# Morphofunctional changes at the active zone during synaptic vesicle exocytosis

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## **Abstract**

The fusion of synaptic vesicles (SVs) with the plasma membrane (PM) proceeds through intermediate steps that remain poorly resolved. Additionally, the effect of persistent high or low exocytosis activity on intermediate steps remains unknown. Through time-resolved cryo-electron tomography, we ordered events into a sequence. Following stimulation, additional SVs are rapidly primed by forming tethers with the PM. Simultaneously, fusion initiation occurs by membrane curvature ('buckling') of the SV and PM. It is followed by the formation of a fusion pore, and the collapse of SV membrane. At this time, membrane-proximal, but not membrane-distal, vesicles loose their interconnections, allowing them to move towards the PM. A SNARE mutation that arrests spontaneous release caused vesicles to reside further from the membrane while forming more tethers, whereas a mutation stimulating spontaneous fusion caused a loss of membrane-proximal SVs with more than two tethers, and loss of intervesicle connectors. Overall, tether formation and connector dissolution is triggered by stimulation and adjusted to the spontaneous fusion rate.

- Need to reformulate the part about 4E mutant
- For 4K mutant: is the loss of tethered SVs restricted to those with more than only one, or to those with more than two tethers?
- Possibly introduce the formation of distal connectors by stimulation

## Introduction

In the central nervous system, neurons communicate through the release of neurotransmitters at synapses. This process relies on synaptic vesicle (SV) exocytosis, i.e. the fusion of SVs with the plasma membrane (PM). This in turn is eminently important for normal brain function such as movement coordination or memory formation. SV exocytosis involves a sequence of steps [1,2]. The vesicle is first docked to the active zone (AZ) PM. Subsequently the exocytosis machinery goes through a maturation process, termed priming, after which the SV is ready to fuse. These SVs form the readily releasable pool (RRP) of SVs. Finally, a calcium influx triggers fusion of the SV with the PM. Docked SVs are defined as the SVs in very close proximity or direct contact with the PM as observed by electron microscopy (EM), whereas priming refers to SV ability to undergo exocytosis immediately upon stimulation. Whether every docked SV is also primed has been debated [1,3]. A recent high-pressure freezing/freeze-substitution EM study of genetically modified synapses has indicated that vesicles that are in direct contact with the PM, i.e. docked, are primed and belong to the RRP and that this situation occurs downstream of vesicle tethering [4]. From a molecular perspective, priming involves several proteins including the SNARE complex (SNAP-25, syntaxin-1, and synaptobrevin-2), Munc13, Munc18, synaptotagmin-1, and complexin [2,5]. All three SNAREs form a highly stable tight four-helix bundle, known as trans-SNARE complex, which brings the SV and the PM [6]. Evidence has suggested that the SNARE complex is only partially zipped in primed SVs [doi:0.1038/sj.emboj.7601003?]. Furthermore, various studies have suggested that at least three SNARE complexes are required for SV to be fusioncompetent [7,8,9]. Synaptotagmin-1 is localized on the SV and was proposed to interact with anionic phospholipids and the t-SNAREs upon Ca<sup>2+</sup>-binding, possibly triggering membrane curvature, followed by a proposed hemifusion step and subsequent fusion[10]. CHARGES ON THE SNARE

Cryo-electron tomography (cryo-ET), which preserves samples to atomic resolution, revealed a somewhat different view, where, under resting conditions, no SV is in direct contact with the PM [11,12]. Furthermore, cryo-EM has shown that the majority of AZ-proximal SVs are connected to the AZ PM by short filaments, termed tethers.

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Italic text

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## Strikethrough

- 1. Ordered list item
- 2. Ordered list item
  - a. Sub-item
  - b. Sub-item
    - i. Sub-sub-item
- 3. Ordered list item
  - a. Sub-item
- List item
- List item
- List item

subscript: H<sub>2</sub>O is a liquid

superscript: 2<sup>10</sup> is 1024.

unicode superscripts<sup>0123456789</sup>

unicode subscripts<sub>0123456789</sub>

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Line break without starting a new paragraph by putting two spaces at end of line.

## **Document organization**

Document section headings:

## **Heading 1**

## **Heading 2**

**Heading 3** 

**Heading 4** 

**Heading 5** 

**Heading 6** 



#### Horizontal rule:

Heading 1's are recommended to be reserved for the title of the manuscript.

Heading 2's are recommended for broad sections such as Abstract, Methods, Conclusion, etc.

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## Referencing figures, tables, equations

Figure 1

Figure 2

```
Figure 3

Figure 4

Table 1

Equation 1

Equation 2
```

## **Quotes and code**

Quoted text

Quoted block of text

Two roads diverged in a wood, and I—I took the one less traveled by, And that has made all the difference.

Code in the middle of normal text, aka inline code.

Code block with Python syntax highlighting:

```
from manubot.cite.doi import expand_short_doi

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    doi = expand_short_doi("10/c3bp")
    # a string too long to fit within page:
    assert doi == "10.25313/2524-2695-2018-3-vliyanie-enhansera-copia-i-
        insulyatora-gypsy-na-sintez-ernk-modifikatsii-hromatina-i-
        svyazyvanie-insulyatornyh-belkov-vtransfetsirovannyh-geneticheskih-
        konstruktsiyah"
```

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```
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## **Figures**



**Figure 1:** A square image at actual size and with a bottom caption. Loaded from the latest version of image on GitHub.



**Figure 2:** An image too wide to fit within page at full size. Loaded from a specific (hashed) version of the image on GitHub.



Figure 3: A tall image with a specified height. Loaded from a specific (hashed) version of the image on GitHub.



**Figure 4:** A vector .svg image loaded from GitHub. The parameter sanitize=true is necessary to properly load SVGs hosted via GitHub URLs. White background specified to serve as a backdrop for transparent sections of the image.

## **Tables**

**Table 1:** A table with a top caption and specified relative column widths.

Bowling Scores	Jane	John	Alice	Bob
Game 1	150	187	210	105
Game 2	98	202	197	102
Game 3	123	180	238	134

**Table 2:** A table too wide to fit within page.

		Digits 1-33	Digits 34-66	Digits 67-99	Ref.
þ	oi	3.14159265358979323 846264338327950	28841971693993751 0582097494459230	78164062862089986 2803482534211706	piday.org
E	<u>)</u>	2.71828182845904523 536028747135266	24977572470936999 5957496696762772	40766303535475945 7138217852516642	nasa.gov

 Table 3: A table with merged cells using the attributes plugin.

	Colors	
Size	Text Color	Background Color
big	blue	orange
small	black	white

## **Equations**

A LaTeX equation:

$$\int_0^\infty e^{-x^2} dx = \frac{\sqrt{\pi}}{2} \tag{1}$$

An equation too long to fit within page:

$$x = a + b + c + d + e + f + g + h + i + j + k + l + m + n + o + p + q + r + s + t + u + v + w + x + y + z + 1 + 2 + 3 + 4 + 5 + 6 + 7 + 8 + 9$$
(2)

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