - [\mathrm{CaY}]) - k^-[\mathrm{CaY}] \end{cases} \tag{5}$$

where $[\mathrm{Y}] = 1 - [\mathrm{CaY}]$ represents the fraction of unbound gates. In this case, the fusion rate is given by the product $\gamma[\mathrm{CaY}]^5$ (identically equal to $\gamma[\mathrm{CaX}]$ in reaction (4)), where [CaY] is the bound fraction (probability) of each gate Y.

Some implementations of vesicle release process postulate the existence of an independent final release-promoting conformational transition reaction occurring after the release sensor is fully $Ca^{2+}$ bound, as, for instance, done in the model of neurotransmitter release at the calyx of Held synaptic terminal by Bollmann and Sakmann (2005) and Bollmann et al. (2000):

$$\mathrm{X} \underset{k^-}{\overset{5[\mathrm{Ca}]\,k^+}{\rightleftharpoons}} \mathrm{CaX} \underset{2k^-}{\overset{4[\mathrm{Ca}]\,k^+}{\rightleftharpoons}} \mathrm{Ca_2X} \ldots \mathrm{Ca_4X} \underset{5k^-}{\overset{[\mathrm{Ca}]\,k^+}{\rightleftharpoons}} \mathrm{Ca_5X} \underset{\delta}{\overset{\gamma}{\rightleftharpoons}} \mathrm{Ca_5X^*} \overset{\rho}{\rightarrow} \mathrm{Fused} \tag{6}$$

DOI 10.1007/978-1-4614-7320-6_178-1


Here ρ is the maximal vesicle fusion rate. In Bollmann et al. (2000), the parameter values that we found to fit well the data from the calyx of Held synaptic terminal are given by $\rho = 40\ \mathrm{ms}^{-1}$, $k^+ = 0.3\ \mu\mathrm{M}^{-1}\mathrm{ms}^{-1}$, $k^- = 3\ \mathrm{ms}^{-1}$, $\gamma = 30\ \mathrm{ms}^{-1}$, and $\delta = 8\ \mathrm{ms}^{-1}$. 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, as seen in experiment (Bollmann et al. 2000; Yamada and Zucker 1992).

## 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 (Felmy et al. 2003; Heidelberger et al. 1994; Schneggenburger and Neher 2000):

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

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 and Neher (2000) and Wolfel and Schneggenburger (2003), quantifying the $Ca^{2+}$ dependence of vesicle release at the calyx of Held synaptic terminal, the following parameter values were obtained: $k^+ = 0.09\ \mu\mathrm{M}^{-1}\ \mathrm{ms}^{-1}$, $k^- = 9.5\ \mathrm{ms}^{-1}$, $\gamma = 6\ \mathrm{ms}^{-1}$, and the cooperativity parameter $b = 0.25$.

Note that the use of the term "cooperativity" is ambiguous in the context of exocytosis mechanisms, since it is used to refer to two different properties of the exocytosis process: cooperativity can either refer to the number of $Ca^{2+}$-binding sites (as inferred from the log-log slope of the $Ca^{2+}$ dose–response curve) or indicate that the $Ca^{2+}$-binding affinity of these sites is not equal and increases 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 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 simulate the exocytosis rate during prolonged $Ca^{2+}$ elevation, for instance, to reproduce caged-$Ca^{2+}$ release experiments, it is sufficient to consider the equilibrium point of the reactions summarized above. At equilibrium, the occupancy of the $Ca^{2+}$-bound states is easily found by equating the right-hand side of the binding reaction to zero. For the case of independent gates, described by Eqs. 4 and 5, the equilibrium is given by

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

DOI 10.1007/978-1-4614-7320-6_178-1


where the ratio of the backward and forward 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. Since the final fusion reaction in Eq. 5 requires the binding of all five sensors, the release rate at equilibrium will be given by the 5th power of the steady-state binding occupancy of gate $Y$:

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

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 $R$ is given by the equilibrium value of fully bound state $Ca_5X$, which obeys a modified form of the Adair-Klotz-Pauling equation (Weiss 1997)

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

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:

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

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 $[\mathrm{Ca}] = K_D = b^2 K$, 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 (reviewed in Neher and Sakaba 2008), although there are reports that highly sensitive vesicle pools exist in some cells, possibly controlled by distinct isoforms of the synaptotagmin sensor with $Ca^{2+}$ affinity of several μM (reviewed in Pedersen and 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. 1. 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 only fit the log-log slope of the experimentally obtained $Ca^{2+}$ sensitivity curve *below* the saturation inflection, which is often done in practice.

