<Title>

Dissection of local Ca^{2+} signals inside cytosol by ER-targeted Ca^{2+} indicator

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Fumihiko Niwa^{1,5}, Shigeo Sakuragi^{2,6}, Ayana Kobayashi², Shin Takagi², Yoichi Oda², Hiroko Bannai^{1, 2, 3, 4 *}, Katsuhiko Mikoshiba^{1, *}

<Affiliations>

1. Laboratory for Developmental Neurobiology, RIKEN Brain Science Institute (BSI), 2-1 Hirosawa, Wako, Saitama 351-0198, Japan

2. Division of Biological Science, Nagoya University, Furo-cho, Chikusa, Nagoya, Aichi, 464-8602, Japan

3. Nagoya Research Center for Brain & Neural Circuits, Nagoya University, Furo-cho, Chikusa, Nagoya, Aichi, 464-8602, Japan

4. Japan Science and Technology Agency, PRESTO, 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, Japan

Current affiliation:

5. École Normale Supérieure, Institut de Biologie de l'ENS (IBENS), INSERM, CNRS, École Normale Supérieure, PSL Research University, 46 rue d'Ulm, 75005 Paris, France

6. Graduate School of Life Sciences, Tohoku University, Sendai, Miyagi, 980-8577, Japan

*corresponding author hbannai@brain.riken.jp [H.B.] mikosiba@brain.riken.jp [K.M.]

<Abstract>

Calcium (Ca^{2+}) is a versatile intracellular second messenger that operates in various signaling pathways leading to multiple biological outputs. The diversity of spatiotemporal patterns of Ca^{2+} signals, generated by the coordination of Ca^{2+} influx from the extracellular space and Ca^{2+} release from the intracellular Ca^{2+} store the endoplasmic reticulum (ER), is considered to underlie the diversity of biological outputs caused by a single signaling molecule. However, such Ca^{2+} signaling diversity has not been well described because of technical limitations. Here, we describe a new method to report Ca^{2+} signals at subcellular resolution. We report that OER-GCaMP6f, a genetically encoded Ca^{2+} indicator (GECI) targeted to the outer ER membrane, can monitor Ca^{2+} release from the ER at higher spatiotemporal resolution than conventional GCaMP6f. OER-GCaMP6f was used for *in vivo* Ca^{2+} imaging of *C. elegans*. We also found that the spontaneous Ca^{2+} elevation in cultured astrocytes reported by OER-GCaMP6f showed a distinct spatiotemporal pattern from that monitored by plasma membrane-targeted GCaMP6f (Lck-GCaMP6f); less frequent Ca^{2+} signal was detected by OER-GCaMP6f, in spite of the fact that Ca^{2+} release from the ER plays important roles in astrocytes. These findings suggest that targeting of GECIs to the ER outer membrane enables sensitive detection of Ca^{2+} release from the ER at subcellular resolution, avoiding the diffusion of GECI and Ca^{2+} . Our results indicate that Ca^{2+} imaging with OER-GCaMP6f in combination with Lck-GCaMP6f can contribute to describing the diversity of Ca^{2+} signals, by enabling dissection of Ca^{2+} signals at subcellular resolution.

<Highlights>

- An outer ER membrane-targeted Ca^{2+} indicator (OER-GCaMP6f) was designed.
- Cytosolic and ER- or membrane-targeted indicators exhibited distinct Ca^{2+} dynamics.
- Astrocytic ER-driven Ca^{2+} signals were observed less frequently than Ca^{2+} influx.

