APPLICATION NOTE

N-STORM Super Resolution Microscope

Super-resolution imaging of double-stained neurons using DNA-Paint

Dr. Noriko Koganezawa (Life Science), Department of Neurobiology and Behavior, Graduate School of Medicine, Gunma University, focuses her research on drebrin, an actin-binding protein that is thought to play an important role in cognitive function. Drebrin accumulates in the posterior synaptic region (dendritic spine) of mature neurons, but when synaptic dysfunction occurs, this accumulation disappears. Also, when the NMDA receptor is activated by glutamate or the like, the accumulation of drebrin is transiently eliminated (Figs.1 and 2). Focusing on the dynamics of drebrin, Dr. Koganezawa is conducting research to verify its effects on cognitive function.

In this Application Note, we introduce examples of image acquisition of changes in the localization of drebrin by glutamate stimulation using the N-STORM super-resolution microscope and quantitative evaluation by cluster analysis.

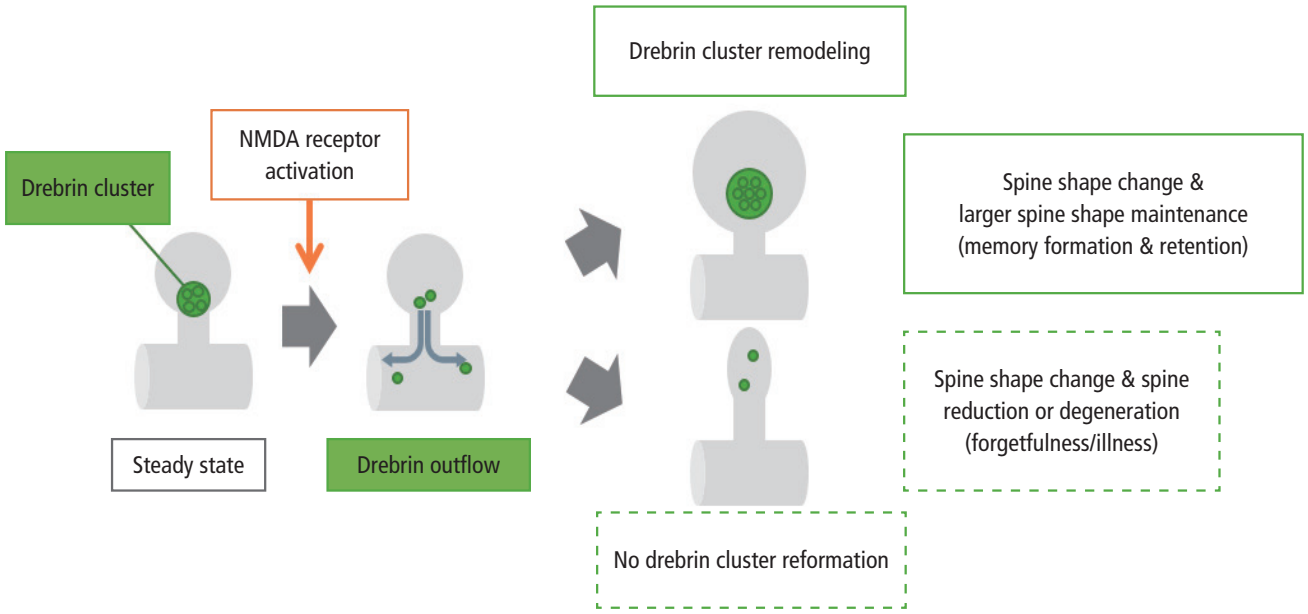

Figure 1. A model of spine shape change caused by drebrin dynamics

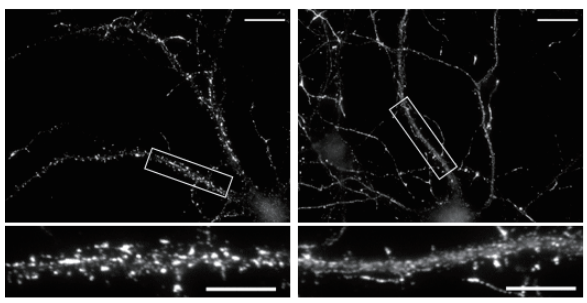

Figure 2. Disappearance of drebrin clusters due to glutamate stimulation
Fluorescent images of immunostained drebrin of DIV 14 rat hippocampal neurons. Upper: neurons (scale bar: 20μm), bottom: dendrites (scale bar: 10μm)

Outline of the experiment

Neurons were double-stained using DNA-Paint (Massive Photonics GmbH, Germany), and dendrites (using anti-MAP2 antibodies) and drebrin were observed. The localization of drebrin with and without glutamate stimulation was observed with the N-STORM super-resolution microscope, and quantitatively evaluated by cluster analysis.

Results

In untreated cells, drebrin was shown to be distributed along the dendrites and accumulated within the dendritic spines, as observed with conventional fluorescence microscopy. On the other hand, in cells stimulated with NMDA receptors by adding glutamate (100 µM, 10 minutes), drebrin was observed to be distributed on the dendrites. This suggests that glutamate stimulation changed the localization of drebrin from the dendritic spines to the dendrites (Fig. 3).
In addition, cluster analysis revealed that glutamate-stimulated cells form smaller clusters of drebrin than untreated cells (Fig. 4).