DOI 10.1007/978-1-4614-7320-6_178-1

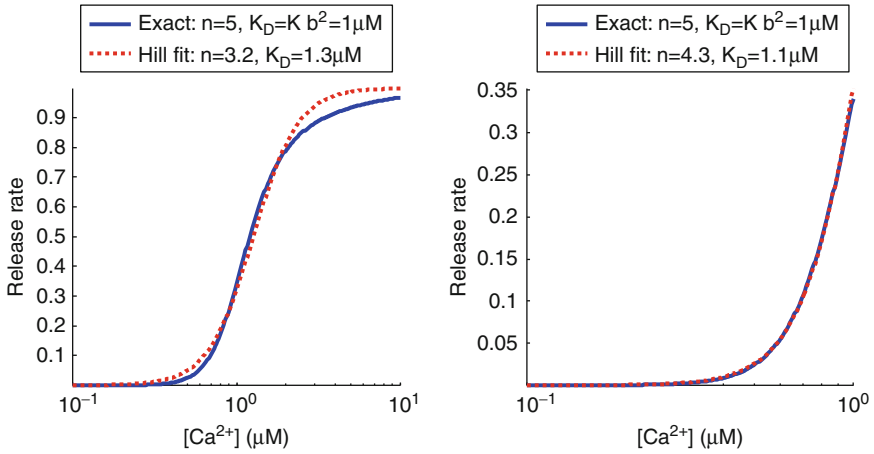




**Fig. 1** Hill function fit underestimates true biochemical cooperativity and affinity of a 5th-order $Ca^{2+}$-binding reaction (Eqs. 7 and 10), even in the case of moderately strong cooperativity parameter ($b = 0.25$). Note that the low quality of the fit is mostly a result of poor matching of the 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 $\Delta t$, the amount of fused vesicles is given by Quastel et al. (1992):

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

where $F_{\max}$ represents the total amount of available exocytosis resources (say, vesicles) and $R_{\mathrm{eq}}([\mathrm{Ca}])$ represents the equilibrium reaction rate at $Ca^{2+}$ concentration [Ca] attained at the vesicle location. The exponential term can be interpreted as the probability of release failure. For pulses raising $[Ca^{2+}]$ to subsaturating levels, given Eq. 1 we have $R \approx k[\mathrm{Ca}]^n$; therefore (Quastel et al. 1992)

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

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:

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

As was explained above, Eqs. 12 and 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 overestimate the true release and could only serve as an upper bound on release rate (Shahrezaei and 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,

DOI 10.1007/978-1-4614-7320-6_178-1


using simulations of $Ca^{2+}$ entry through $Ca^{2+}$ channels, diffusion, and binding to intracellular $Ca^{2+}$ buffers.

## Release at Low $Ca^{2+}$ Concentration: Asynchronous and Spontaneous Transmission

At very low $Ca^{2+}$ concentration, depolarization-evoked exocytosis is more stochastic and less synchronized with the stimulus, and its rate decreases, becoming comparable to the spontaneous transmission observed in the absence of stimulation. This delayed component of exocytosis is termed asynchronous or delayed exocytosis and at some synapses represents a significant contribution to the overall neurotransmitter release under physiological conditions. A prominent feature of asynchronous release is that it seems to exhibit a lower, almost linear $Ca^{2+}$ dependence (Lou et al. 2005; Sun et al. 2007). As will be discussed further below, there is some debate whether the distinct components of synaptic transmission are all manifestations of the same $Ca^{2+}$-dependent process or are in fact caused by distinct vesicle pools released via independent mechanisms (Chung and Raingo 2013; Smith et al. 2012). However, it should be noted that the mechanisms of asynchronous release may vary across distinct types of synapses (Kaeser and Regehr 2014).

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 $f < 1$:

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

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:

DOI 10.1007/978-1-4614-7320-6_178-1


$$
\begin{array}{ccccccccc}
\text{Spontaneoous} & & & & & & & & \\
\text{Release} & & & & & & & & \\
\rho\uparrow & & & & & & & & \\
\text{RRP} & & & & & & & & \\
\mathbf{X_0Y_0} & \underset{\beta}{\overset{5\alpha\,\mathrm{Ca}}{\rightleftharpoons}} & \mathbf{X_1Y_0} & \underset{2\,b\beta}{\overset{4\alpha\,\mathrm{Ca}}{\rightleftharpoons}} & \cdots & \underset{5\,b^4\beta}{\overset{\alpha\,\mathrm{Ca}}{\rightleftharpoons}} & \mathbf{X_5Y_0} & \overset{\gamma}{\rightarrow} & \text{Synchronous release} \\
\delta\uparrow\downarrow 2f\,\mathrm{Ca} & & \delta\uparrow\downarrow 2f\,\mathrm{Ca} & & & & \delta\uparrow\downarrow 2f\mathrm{Ca} & & \\
\mathbf{X_0Y_1} & \underset{\beta}{\overset{5\alpha\,\mathrm{Ca}}{\rightleftharpoons}} & \mathbf{X_1Y_1} & \underset{2\,b\beta}{\overset{4\alpha\,\mathrm{Ca}}{\rightleftharpoons}} & \cdots & \underset{5\,b^4\beta}{\overset{\alpha\,\mathrm{Ca}}{\rightleftharpoons}} & \mathbf{X_5Y_1} & \overset{\gamma}{\rightarrow} & \text{Synchronous release} \\
2b\delta\uparrow\downarrow f\,\mathrm{Ca} & & 2b\delta\uparrow\downarrow f\,\mathrm{Ca} & & & & 2b\delta\uparrow\downarrow f\mathrm{Ca} & & \\
\mathbf{X_0Y_2} & \underset{\beta}{\overset{5\alpha\,\mathrm{Ca}}{\rightleftharpoons}} & \mathbf{X_1Y_2} & \underset{2\,b\beta}{\overset{4\alpha\,\mathrm{Ca}}{\rightleftharpoons}} & \cdots & \underset{5\,b^4\beta}{\overset{\alpha\,\mathrm{Ca}}{\rightleftharpoons}} & \mathbf{X_5Y_2} & \overset{\gamma}{\rightarrow} & \text{Synchronous release} \\
\downarrow\gamma & & \downarrow\gamma & & & & \downarrow\gamma & & \\
\text{Asynchronous release} & & \text{Asynchronous release} & & & & \text{Asynchronous release} & &
\end{array}
\tag{16}
$$