<keywords>

Local Ca^{2+} ; Genetically encoded Ca^{2+} indicator; Endoplasmic Reticulum; astrocyte; *C. elegans*

<abbreviations>

ER: Endoplasmic Reticulum

GABA_A receptor: gamma amino-butylic acid receptor type A

IP₃: Inositol triphosphate

GEI: Genetically encoded Ca^{2+} indicator

ATP: Adenosine triphosphate

STIM1: Stromal interaction molecule 1

1. Introduction

Calcium (Ca^{2+}) is a versatile intracellular second messenger that operates in various signaling pathways. Multiple intracellular Ca^{2+} signaling pathways exist, and these produce diverse biological outputs. In neurons, distinct Ca^{2+} signals regulate release of synaptic vesicles in the pre-synapse, modulation of gene expression in the nucleus and regulation of synaptic plasticity in dendrites [1], by activating downstream enzymes such as kinases and phosphatases at the right place and with precise timing. This complex regulation is enabled by the diversity of spatiotemporal regulation of Ca^{2+} signaling, originating from differences in the source of Ca^{2+} signals, i.e., Ca^{2+} influx from the extracellular space and Ca^{2+} release from the intracellular Ca^{2+} store, the endoplasmic reticulum (ER) [2,3]. Indeed, lines of evidence support that the source of Ca^{2+} signal is one of the crucial determinants of the different outputs. Growth cone turning direction depends critically on the source of Ca^{2+} : Ca^{2+} release from the ER guides attraction, whereas Ca^{2+} influx from the extracellular space induces repulsion if unaccompanied by Ca^{2+} release from the ER [4]. We also reported that Ca^{2+} release from the ER is required for the clustering of GABA_A receptors at inhibitory

GABAergic synapses; in contrast, Ca^{2+} influx from the extracellular space enhances the de-clustering of GABA_A receptors at the synapse [5]. Cells have an ability to decode distinct spatiotemporal patterns of Ca^{2+} generated by the coordination between Ca^{2+} influx and Ca^{2+} release. It is important to describe the diversity of Ca^{2+} signals at subcellular resolution to completely understand the signaling pathways underlying Ca^{2+} signal-dependent cellular outputs. However, the spatiotemporal resolution has not been fully achieved by conventional Ca^{2+} imaging techniques using chemical Ca^{2+} sensors or genetically encoded Ca^{2+} indicators (GECIs).

In this study, we propose a new method to accurately report Ca^{2+} signals at subcellular spatial resolution. Local Ca^{2+} microdomains have been successfully monitored by targeting GECIs to the plasma membrane [6]. Here, we localized the GECI GCaMP6f to the cytosolic side of the ER membrane using part of an ER membrane protein, to detect the patterns of Ca^{2+} signals in the vicinity of the ER. Using this sensor, we succeeded in detecting different Ca^{2+} patterns from non-targeted or membrane-targeted GCaMP6f [6].

2. Materials and Methods

2-1. Ethics statement

All animal procedures were performed in accordance with the guidelines issued by the Japanese Ministry of Education, Culture, Sports, Science and Technology, and were approved by the Animal Experiment Committee of RIKEN and Nagoya University.

2-2. Plasmids

Human STIM1 (hSTIM1), G-CEPIA1*er* [7], pGP-CMV-GCaMP6f (cytosolic GCaMP6f; Addgene # 40755) [8], and pN1-Lck-GCaMP5G (plasma membrane-targeted GCaMP5F; Addgene # 34924) [9] were kindly provided by Akihiro Mizutani, Masamitsu Iino, Douglas Kim and Baljit Khakh, respectively. OER-GCaMP6f was created by inserting the luminal and

transmembrane domains (aa 1–250) of hSTIM1 without the EF-SAM domain (aa 58–201) [10,11] into the N-terminal of GCaMP6f in pGP-CMV-GCaMP6f (Fig. 1A). For Lck-GCaMP6f, GCaMP5G in pN1-Lck-GCaMP6f was replaced by GCaMP6f from pGP-CMV-GCaMP6f. Creation of the mRFP-KDEL plasmid is described in [12,13]. The plasmid used to generate OER-GCaMP6f-expressing *C. elegans* was created as follows. The *lin-17* promoter [14], which drives gene expression in several cell types in the posterior body of worms, was cloned into the pDEST-PL vector [15] to generate pDEST-lin17p. The cDNA for OER-GCaMP6f was cloned into the pENTR/D TOPO vector (Invitrogen) after amplification by polymerase chain reaction. pDEST-lin-17p::OER-GCaMP6f was generated using the LR cloning kit (Invitrogen).

