

Featured Publication Note

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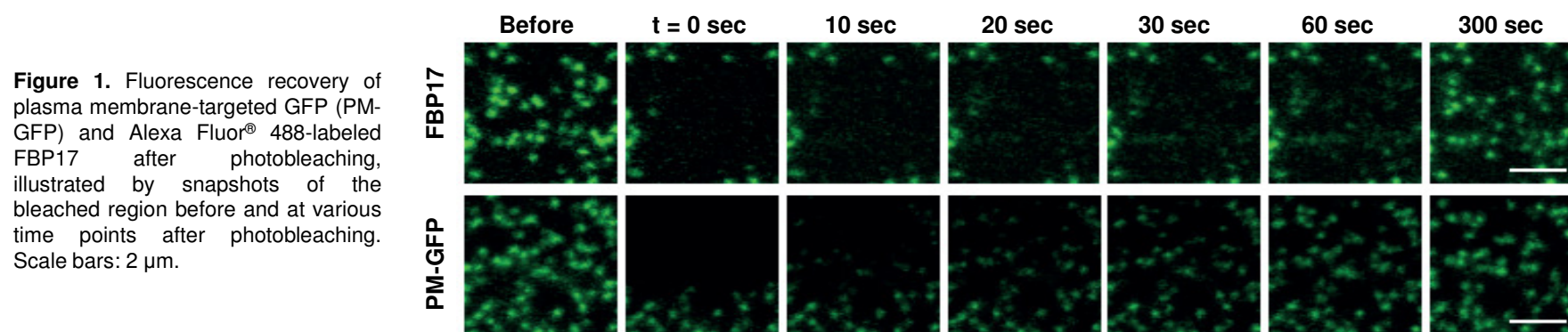


UltraVIEW VoX FRAP studies offer new insights into endocytosis

In clathrin-mediated endocytosis, a clathrin-coated pit is formed at the plasma membrane which then invaginates and undergoes fission (pinching off) to form a coated vesicle. In this recent study, published in *Nature Cell Biology*, researchers have gained new insights into the mechanism of endocytosis by reconstituting vesicle budding and fission from isolated plasma membrane sheets and imaging these events.

Using a combination of electron and fluorescence microscopy, researchers revealed that when fission was blocked, deep tubular plasma membrane invaginations were present which were nucleated by clathrin-coated buds. These tubules were shown to be surrounded by a stabilizing scaffold containing an F-BAR (FBP17) domain, the assembly of which is thought to be facilitated by actin. The researchers proposed that intermolecular interactions within this scaffold may contribute to tubule stability after actin disassembly. This was supported by Fluorescence Recovery after Photobleaching (FRAP) experiments which were performed to investigate the turnover rate of FBP17 on tubules.

FRAP was performed on an **UltraVIEW® VoX** spinning disk confocal microscope controlled by **Volocity®** software. A region of interest (50 x 50 pixels, 14 μm^2) was chosen on the plasma membrane sheets and this region was bleached for 100 cycles. Images were acquired at 10 sec intervals and at least 10 frames were acquired before bleaching (Figure 1). Quantification of FRAP was done by calculating the average intensity of the bleached region, which was corrected with background and normalized with the average intensity of a neighboring non-bleached fluorescent region to account for global intensity changes. The subsequent recovery curve was fitted with the Soumpasis equation, one of the FRAP analytical equations offered by **Volocity**. The results revealed a slow turnover rate of FBP17 on the tubules.



Researchers also found that triggering fission led not only to the separation of the clathrin-coated buds, but also to vesicle formation by fragmentation of the FBP17-coated tubules. Together, these results suggested a functional link between FBP17-dependent membrane tubulation and clathrin-dependent budding.