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 requires a special non-synaptotagmin sensor Doc2 (Yao et al. 2011). However, the mechanisms of asynchronous and spontaneous release are still under debate and may vary across synaptic types (Chung and Raingo 2013; Kaeser and 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 and Sakaba 2008; Pedersen and Sherman 2009; Verhage and Toonen 2007). 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) or by differences in the arrangement of the molecular machinery needed for exocytosis ("molecular priming" and "super-priming" (Chung and Raingo 2013; Lee et al. 2013; Sorensen 2004; Verhage and Toonen 2007)). 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), and Voets et al. (1999) that was first put forward to quantify two temporal components of secretory vesicle release in adrenal chromaffin cells. Here is a recent implementation of such a two-pool model explored by Sorensen (2004):

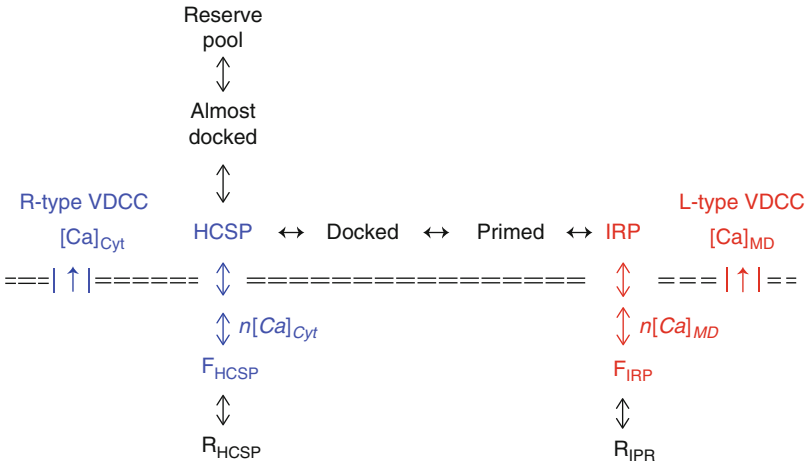
DOI 10.1007/978-1-4614-7320-6_178-1


$$\begin{array}{ccccccc} & \text{Docking} & & \text{Priming} & & \sim 10\text{s} & \\ & \sim 300\text{s} & & \sim 30\text{s} & & & \\ \text{Reserve} & \underset{k_o^-}{\overset{k_o}{\rightleftharpoons}} & \text{Docked} & \underset{k_1^-}{\overset{k_1}{\rightleftharpoons}} & \text{Slow} & \underset{k_2^-}{\overset{k_2}{\rightleftharpoons}} & \text{Fast} \\ \text{pool} & & \text{pool} & & \text{pool (S)} & & \text{pool (F)} \\ & & & & \beta_s \uparrow\downarrow 3\alpha_s\text{Ca} & & \beta_f \uparrow\downarrow 3\alpha_f\text{Ca} \\ & & & & \text{CaS} & & \text{CaF} \\ & & & & 2\beta_s \uparrow\downarrow 2\alpha_s\text{Ca} & & 2\beta_f \uparrow\downarrow 2\alpha_f\text{Ca} \\ & & & & \text{Ca}_2S & & \text{Ca}_2\text{F} \\ & & & & 3\beta_s \uparrow\downarrow \alpha_s\text{Ca} & & 3\beta_f \uparrow\downarrow \alpha_f\text{Ca} \\ & & & & \text{Ca}_3S & & \text{Ca}_3\text{F} \\ & & & & \downarrow \gamma_s & & \downarrow \gamma_f \\ & & & & \text{Slow} & & \text{Fast} \\ & & & & \text{Release} & & \text{Release} \end{array} \tag{17}$$

In this model exocytosis proceeds with cooperativity 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 10 s of μM, which allows it to participate in exocytosis despite a much greater separation from the $Ca^{2+}$ channels compared to the immediately releasable, fast pool (reviewed in Pedersen and 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 insulin-secreting beta cells (Yang and 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 and Sherman (2009). This model is based on an earlier model of Chen et al. (2008), and it accurately predicts the characteristic biphasic release of insulin from pancreatic beta cells, with the first phase of secretion mostly due to the docked vesicles that are rapidly converted to immediately releasable pool (IRP) and exocytosed in response to local "microdomain" $[Ca^{2+}]$ ($[Ca]_{MD}$) entering the cell through L-type $Ca^{2+}$ channels, whereas 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:

DOI 10.1007/978-1-4614-7320-6_178-1


(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.