2-3. Cell Culture

COS7 cells and HeLa cells (RIKEN BioResource Center, Ibaraki, Japan) were plated onto 18-mm-diameter glass coverslips and cultured in Dulbecco's modified Eagle's medium (Nacalai Tesque, Kyoto, Japan) supplemented with 10% fetal bovine serum and antibiotics. Primary cultures of hippocampal astrocytes co-cultured with neurons were prepared from E18–21 Wistar rat embryos as previously described [16]. At least three independent cultures were used for each experiment.

2-4. Transfection

COS7 or HeLa cells were transfected with plasmid DNA (250 ng for Ca²⁺ indicators, 100 ng for mRFP-KDEL) using Extremegene HP (Sigma-Aldrich, Tokyo, Japan) according to the manufacturer's protocol for 24 h before observation. Astrocytes were transfected at 3-7 days in vitro with 0.5 µg OER-GCaMP6f DNA using Lipofectamine 3000 (Thermo Fisher Scientific) according to the manufacturer's protocol, and imaged 48 h after transfection [17].

2-5. Transgenic *C. elegans*

Transgenic animals carrying *lin17p-OER-GCaMP6f* in an extrachromosomal array were generated by injection of *lin-17p::ER:GCaMP6f* (0.07 µg/µl) mixed with transformation markers *lin-17p::PH::mCherry* (0.008 µg/µl) and *pRF4(rol-6d)* (0.07 µg/µl) into the gonads of adult N2 hermaphrodites [18]. Worms exhibiting the roller phenotype were irradiated with γ-rays to generate an integrant line bearing *ncIs61[lin17p-OER-GCaMP6f; lin-17p-mCherry-PH; pRF4]* [19].

2-6. Immunocytochemistry

To characterize the distribution of OER-GCaMP6f, cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. After blocking with 5% BSA, cells were incubated with anti-GFP antibody B-2 (Santa Cruz Biotechnology, CA, USA) in the presence of 2.5% BSA for 90 min and subsequently labeled with goat anti-mouse IgG (H+L) secondary antibody, Alexa Fluor® 488 conjugate (Thermo Fisher Scientific). Immunofluorescence was observed using a FV-1000 confocal microscope (Olympus, Tokyo, Japan) equipped with a Plan Apo 60× oil immersion objective with a numerical aperture (NA) of 1.42 (Olympus).

2-7. Ca²⁺ imaging

Ca²⁺ imaging in COS7 cells was performed in Krebs-Ringer Solution (2 mM Ca²⁺) as described [20]. Fluorescence from GCaMP6f in HeLa and COS7 cells was measured at 37°C with an inverted microscope (IX-70; Olympus) equipped with a 40× objective (NA 0.85, UPlanApo; Olympus), an EM-CCD camera (ImagEM, Hamamatsu Photonics) and appropriate filters (Ex, 480 ± 10 nm; Em, 530 ± 20 nm). Time-lapse images were acquired for 5 min at 2 Hz with 100 ms exposure. *C. elegans in vivo* imaging was carried out under an upright microscope (Olympus BX51W1) equipped with a spinning disk confocal scanner unit (Yokogawa CSU-W1), an oil-immersion objective (Olympus 100×, NA 1.35) and an EM-CCD camera (Andor iXon3). OER-GCaMP6f signal was excited with a 488-nm laser (Coherent OBIS), and emitted with a

525–550-nm band pass filter. Images were acquired every 200 ms for 100 s under the continuous illumination. Ca^{2+} imaging of astrocytes was carried out using an inverted fluorescence microscope (IX73, Olympus) equipped with an oil-immersion objective lens (60 $\times$, NA 1.42, Olympus), LED illumination (Cool LED, Andover, UK) and an EM-CCD camera (ImagEM). Time-lapse images were acquired at 2 Hz with 100 ms exposure, in MEM supplemented with 20 mM HEPES pH 7.4, 2 mM glutamine and 1 mM sodium pyruvate at 37°C.

