# Visualizing Ultrasound Responsiveness of Ion Channel Receptors for Sonogenetics

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## 1. Introduction

Exogenous stimulation to neurons is potentially valuable for the treatment of psychiatric disorders such as depression and bipolar disorder, and neurological disorders such as Parkinson's disease and epilepsy[1)]. Conventional electrical methods of neuronal stimulation have high target specificity, but are invasive due to the insertion of electrodes into the tissue[2)]. Optogenetics using light-activated proteins can stimulate specific neurons with high temporal resolution[3)]. Still, optogenetics also needs an insertion of electrodes and is difficult to stimulate deep tissue[2)] Therefore, sonogenetics using ultrasound-activated proteins has been getting attention. Sonogenetics is expected to stimulate deep tissue noninvasively[4)]. Transient receptor potential A1 (TRPA1) was recently reported to be activated by mechanical stimulation caused by ultrasound[5)]. TRPA1 is a non-selective cation channel, especially permeable to calcium ion. This TRPA1 is one of the ion channel-type receptors known to respond to cold sensation and allyl isothiocyanate (AITC), an intense component of wasabi. It shows the possibility of sonogenetics manipulating neurons. However, the ultrasound stimulation conditions for effective reception of mechanical stimulation, including frequency and environmental effects, have not been clarified.

Here, we fabricated an ultrasound irradiation device to effectively activate TRPA1 with a piezoelectric single crystal, lithium niobate (LN). Moreover, to evaluate the characteristics of mechanoreceptors, the stimulus responsiveness of TRPA1-expressing HEK293 cells was visualized by calcium indicator proteins (GCaMP6s).

## 2. Experiment

### 2.1 Fabrication of an ultrasound irradiation device

For microfabrication, LN with 127.86° Y-rotated cut of 0.5 mm thickness was ultrasonically cleaned with acetone and ethanol each for 1 minute. Then, chromium and aluminum were evaporated on both sides of the LN. After the evaporation, the LN transducer was fabricated by cutting the four sides of the LN into 15 mm × 30 mm pieces to remove the aluminum evaporated on the sides.

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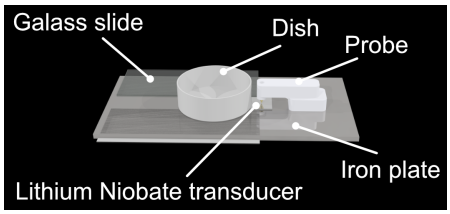


Fig. 1 Ultrasound irradiation device with LN transducer.

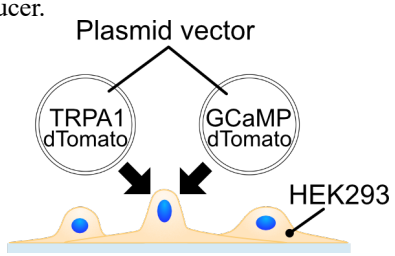


Fig. 2 Gene transfection of TRPA1 with dTomato and GCaMP6s with dTomato in HEK293 cells.

An ultrasound irradiation device was also constructed with a fabricated LN transducer (**Fig. 1**). The LN transducer was placed on a steel plate and energized with a probe. A dish was placed on top of the transducer. Then, the space between the transducer and the dish was filled with water so that the ultrasound stimulus would propagate into the dish. A glass slide was placed between the dish and the steel plate to keep the dish parallel.

### 2.2 Gene transfection into HEK293 cells and visualization

To visualize the stimulus-responsiveness of TRPA1, pcDNA3.1, a plasmid vector, was used to transfect the gene into HEK293 cells (**Fig. 2**). To visualize the activation of TRPA1 ion channels by stimulation, a calcium indicator protein GCaMP6s, which binds to calcium to enhance green fluorescence, was transfected. By using the 2A peptide sequence, which is capable of translating two proteins from one mRNA, dTomato was expressed together with TRPA1 and GCaMP6s, to confirm transfected cells with red fluorescence.

First, AITC (30 μM), a ligand for TRPA1, was added to HEK293 cells expressing TRPA1 and GCaMP6s, to observe TRPA1 activation under a fluorescence microscope. Furthermore, the ultrasound irradiation device was used to visualize

the response of TRPA1 to ultrasound stimulation by irradiating HEK293 cells expressing TRPA1 and GCaMP6s.