Although the existence of multiple vesicle pools can also be considered as a potential explanation for asynchronous release (Chung and Raingo 2013), it is likely that intrinsic sources of heterogeneity in release properties captured by the allosteric and the dual-sensor models (see above) are also present in each vesicle (Kaeser and Regehr 2014; Sun et al. 2007). For instance, experiments that disrupt specific molecular components of release machinery indicate that at some synapses synchronous and asynchronous release can be independently manipulated and that asynchronous release is mediated by a distinct $Ca^{2+}$ sensor, Doc2 (Yao et al. 2011), and possibly involves a distinct isoform of the vesicle SNARE protein VAMP4 (Raingo et al. 2012). 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 (distinct pools), seem to be relevant in many synapses (Kaeser and 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 and Schneggenburger (2003), who considered allosteric $Ca^{2+}$ binding for both pools, as in reaction (15) (neglecting however the exchange between the two pools due to the short timescales 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. Including more than two pools would most likely lead to an underdetermined model and to 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 is still under investigation (Lee et al. 2013; Verhage and Toonen 2007). Experimental evidence suggests that at least some of these vesicle priming steps are $Ca^{2+}$

DOI 10.1007/978-1-4614-7320-6_178-1


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 and 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 and 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 and 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 and Regehr 1998; Hosoi et al. 2007; Stevens and Wesseling 1998; von Ruden and Neher 1993; Wang and 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) and Sakaba (2008) for a slightly modified version of this scheme):

$$\underbrace{U \underset{k_{\text{unprime}}}{\overset{[\text{Ca}]\,k_{\text{prime}}}{\rightleftharpoons}} U_{\text{Ca}} \underset{k_{\text{unfill}}}{\overset{k_{\text{fill}}}{\rightleftharpoons}} X}_{\text{Priming}} \underbrace{\overset{5[\text{Ca}]\,k^+}{\underset{k^-}{\rightleftharpoons}} \text{CaX} \overset{4[\text{Ca}]\,k^+}{\underset{2bk^-}{\rightleftharpoons}} \ldots \overset{[\text{Ca}]\,k^+}{\underset{5b^4k^-}{\rightleftharpoons}} \text{Ca}_5\text{X} \xrightarrow{\gamma} \text{Fused}}_{\text{Fast } Ca^{2+}\text{-triggered secretion}} \tag{19}$$

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 the above (see also Pan and Zucker 2009):

$$\begin{array}{ccccccc}
 & & \mathbf{U} & & & & \\
 & & k_{\text{unprime}} \uparrow\downarrow [\text{Ca}]\,k_{\text{prime}} & & & & \\
 & & \mathbf{U_{Ca}} & & & & \\
 & & k_{\text{unfill}} \uparrow\downarrow [\text{Ca}]\,k_{\text{fill}} & & & & \\
\mathbf{V_{0,0}} & \overset{3\,k_1^+[\text{Ca}]}{\underset{k_1^-}{\rightleftharpoons}} & \mathbf{V_{0,1}} & \overset{2\,k_1^+[\text{Ca}]}{\underset{2\,b_1\,k_1^-}{\rightleftharpoons}} & \mathbf{V_{0,2}} & \overset{k_1^+[\text{Ca}]}{\underset{3\,b_1^2\,k_1^-}{\rightleftharpoons}} & \mathbf{V_{0,3}} \\
k_2^- \uparrow\downarrow 2k_2^+[\text{Ca}] & & k_2^- \uparrow\downarrow 2k_2^+[\text{Ca}] & & k_2^- \uparrow\downarrow 2k_2^+[\text{Ca}] & & \\
\mathbf{V_{1,0}} & \overset{3\,k_1^+[\text{Ca}]}{\underset{k_1^-}{\rightleftharpoons}} & \mathbf{V_{1,1}} & \overset{2\,k_1^+[\text{Ca}]}{\underset{2\,b_1\,k_1^-}{\rightleftharpoons}} & \mathbf{V_{1,2}} & \overset{k_1^+[\text{Ca}]}{\underset{3\,b_1^2\,k_1^-}{\rightleftharpoons}} & \mathbf{V_{1,3}} \\
2\,b_2\,k_2^- \uparrow\downarrow 2\,k_2^+\,[\text{Ca}] & & 2\,b_2\,k_2^- \uparrow\downarrow 2\,k_2^+\,[\text{Ca}] & & 2\,b_2\,k_2^- \uparrow\downarrow 2\,k_2^+\,[\text{Ca}] & & \\
\mathbf{V_{2,0}} & \overset{3\,k_1^+[\text{Ca}]}{\underset{k_1^-}{\rightleftharpoons}} & \mathbf{V_{2,1}} & \overset{2\,k_1^+[\text{Ca}]}{\underset{2\,b_1\,k_1^-}{\rightleftharpoons}} & \mathbf{V_{2,2}} & \overset{k_1^+[\text{Ca}]}{\underset{3\,b_1^2\,k_1^-}{\rightleftharpoons}} & \mathbf{V_{2,3}} \xrightarrow{\gamma} \mathbf{Fused}
\end{array} \tag{20}$$