For the automatic analysis and comparison of OER-GCaMP6f and GCaMP6f, we used the LC-pro plugin for ImageJ (NIH) software [21,22]. Elevations with peaks existing prior to the application of ATP were excluded from the analysis. For other analyses, data were analyzed using a TI workbench [23].

3. Results

3-1. Development and characterization of outer ER membrane-targeted GCaMP6f (OER-GCaMP6f)

To observe Ca^{2+} signals in the vicinity of the ER, we designed a GECI targeted to the cytosolic side of the ER membrane (outer ER membrane-targeted GCaMP6f, OER-GCaMP6f). GCaMP6f was attached to a part of the transmembrane ER protein STIM1. The luminal and transmembrane domains (aa 1–250) of human STIM1 (hSTIM1) with the EF-SAM domain (aa 58–201) [10,11] removed were attached to the N-terminal of GCaMP6f (Fig. 1A). To confirm that OER-GCaMP6f was expressed on the ER, we performed immunocytochemistry on COS7 (Fig. 1B, C) and HeLa cells (Fig. 1D) co-transfected with OER-GCaMP6f and the ER marker protein mRFP-KDEL [12]. The OER-GCaMP6f signal, highlighted with the anti-GFP antibody, was co-localized with the ER marker mRFP-KDEL (Fig. 1B, C, D), indicating that OER-GCaMP6f was targeted to the ER.

Next, we examined whether GCaMP6f was located on the cytosolic side of the ER. COS7 cells expressing OER-GCaMP6f were stimulated with ATP to induce Ca^{2+} release from the ER into the cytosol via IP_3 receptors. 125 nM ATP induced enhancement of the signal from OER-GCaMP6f (Fig. 2A), suggesting that OER-GCaMP6f successfully reported the elevation of Ca^{2+} in the cytoplasm. Therefore, we concluded that OER-GCaMP6f was located on the cytosolic side of the ER membrane and worked as a Ca^{2+} sensor. Although Krebs-Ringer Solution contains 2 mM Ca^{2+} , elevation of the OER-GCaMP6f signal was due to Ca^{2+} release from the ER, not Ca^{2+} influx, because U73122, a blocker of the IP_3 -producing enzyme phospholipase C, completely blocked the ATP-induced OER-GCaMP6f signal increase (data not shown).

Calcium-measuring organelle-entrapped protein indicators (CEPIA) are the GECI targeted to the lumen of intracellular Ca^{2+} stores. We used G-CEPIA1*er*, which is a green variant of CEPIA, to specifically detect Ca^{2+} release from the ER as a decrease of signal [7]. As expected, the G-CEPIA1*er* signal expressed in COS7 cells decreased when stimulated with 250 nM ATP (Fig. 2B). However, a decrease of the G-CEPIA1*er* signal was not detected with stimulation by 100 nM ATP (Fig. 2C). This result indicated that G-CEPIA1*er* could detect Ca^{2+} release from the ER which was induced by 250 nM ATP stimulation, but not that induced by 100 nM ATP. In contrast, the fluorescent signal of OER-GCaMP6f was increased by 100 nM ATP stimulation (Fig. 2D). This result was also confirmed by averaging of signals from multiple cells (Fig. 2E; CEPIA1*er*, n = 40; OER-GCaMP6f, n = 38 cells).

To examine the potential application of OER-GCaMP6f for *in vivo* imaging, we monitored OER-GCaMP6f signals in the living nematode *C. elegans* using a spinning disk confocal microscope. OER-GCaMP6f was expressed in worms using the *lin-17* promoter [14], and the expression of OER-GCaMP6f was confirmed in VD motor neurons in the ventral nerve cord and somatic cells (Fig. 2F). Significant elevation of the OER-GCaMP6f signal was observed predominantly in VD motor neurons (Fig. 2F, G). This result indicates that the application of

OER-GCaMP6f for *in vivo* Ca^{2+} imaging is possible, at least for monitoring Ca^{2+} dynamics in *C. elegans*.