## 3. Results and Discussion

### 3.1 Confirmation of acoustic streaming by ultrasonic irradiation device

For confirming the ultrasound irradiation by the fabricated device, acoustic streaming was observed when the ultrasound irradiation device was used to irradiate the water-filled dish with ultrasound. This enabled us to induce TRPA1 activation by ultrasound (**Fig. 3**).

### 3.2 Visualization of stimulus responsiveness of TRPA1

For confirming gene expression, the red fluorescence of dTomato was observed by fluorescence microscopy of the transfected cells, confirming that TRPA1 and GCaMP6s had been transfected into HEK293 cells (**Fig. 4**). Furthermore, the gene expression rate was calculated by analyzing the image analysis software Fiji (ImageJ) [5]. The gene expression rate in the cells was 43.4%.

For TRPA1-expression, AITC was added to the cells, which were confirmed to be transfected with dTomato. The cells were observed under a fluorescence microscope before and after the addition of AITC (**Fig. 5**). The number of green fluorescent cells and the intensity of green fluorescence increased after the addition of AITC compared to those before the addition of AITC. In other words, the intracellular calcium concentration in the cells was confirmed. This indicates that TRPA1 was transfected in HEK293 cells.

In addition, the cells were irradiated with an ultrasound irradiation device and then observed before and after irradiation with a fluorescence microscope (**Fig. 6**). When the cells were irradiated with ultrasound at a frequency of 6.6 MHz at an acoustic pressure of 3.5 MPa, the number of green fluorescent cells increased before and after ultrasound irradiation. The intensity of green fluorescence also increased in each case. This confirmed the increase in intracellular calcium concentration induced by the activation of TRPA1 triggered by ultrasound irradiation. This suggests that we have set up an environment for evaluating the stimulus-responsiveness of TRPA1.

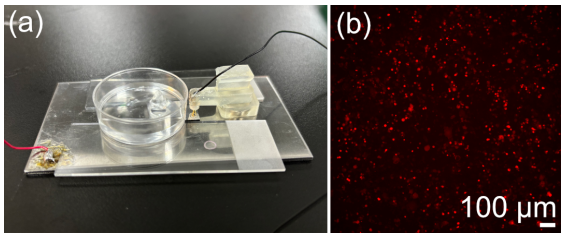


Fig. 3 Setup of environment for TRPA1 activation. (a) Acoustic streaming with ultrasound irradiation device. (b) TRPA1 and GCaMP6s gene expression monitored by dTomato in HEK293 cells.

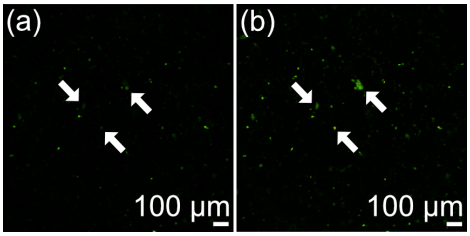


Fig. 5 AITC-responsive activation of GCaMP6s in HEK293 cells expressing TRPA1. (a) Before AITC addition. (b) After AITC addition.

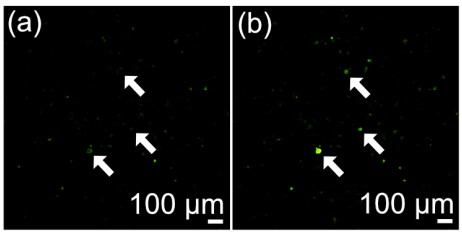


Fig. 6 Ultrasound-responsive activation of GCaMP6s in HEK293 cells expressing TRPA1. (a) Before ultrasound stimulation. (b) After ultrasound stimulation.

## 4. Conclusion

In this study, we fabricated an ultrasound device to activate TRPA1. Gene transfection of TRPA1 and GCaMP6s was confirmed by dTomato and AITC. Then, TRPA1 was activated by ultrasound to increase the intracellular calcium concentration. In the future, the effects of environmental factors including the cell scaffold and frequency on the stimulus responsiveness of TRPA1 will be investigated.


## Acknowledgment

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## References


1) Timothy Wagner, et al., *Annu. Rev. Biomed.* Eng., **9**, (2007), 527-565
2) Aditya Vasan, et al., *Adv. Sci.*, **9**, 2022, 2101950
3) Watanabe Hidenori, et al., *Nat. Commun.*, **11**, (2020), 3253
4) Johannes Hahmann, et al., *Angew. Chem.*, **63**, (2024), e202317112
5) Marc Duque, et al., *Nat. Commun.*, **13**, (2022), 600