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

DOI 10.1007/978-1-4614-7320-6_178-1


explaining the difference between release properties of the facilitating "tonic" and depressing "phasic" crustacean neuromuscular junctions (Millar et al. 2005; Pan and 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 scheme (17) leads to the following class of models (Verhage and Toonen 2007):

$$\begin{array}{ccccccc} & \text{Docking} & & \text{Priming} & & \text{Super-priming} & \\ \text{Reserve} & \rightleftharpoons & \text{Docked} & \overset{\text{Ca}}{\rightleftharpoons} & \text{Slow} & \overset{\text{Ca (?)}}{\rightleftharpoons} & \text{Fast} \\ \text{pool} & & \text{pool} & & \text{pool} & & \text{pool} \\ & & & & \uparrow\downarrow n\text{Ca} & & \uparrow\downarrow m\text{Ca} \\ & & & & \text{Ca}_n\text{S} & & \text{Ca}_m\text{F} \\ & & & & \downarrow & & \downarrow \\ & & & & \text{Slow} & & \text{Fast} \\ & & & & \text{Release} & & \text{Release} \end{array} \tag{21}$$

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 and 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 and Sakaba 2008).

## Comprehensive Biophysical Models of $Ca^{2+}$-Dependent Exocytosis

Several particularly detailed recent modeling studies include simulations of $Ca^{2+}$ dynamics and combine together many of the mechanisms reviewed above to build a more comprehensive model of vesicle exocytosis:

1. The model of Dittrich et al. (2013) is one of the most complete recent models of exocytosis that includes stochastic simulations of $Ca^{2+}$ ions diffusion, buffering, and binding to multiple synaptotagmin molecules and takes into account most recent data on 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 it examines the effective $Ca^{2+}$ cooperativity of exocytosis as an emergent characteristic of such partial binding of a subset of $Ca^{2+}$-binding sites.
2. The study of Pan and Zucker (2009) builds a comprehensive model of release and short-term plasticity at tonic and phasic crustacean neuromuscular junctions, based on the scheme (20) reviewed 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.

The latter model of Pan and Zucker (2009) is a more detailed example 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 build a comprehensive model of short-term changes in synaptic transmission strength in response to repeated stimulation. Such modeling work is reviewed in more detail in the entry "► Facilitation, Biophysical Models."

## Spatial Coupling Between Vesicle and $Ca^{2+}$ Channels

Building a comprehensive model of $Ca^{2+}$-dependent exocytosis at a particular class of synaptic terminal requires accurate description of $Ca^{2+}$ dynamics at the release site, which in turn requires taking into account the latest data on the spatial coupling between voltage-dependent $Ca^{2+}$ channels and synaptic vesicles (Eggermann et al. 2012; Gentile and Stanley 2005; Moser et al. 2006; Oheim et al. 2006; Stanley 1997). The geometric arrangement of channels and vesicles at the release site has been recently a subject of intensive modeling effort, using both stochastic simulation of $Ca^{2+}$ diffusion and buffering (Bennett et al. 2004; Glavinovic and Rabie 2001; Nadkarni et al. 2012; Scimemi and Diamond 2012; Shahrezaei et al. 2006; Shahrezaei and Delaney 2005) as well as deterministic solution of continuous reaction–diffusion equations (Bucurenciu et al. 2008; Ermolyuk et al. 2013; Matveev et al. 2009, 2011; Meinrenken et al. 2002, 2003; Schmidt et al. 2013; Zucker and Fogelson 1986) and steady-state approximations of $Ca^{2+}$ distribution near an array of open $Ca^{2+}$ channels (Bertram et al. 1999; Coggins and Zenisek 2009).

## References

Barg S, Ma X, Eliasson L, Galvanovskis J, Gopel SO, Obermuller S, Platzer J, Renstrom E, Trus M, Atlas D, Striessnig J, Rorsman P (2001) Fast exocytosis with few Ca($^{2+}$) channels in insulin-secreting mouse pancreatic B cells. Biophys J 81:3308–3323

Bennett MR, Farnell L, Gibson WG (2004) The facilitated probability of quantal secretion within an array of calcium channels of an active zone at the amphibian neuromuscular junction. Biophys J 86:2674–2690

Bertram R, Sherman A, Stanley EF (1996) Single-domain/bound calcium hypothesis of transmitter release and facilitation. J Neurophysiol 75:1919–1931

Bertram R, Smith GD, Sherman A (1999) Modeling study of the effects of overlapping $Ca^{2+}$ microdomains on neurotransmitter release. Biophys J 76:735–750