3-2. OER-GCaMP6f shows higher temporal resolution than non-targeted GCaMP6f

Next, we compared the Ca^{2+} signals reported by OER-GCaMP6f with those reported by conventional cytosolic GCaMP6f lacking a localization tag (Fig. 3A). Ca^{2+} spikes were induced by 100 nM ATP and signals were automatically detected and analyzed using the LC-pro plugin for ImageJ software [21,22]. The averaged F/F_0 time-course suggested that lower magnitude and shorter lasting ATP-induced elevation was reported by OER-GCaMP6f than that obtained from cytosolic GCaMP6f (Fig. 3B). OER-GCaMP6f reported significantly smaller amplitude (Fig. 3C) and area under the curve (A.U.C, Fig. 3D) of Ca^{2+} transients than cytosolic GCaMP6f. The maximal spreading area of Ca^{2+} transients (spread) was also smaller with OER-GCaMP6f than cytosolic GCaMP6f (Fig. 3E). There were remarkable differences in the kinetics of Ca^{2+} transients between OER-GCaMP6f and cytosolic GCaMP6f. The duration of ATP-induced Ca^{2+} transients reported by OER-GCaMP6f was shorter than that reported by cytosolic GCaMP6f (Fig. 3F; median duration 12 s for OER-GCaMP6f, 17.5 s for cytosolic GCaMP6f). Detailed analysis of the time-course revealed that the shorter duration of OER-GCaMP6f-reported Ca^{2+} transients was due to reduced decay time (the time in which Ca^{2+} transients return to normal from maximal levels). There was no significant difference in the rate at which Ca^{2+} transients reached their maximum amplitude (Fig. 3G; attack), but OER-GCaMP6f showed shorter decay of Ca^{2+} transients (Fig. 3H; median decay 6.5 s for OER-GCaMP6f, 14 s for cytosolic GCaMP6f). OER-GCaMP6f showed longer decay when cells were treated with 150 nM Thapsigargin (TG) (Fig. 3B OER-GCaMP6f-TG). This result suggests that OER-GCaMP6f has an ability to report longer Ca^{2+} transients, and shorter Ca^{2+} transient reported by 100 nM ATP is less likely to be due to reduced Ca^{2+} affinity or reduced kinetics for this indicator.

3-3. OER-GCaMP6f shows distinct Ca^{2+} signals from plasma membrane-targeted GCaMP6f

Astrocytes, which are the predominant glial cell type in the central nervous system, use intra/intercellular Ca^{2+} signals for various types of information processing [24,25]. We examined whether the subcellular diversity of Ca^{2+} signals could be distinguished by targeting GECI to the subcellular components in astrocytes. Here, spontaneous Ca^{2+} transients in cultured astrocytes [17] were monitored using membrane-targeted GCaMP6f (Lck-GCaMP6f) [6] (Fig. 4A) or OER-GCaMP6f (Fig. 4B). Lck-GCaMP6f distributed to the peripheral processes (Fig. 4A, left), while in contrast the distribution of OER-GCaMP6f was limited to the main branches of astrocytic processes (Fig. 4B, left). Both Lck-GCaMP6f and OER-GCaMP6f showed Ca^{2+} transients (Fig. 4A, B, right); however, more frequent Ca^{2+} transients were observed in Lck-GCaMP6f-expressing astrocytes (Fig. 4C) compared with those expressing OER-GCaMP6f (Fig. 4D). The frequency of Ca^{2+} transients was significantly different between Lck-GCaMP6f and OER-GCaMP6f (Fig. 4E). The finding that OER-GCaMP6f detected fewer Ca^{2+} transients compared with Lck-GCaMP6f indicates that ER-targeted OER-GCaMP6f and plasma membrane-targeted Lck-GCaMP6f could exhibit different temporal patterns of Ca^{2+} transients, even though both indicators are present within the same cytosol. This result also suggests that the Ca^{2+} influx reported by Lck-GCaMP6f was more frequent than Ca^{2+} release from the ER, monitored by OER-GCaMP6f.

