# Ultrasound modulates Piezo1 ion channel activity

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## Background, Motivation, Hypothesis and Objectives

Ultrasound can induce mechanosensing bioeffects to accelerate several kinds of noninvasive therapies. Further understanding of the regulatory mechanism at the molecular or the biophysical level allows precise modulation of targeted and clinically translatable ultrasound-based treatment. One of the bioeffects elicited by ultrasound is the induction of intracellular calcium transients through activating ion channels. Recently, Piezo1 was characterized as a member of mechanosensitive ion channels and its ion channel activity can be mediated through stimulation of substrate stiffness, shear stress, osmotic pressure, compression, and potentially through ultrasound. In this study, we explore the capability of ultrasound in mediating Piezo1 ion channel activity and the underlying biophysical mechanisms.

## Statement of Contribution/Methods

HEK293T cells were used for creating Piezo1-overexpressed and Piezo1-knockdown cell models. Before calcium imaging, cells were loaded with Fluo4-AM to label intracellular calcium ion at 37°C incubation. A 10-MHz focused ultrasound transducer was connected to a pulser-receiver for ultrasound stimulation to the cells (figure A). The calcium imaging was carried out with a total internal reflection fluorescence microscopy at a frame rate of 1 fps. Acoustic streaming velocity was determined by using fluorescence microscopy to trace the movement of fluorescent beads during ultrasound stimulation.

## Results, Discussion and Conclusion

Comparing to wild-type cells, intracellular calcium influx was increased in Piezo1-overexpressed cells after ultrasound stimulation (figure B), and the increase was reversed when introduced Piezo1 small interfering RNA into Piezo1-overexpressed cells (mPz1+siPz1) to knock down the Piezo1 expression. Furthermore, it was found that the average acoustic streaming velocity was higher at the height far from the cell membrane, and the average velocity for generating the highest local shear stress at proximity of a solid boundary (i.e. 70 μm above the cell membrane) to activate ion channel activity was 234.2 μm/s (figure C). In conclusion, the capability of ultrasound to induce Piezo1 ion channel activity was demonstrated and acoustic streaming-derived shear stress can be one of the responsive biophysical mechanisms.

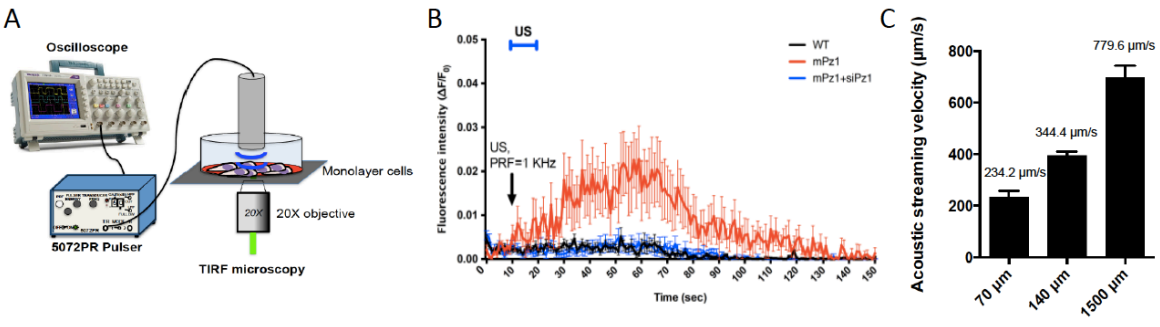


**Figure:** Increased calcium influx in US-modulated Piezo1-overexpressed cells. (A) Ultrasound modulation system setup. (B) Ultrasound- mediated calcium influx in Piezo1-overexpressed HEK293T cells. (C) Acoustic streaming velocity as a function of the height above the cell membrane.