Summary

By observation with double staining, it was possible to visualize whether drebrin is localized on the dendrites or in the surrounding area with single molecule level accuracy. In addition to observing the localization of drebrin with and without glutamate stimulation, quantitative evaluation of differences in distribution was possible.
STORM observation is a method of controlling the blinking phenomenon of fluorescent molecules when acquiring images, but since the fluorescent dyes that efficiently cause the blinking phenomenon are limited, selection of dyes is one of the issues in multicolor STORM observation. By using DNA-Paint, it is possible to capture the correlation between two types of molecules and to perform imaging without photobleaching.

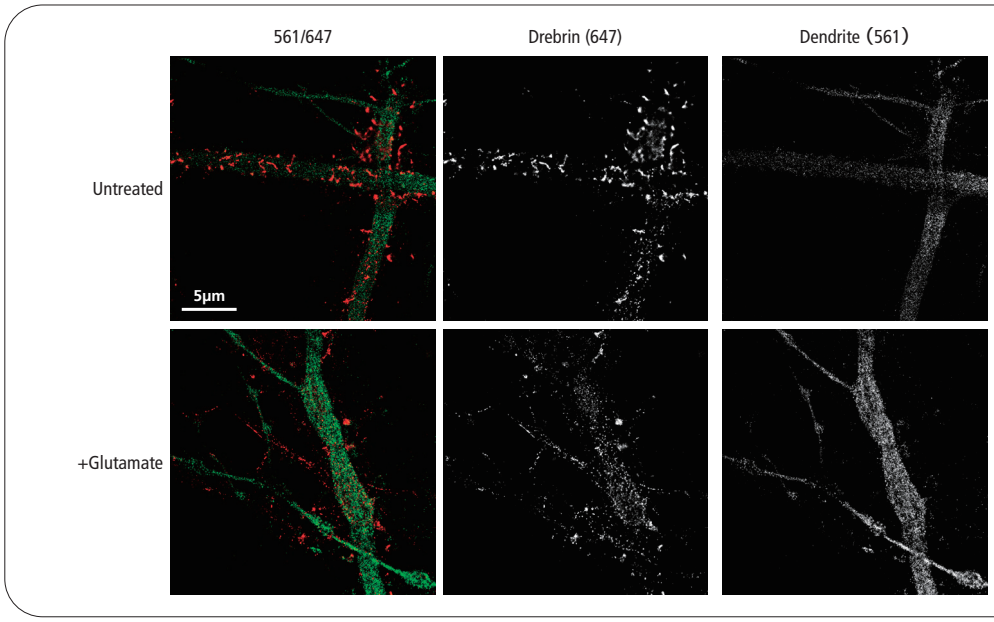

Figure 3. Dynamic changes in the localization of drebrin by glutamate stimulation
Drebrin and dendrites of rat hippocampal-derived primary cultured neurons were stained using DNA-Paint immunostaining kit and captured with 2D-STORM.
(left) merged image, (center) drebrin acquired with ex. 647nm, primary antibody: anti-drebrin antibody, secondary antibody: anti-mouse AB+docking site, imager: ATTO655, (right) dendrites acquired with ex. 561nm, primary antibody: anti-MAP2 antibody, secondary antibody: anti-rabbit AB+docking site, imager: Cy3B
Objective: CFI SR HP Apochromat TIRF 100XC Oil
Images courtesy of Dr. Noriko Koganezawa, Graduate School of Medicine, Gunma University

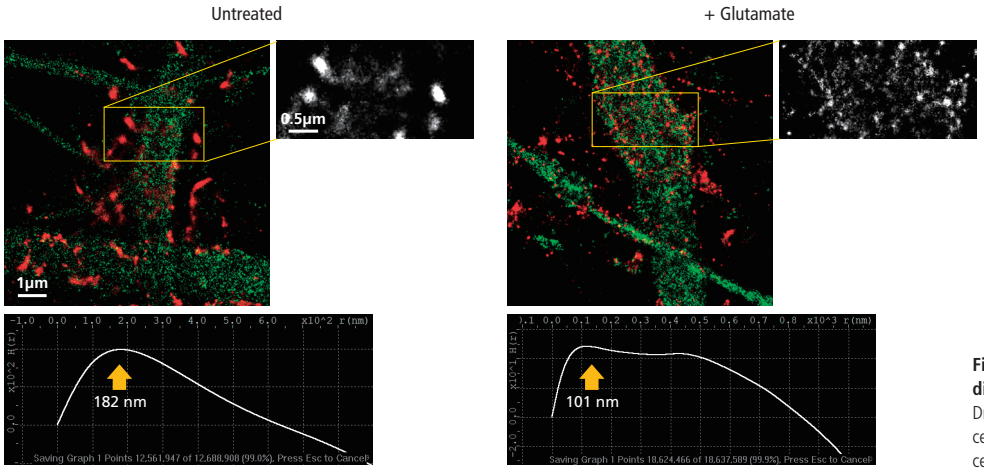

Figure 4. Quantification of drebrin distribution by cluster analysis
Drebrin forms clusters (r = 182 nm) in untreated cells, and forms smaller clusters (r = 101 nm) in cells supplemented with glutamate.

Product Information

N-STORM Super Resolution Microscope

The N-STORM utilizes a localization technique called STochastic Optical Reconstruction Microscopy (STORM) to achieve a resolution of ten times that of conventional light microscopes. It allows imaging of the structure of cell organelles on a molecular level.

- Lateral resolution: Approx. 20 nm
- Axial resolution: Approx. 50 nm

Cluster Analysis Function

Allows automatic analysis of the distribution patterns of fluorescent spots by specifying the target area with the ROI in the image captured by the super-resolution microscope.
With quantitative evaluation added to image acquisition of nanoscale accuracy, the reliability of the information obtained from images is significantly increased.