4. Discussion

In this study we developed an ER membrane-targeted cytosolic Ca^{2+} indicator “OER-GCaMP6f”, which is available both *in vitro* and *in vivo*. OER-GCaMP6f showed higher sensitivity than ER lumen Ca^{2+} sensor CEPIA in the case of stimulation with low concentrations of ATP. In addition, OER-GCaMP6f reported different Ca^{2+} dynamics to those detected by cytosolic or plasma membrane-targeted GCaMP6f.

Despite the only difference between OER-GCaMP6f and cytosolic GCaMP6f being local targeting, OER-GCaMP6f showed smaller amplitude and area of Ca^{2+} elevation compared with GCaMP6f. This could be explained by the distribution of the indicators: cytosolic GCaMP6f can disperse more widely across the cytosol. We also found significant differences in the decay of Ca^{2+} signal between OER-GCaMP6f and cytosolic GCaMP6f. These results suggest that cytosolic GCaMP6f could not faithfully report rapid removal of Ca^{2+} around the ER, because of Ca^{2+} signal remaining widely distributed in the cytosol, where cytosolic GCaMP6f is located. Therefore, OER-GCaMP6f is considered to be more suitable to detect the Ca^{2+} dynamics in the vicinity of the ER and to report the real time-course of the opening of Ca^{2+} release channels. Furthermore, we revealed differences in Ca^{2+} dynamics close to the ER or plasma membrane in astrocytes using OER-GCaMP6f and Lck-GCaMP6f. Astrocytes express various types of G-protein-coupled receptors, and can respond to neural activity and potentially modulate neurotransmission [26]. Previous studies reported that Ca^{2+} signals in astrocytes depend on IP_3 -induced calcium release [24,25]. Although recent studies have also shown that Ca^{2+} influx in astrocytes occurs via transient receptor potential (TRP) A1, TRPC and TRPV4 [27–31], the role of Ca^{2+} influx in astrocytes has not yet been studied in detail. Different patterns of Ca^{2+} spikes detected by OER-GCaMP6f and Lck-GCaMP6f imply the possibility that the frequency of Ca^{2+} influx outnumbered Ca^{2+} release. In other words, this result suggests a potentially important role of Ca^{2+} influx in astrocytes. Future studies to examine the relationship between the source of Ca^{2+} signals and the resultant biological outputs will be important to understand the diversity of Ca^{2+} signaling pathways in astrocytes.

The existence of Ca^{2+} microdomains inside the continuous cytosol is essential to explain the multiple Ca^{2+} -dependent processes that can occur in a single cell. Ca^{2+} release from the intracellular ER Ca^{2+} store and Ca^{2+} influx from the extracellular space could result in differences in Ca^{2+} signals in terms of Ca^{2+} concentration, temporal patterns and spatial

spreading patterns. This possibility should be confirmed in future studies by further characterizing the origin of local Ca^{2+} microdomains in various cell types.

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Figure legends.

Figure 1. Structure and distribution of the ER-targeted GCaMP6f, OER-GCaMP6f

A: Illustration of the structure of the ER membrane protein hSTIM1 and OER-GCaMP6f.

Signal sequence and truncated ER luminal domain attached to the N-terminal of GCaMP6f.

B-D: Expression pattern of OER-GCaMP6f, ER marker mRFP-KDEL and merged images in COS7 (**B, C**) and HeLa cells (**D**). **C** shows the enlarged image of the area indicated by a rectangle in **B**. Scale bars: 20 μm .