Bollmann JH, Sakmann B (2005) Control of synaptic strength and timing by the release-site $Ca^{2+}$ signal. Nat Neurosci 8:426–434

Bollmann JH, Sakmann B, Borst JG (2000) Calcium sensitivity of glutamate release in a calyx-type terminal. Science 289:953–957

Bornschein G, Arendt O, Hallermann S, Brachtendorf S, Eilers J, Schmidt H (2013) Paired-pulse facilitation at recurrent Purkinje neuron synapses is independent of calbindin and parvalbumin during high-frequency activation. J Physiol 591:3355–3370

Bucurenciu I, Kulik A, Schwaller B, Frotscher M, Jonas P (2008) Nanodomain coupling between $Ca^{2+}$ channels and $Ca^{2+}$ sensors promotes fast and efficient transmitter release at a cortical GABAergic synapse. Neuron 57:536–545

Chapman ER (2002) Synaptotagmin: a Ca($^{2+}$) sensor that triggers exocytosis? Nat Rev Mol Cell Biol 3:498–508

Chen YD, Wang S, Sherman A (2008) Identifying the targets of the amplifying pathway for insulin secretion in pancreatic beta-cells by kinetic modeling of granule exocytosis. Biophys J 95:2226–2241

Cho S, von Gersdorff H (2012) Ca($^{2+}$) influx and neurotransmitter release at ribbon synapses. Cell Calcium 52:208–216

Chow RH, Lv R, Neher E (1992) Delay in vesicle fusion revealed by electrochemical monitoring of single secretory events in adrenal chromaffin cells. Nature 356:60–63

Chung C, Raingo J (2013) Vesicle dynamics: how synaptic proteins regulate different modes of neurotransmission. J Neurochem 126:146–154

Coggins M, Zenisek D (2009) Evidence that exocytosis is driven by calcium entry through multiple calcium channels in goldfish retinal bipolar cells. J Neurophysiol 101:2601–2619

Dittman JS, Regehr WG (1998) Calcium dependence and recovery kinetics of presynaptic depression at the climbing fiber to Purkinje cell synapse. J Neurosci 18:6147–6162

Dittman JS, Kreitzer AC, Regehr WG (2000) Interplay between facilitation, depression, and residual calcium at three presynaptic terminals. J Neurosci 20:1374–1385

Dittrich M, Pattillo JM, King JD, Cho S, Stiles JR, Meriney SD (2013) An excess-calcium-binding-site model predicts neurotransmitter release at the neuromuscular junction. Biophys J 104:2751–2763

Dodge FA, Rahamimoff R (1967) Cooperative action of calcium ions in transmitter release at the neuromuscular junction. J Physiol 193:419–432

Eggermann E, Bucurenciu I, Goswami SP, Jonas P (2012) Nanodomain coupling between Ca(2)(+) channels and sensors of exocytosis at fast mammalian synapses. Nat Rev Neurosci 13:7–21

Ermolyuk YS, Adler FG, Surges R, Pavlov IY, Timofeeva Y, Kullman DM, Volynski KE (2013) Differential triggering of spontaneous glutamate release by P/Q-, N- and R-type $Ca^{2+}$ channels. Nature Neurosci 16:1754–1763

Felmy F, Neher E, Schneggenburger R (2003) Probing the intracellular calcium sensitivity of transmitter release during synaptic facilitation. Neuron 37:801–811

Gentile L, Stanley EF (2005) A unified model of presynaptic release site gating by calcium channel domains. Eur J Neurosci 21:278–282

Glavinovic MI, Rabie HR (2001) Monte Carlo evaluation of quantal analysis in the light of $Ca^{2+}$ dynamics and the geometry of secretion. Pflugers Arch 443:132–145

Han X, Wang CT, Bai J, Chapman ER, Jackson MB (2004) Transmembrane segments of syntaxin line the fusion pore of $Ca^{2+}$-triggered exocytosis. Science 304:289–292

Heidelberger R, Heinemann C, Neher E, Matthews G (1994) Calcium dependence of the rate of exocytosis in a synaptic terminal. Nature 371:513–515

Heil P, Neubauer H (2010) Summing across different active zones can explain the quasi-linear Ca-dependencies of exocytosis by receptor cells. Front Synaptic Neurosci 2:148

Heinemann C, von Ruden L, Chow RH, Neher E (1993) A two-step model of secretion control in neuroendocrine cells. Pflugers Arch 424:105–112

Hosoi N, Sakaba T, Neher E (2007) Quantitative analysis of calcium-dependent vesicle recruitment and its functional role at the calyx of Held synapse. J Neurosci 27:14286–14298

Jahn R, Fasshauer D (2012) Molecular machines governing exocytosis of synaptic vesicles. Nature 490:201–207

Johnson SL, Franz C, Kuhn S, Furness DN, Ruttiger L, Munkner S, Rivolta MN, Seward EP, Herschman HR, Engel J, Knipper M, Marcotti W (2010) Synaptotagmin IV determines the linear $Ca^{2+}$ dependence of vesicle fusion at auditory ribbon synapses. Nat Neurosci 13:45–52

