Review of: "Visualizing sequential compound fusion and kiss-and-run in live excitable cells"

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In this manuscript, Ge, Shin, and Wu examine exocytosis in chromaffin cells to quantitatively explore compound fusion events, using STED microscopy to image plasma membrane, and either a Atto532 added to media or fluorescently labeled neurotransmitter to mark Ω structures. They demonstrate that the vast majority of fusion evens are single fusion events that occur at the plasma membrane, but that around 24% of fusion evens are sequential compound. They then go on to more carefully inspect the compound fusion events, and characterize them as stay (open), close, or dilate. These are novel characterization of compound fusion events. Examination of the difference in fluorescence decay between the first and sequential compound events, and the onset of first and second fusions provides kinetic information, that leads the authors to suggest that fluorescence decay is similar, but a lag in fusion of the compound event may involve diffusion and availability of tSNARE. Overall this a nice contribution describing novel behaviors of compound exocytosis in primary cells.

Experiments were performed at room temperature, even though it is a primary bovine cell. Authors are encouraged to determine if physiological temperature alters the kinetics or distribution of fusion modes. For example, it is surprising that only 336 fusion events were visualized in 1211 cells.

The authors are encouraged to clarify the imaging paradigm, which is confusing as written. For example, does the voltage clamp synchronize and increase the frequency of fusion? And the range of time 26-300 ms I think you mean that is the timelapse window, but then say single image frame, please clarify this “To provide more conclusive proof and to characterize Fusionseq-comp, STED PHG/A532 imaging data were collected from a large number of cells, 1211 cells, at the voltage-clamp configuration. A total of 336 PHG-labelled □-shape profiles filled with A532 (□PH) appeared within a single image frame (26-300□ms), reflecting vesicle fusion that allowed for PHG/A532 diffusion from PM/bath into the fusion-generated □-profile (Fig.□1c-f).”

Similar to Figure 1f, authors are encouraged to plot the overall distributrion of fusion modes for the FFN in Figure 1g-j. Is this similar to the atto532?

Demonstrating the timecourse of signal photobleaching would be helpful in confirming the definition categorization of close fusion. In Close Fusion (Figure 2B), that no longer has fluorescent A532 in it, what is the time course of bleaching?

“The 20-80% FFN511 fluorescence decay time was similar between the 2nd and the 1st □PH during Fusionseq-comp” is confusing. Does this mean the time it takes to go from 20% bleached to 80% bleached. And why were these numbers chosen, why not 0-80?

Some of the data did not appear normally distributed IFigure 3C, yet a mean was reported. A median may be better, and nonparametric statistical tests.

Although potentially outside the context of a short report characterizing compound fusion, authors are encouraged to test their hypothesis about tSNARE availability. For example, would over expression of tSNAREs decrease the lag in onset time between first and 2nd fusion?