Figure 2. OER-GCaMP6f reports Ca^{2+} elevation *in vitro* and *in vivo* with high sensitivity

A: Top: Sequential images of OER-GCaMP6f signal expressed in COS7 cells stimulated with 125 nM ATP at $t = 0$ s. Bottom: time-course plot of OER-GCaMP6f dF/F_0 ratio monitored in the region of interest (ROI) indicated in the top image. ATP was applied as indicated by the gray bar.

B-D: Representative COS7 cells expressing G-CEPIA1er (**B, C**) or OER-GCaMP6f (**D**) and dF/F_0 ratio time-course reported in the ROI indicated in the left image. Cells were

stimulated with 250 nM (**B**) or 100 nM (**C, D**) ATP as shown by the gray bar. **F:** *In vivo*

imaging of *C. elegans* expressing OER-GCaMP6f under control of the *lin-17p* promoter. Left: expression pattern of OER-GCaMP6f in the posterior body of an *ncls61* hermaphrodite at the 4th larval stage. Lateral view, posterior to the top, dorsal to the right. Right: sequential F/F_0 images.

The ventral nerve cord, VD motor neurons and some somatic cells in the tail showed

fluorescence signals. Arrowheads indicate the elevation of F/F_0 signal. **G:** Time-course of dF/F_0 elevation in VD motor neurons. dF/F_0 traces are from the ROIs indicated in **F**. Scale bars: 20 μm

for **A-D** and 10 μm for **F**.

Figure 3. Distinct temporal pattern of ATP-evoked Ca^{2+} elevation reported by OER-GCaMP6f and cytosolic GCaMP6f in COS7 cells

A: Representative pseudo-color image of Ca^{2+} elevation evoked by 100 nM ATP in COS7 cells. Scale bar: 20 μm . **B:** Time-course plot of average F/F_0 ratio of Ca^{2+} elevation induced by 100 nM ATP detected by OER-GCaMP6f (red; $n = 101$) or GCaMP6f (blue; $n = 96$). Yellow trace shows Ca^{2+} elevation induced by 150 nM Thapsigargin (TG) reported by OER-GCaMP6f ($n = 35$). Gray bar indicates the timing of drug application. **C–H:** Parameters of Ca^{2+} elevation obtained from OER-GCaMP6f (red) or GCaMP6f (blue). Each graph shows distribution of amplitude (**C**), area under the curve (A.U.C) (**D**), spread (**E**), duration (**F**), attack (**G**) and decay (**H**) analyzed using the LC-pro plugin of ImageJ. $n = 121$ for OER-GCaMP6f and $n = 99$ for GCaMP6f. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, Mann-Whitney U test.

Figure 4. Distinct temporal patterns of spontaneous Ca^{2+} elevation in astrocytes reported by Lck-GCaMP6f and OER-GCaMP6f

A, B: Spontaneous Ca^{2+} elevation in astrocytes monitored with plasma membrane-targeted GCaMP6f (Lck-GCaMP6f; **A**) and OER-GCaMP6f (**B**). Left, representative expression pattern of indicators; right, sequential F/F_0 images. Arrowheads indicate the elevation of F/F_0 signal. Scale bar: 20 μm . **C, D:** dF/F_0 time-course of Lck-GCaMP6f (**C**) and OER-GCaMP6f (**D**), monitored in the regions of interest in **A** and **B**, respectively. **E:** The average Ca^{2+} event number ($\pm$ SEM) during 2 min recording time. $n = 26$ for Lck-GCaMP (Lck); $n = 26$ for OER-GCaMP6f (OER). *** $P < 0.001$, Student's t -test.

Figure1

Figure1. Niwa et. al.

Figure2

Figure 2 Niwa et al.

Figure3

Figure 3. Niwa et. al.

Figure4

Figure 4 Niwa et al.

Dissection of local Ca^{2+} signals inside cytosol by ER-targeted Ca^{2+} indicator

Same indicator showed different Ca^{2+} patterns inside cytosol by localization of Ca^{2+} indicator