Kaeser PS, Regehr WG (2014) Molecular mechanisms for synchronous, asynchronous, and spontaneous neurotransmitter release. Annu Rev Physiol 76:333–363

Lee JS, Ho WK, Neher E, Lee SH (2013) Superpriming of synaptic vesicles after their recruitment to the readily releasable pool. Proc Natl Acad Sci USA 110:15079–15084

Lou X, Scheuss V, Schneggenburger R (2005) Allosteric modulation of the presynaptic $Ca^{2+}$ sensor for vesicle fusion. Nature 435:497–501

Matveev V, Bertram R, Sherman A (2006) Residual bound $Ca^{2+}$ can account for the effects of $Ca^{2+}$ buffers on synaptic facilitation. J Neurophysiol 96:3389–3397

Matveev V, Bertram R, Sherman A (2009) $Ca^{2+}$ current versus $Ca^{2+}$ channel cooperativity of exocytosis. J Neurosci 29:12196–12209

Matveev V, Bertram R, Sherman A (2011) Calcium cooperativity of exocytosis as a measure of Ca (2)+ channel domain overlap. Brain Res 1398:126–138

Meinrenken CJ, Borst JG, Sakmann B (2002) Calcium secretion coupling at calyx of held governed by nonuniform channel-vesicle topography. J Neurosci 22:1648–1667

Meinrenken CJ, Borst JG, Sakmann B (2003) Local routes revisited: the space and time dependence of the $Ca^{2+}$ signal for phasic transmitter release at the rat calyx of Held. J Physiol 547:665–689

Millar AG, Zucker RS, Ellis-Davies GC, Charlton MP, Atwood HL (2005) Calcium sensitivity of neurotransmitter release differs at phasic and tonic synapses. J Neurosci 25:3113–3125

Moser T, Neef A, Khimich D (2006) Mechanisms underlying the temporal precision of sound coding at the inner hair cell ribbon synapse. J Physiol 576:55–62

Mutch SA, Kensel-Hammes P, Gadd JC, Fujimoto BS, Allen RW, Schiro PG, Lorenz RM, Kuyper CL, Kuo JS, Bajjalieh SM, Chiu DT (2011) Protein quantification at the single vesicle level reveals that a subset of synaptic vesicle proteins are trafficked with high precision. J Neurosci 31:1461–1470

Nadkarni S, Bartol TM, Stevens CF, Sejnowski TJ, Levine H (2012) Short-term plasticity constrains spatial organization of a hippocampal presynaptic terminal. Proc Natl Acad Sci USA 109:14657–14662

Neher E (2012) Introduction: regulated exocytosis. Cell Calcium 52:196–198

Neher E, Sakaba T (2008) Multiple roles of calcium ions in the regulation of neurotransmitter release. Neuron 59:861–872

Nouvian R, Neef J, Bulankina AV, Reisinger E, Pangrsic T, Frank T, Sikorra S, Brose N, Binz T, Moser T (2011) Exocytosis at the hair cell ribbon synapse apparently operates without neuronal SNARE proteins. Nat Neurosci 14:411–413

Oheim M, Kirchhoff F, Stuhmer W (2006) Calcium microdomains in regulated exocytosis. Cell Calcium 40:423–439

Pan B, Zucker RS (2009) A general model of synaptic transmission and short-term plasticity. Neuron 62:539–554

Pangrsic T, Reisinger E, Moser T (2012) Otoferlin: a multi-C2 domain protein essential for hearing. Trends Neurosci 35:671–680

Pedersen MG, Sherman A (2009) Newcomer insulin secretory granules as a highly calcium-sensitive pool. Proc Natl Acad Sci USA 106:7432–7436

Quastel DM, Guan YY, Saint DA (1992) The relation between transmitter release and $Ca^{2+}$ entry at the mouse motor nerve terminal: role of stochastic factors causing heterogeneity. Neuroscience 51:657–671

Raingo J, Khvotchev M, Liu P, Darios F, Li YC, Ramirez DM, Adachi M, Lemieux P, Toth K, Davletov B, Kavalali ET (2012) VAMP4 directs synaptic vesicles to a pool that selectively maintains asynchronous neurotransmission. Nat Neurosci 15:738–745

Roux I, Safieddine S, Nouvian R, Grati M, Simmler MC, Bahloul A, Perfettini I, Le Gall M, Rostaing P, Hamard G, Triller A, Avan P, Moser T, Petit C (2006) Otoferlin, defective in a human deafness form, is essential for exocytosis at the auditory ribbon synapse. Cell 127:277–289

Sakaba T (2008) Two Ca(2+)-dependent steps controlling synaptic vesicle fusion and replenishment at the cerebellar basket cell terminal. Neuron 57:406–419

Schmidt H, Brachtendorf S, Arendt O, Hallermann S, Ishiyama S, Bornschein G, Gall D, Schiffmann SN, Heckmann M, Eilers J (2013) Nanodomain coupling at an excitatory cortical synapse. Curr Biol 23:244–249

Schneggenburger R, Neher E (2000) Intracellular calcium dependence of transmitter release rates at a fast central synapse. Nature 406:889–893

Scimemi A, Diamond JS (2012) The number and organization of $Ca^{2+}$ channels in the active zone shapes neurotransmitter release from Schaffer collateral synapses. J Neurosci 32:18157–18176

Shahrezaei V, Delaney KR (2005) Brevity of the $Ca^{2+}$ microdomain and active zone geometry prevent $Ca^{2+}$-sensor saturation for neurotransmitter release. J Neurophysiol 94:1912–1919

Shahrezaei V, Cao A, Delaney KR (2006) $Ca^{2+}$ from one or two channels controls fusion of a single vesicle at the frog neuromuscular junction. J Neurosci 26:13240–13249

Smith SM, Chen W, Vyleta NP, Williams C, Lee CH, Phillips C, Andresen MC (2012) Calcium regulation of spontaneous and asynchronous neurotransmitter release. Cell Calcium 52:226–233

Sorensen JB (2004) Formation, stabilisation and fusion of the readily releasable pool of secretory vesicles. Pflugers Arch 448:347–362

Stanley EF (1997) The calcium channel and the organization of the presynaptic transmitter release face. Trends Neurosci 20:404–409

Sterling P, Matthews G (2005) Structure and function of ribbon synapses. Trends Neurosci 28:20–29

Stevens CF, Wesseling JF (1998) Activity-dependent modulation of the rate at which synaptic vesicles become available to undergo exocytosis. Neuron 21:415–424

Sun J, Pang ZP, Qin D, Fahim AT, Adachi R, Sudhof TC (2007) A dual-$Ca^{2+}$-sensor model for neurotransmitter release in a central synapse. Nature 450:676–682

Thoreson WB, Rabl K, Townes-Anderson E, Heidelberger R (2004) A highly $Ca^{2+}$-sensitive pool of vesicles contributes to linearity at the rod photoreceptor ribbon synapse. Neuron 42:595–605

Verhage M, Toonen RF (2007) Regulated exocytosis: merging ideas on fusing membranes. Curr Opin Cell Biol 19:402–408

Voets T (2000) Dissection of three $Ca^{2+}$-dependent steps leading to secretion in chromaffin cells from mouse adrenal slices. Neuron 28:537–545

Voets T, Neher E, Moser T (1999) Mechanisms underlying phasic and sustained secretion in chromaffin cells from mouse adrenal slices. Neuron 23:607–615

von Ruden L, Neher E (1993) A Ca-dependent early step in the release of catecholamines from adrenal chromaffin cells. Science 262:1061–1065

Wadel K, Neher E, Sakaba T (2007) The coupling between synaptic vesicles and $Ca^{2+}$ channels determines fast neurotransmitter release. Neuron 53:563–575

Wang LY, Kaczmarek LK (1998) High-frequency firing helps replenish the readily releasable pool of synaptic vesicles. Nature 394:384–388

Weiss JN (1997) The Hill equation revisited: uses and misuses. FASEB J 11:835–841
Wolfel M, Schneggenburger R (2003) Presynaptic capacitance measurements and $Ca^{2+}$ uncaging reveal submillisecond exocytosis kinetics and characterize the $Ca^{2+}$ sensitivity of vesicle pool depletion at a fast CNS synapse. J Neurosci 23:7059–7068
Wolfel M, Lou X, Schneggenburger R (2007) A mechanism intrinsic to the vesicle fusion machinery determines fast and slow transmitter release at a large CNS synapse. J Neurosci 27:3198–3210
Worden MK, Bykhovskaia M, Hackett JT (1997) Facilitation at the lobster neuromuscular junction: a stimulus-dependent mobilization model. J Neurophysiol 78:417–428
Wu MM, Llobet A, Lagnado L (2009) Loose coupling between calcium channels and sites of exocytosis in chromaffin cells. J Physiol 587:5377–5391
Yamada MW, Zucker RS (1992) Time course of transmitter release calculated from stimulations of a calcium diffusion model. Biophys J 61:671–682
Yang Y, Gillis KD (2004) A highly $Ca^{2+}$-sensitive pool of granules is regulated by glucose and protein kinases in insulin-secreting INS-1 cells. J Gen Physiol 124:641–651
Yang Y, Udayasankar S, Dunning J, Chen P, Gillis KD (2002) A highly $Ca^{2+}$-sensitive pool of vesicles is regulated by protein kinase C in adrenal chromaffin cells. Proc Natl Acad Sci USA 99:17060–17065
Yao J, Gaffaney JD, Kwon SE, Chapman ER (2011) Doc2 is a $Ca^{2+}$ sensor required for asynchronous neurotransmitter release. Cell 147:666–677
Zucker RS, Fogelson AL (1986) Relationship between transmitter release and presynaptic calcium influx when calcium enters through discrete channels. Proc Natl Acad Sci USA 83:3032–3036
Zucker RS, Regehr WG (2002) Short-term synaptic plasticity. Annu Rev Physiol 64:355–405