Modulation of cellular activities using an engineered auditory-sensing protein with ultrasound

Ching-Hsiang Fan[1], Yao-Shen Huang [2], Yu-Chun Lin[2] and Chih-Kuang Yeh[1,3] ,[1] Department of Biomedical Engineering and Environmental Sciences, National Tsing Hua University, Hsinchu, Taiwan, [2] Institute of Molecular Medicine, National Tsing Hua University, Hsinchu, Taiwan, [3] Institute of Nuclear Engineering and Sciences, National Tsing Hua University, Hsinchu, Taiwan

Background, Motivation, and Objective:
Biomolecules that respond to different external stimuli enable the remote control of genetically modified cells. Chemogenetics and optogenetics, two tools that can control cellular activities via synthetic chemicals or photons, respectively, have been widely used to elucidate underlying physiological processes. However, these methods are very invasive, have poor penetrability, or low spatiotemporal precision, attributes that hinder their use in therapeutic applications. The goal of this study is the identification of proteins that sense ultrasound (US), a focused and non-invasive stimulus with good depth of penetration. We showed that Prestin, a conserved membrane protein that highly expresses in mammalian auditory system, can function as an "US amplifier" that endows transfected mammalian cells with US sensing. Moreover, its US sensitivity could be largely enhanced by introducing specific amino acid substitution which frequently occurring in sonar species into mouse wild-type prestin.

Statement of Contribution/Methods:
Yellow fluorescence protein (Venus)-tagged wild-type prestin and engineered prestin were transfected in Human HEK293T cells. Each construct was co-transfected with the calcium biosensor cyan fluorescence protein (CFP)-R-GECO into the cell. The calcium influx of transfected cells was used as a readout in response to the mechanical stimulation of US. The cellular response resulted from prestin with US (frequency = 80 kHz - 3.5 MHz, pressure = 0.5MPa, cycle = 2000, pulse repetition frequency (PRF): 10 Hz, sonication time =3 s) was monitored by live-cell imaging. The successful transgene expressions were verified by fluorescence protein expression.

Results/Discussion:
The data indicated that US induced a 351 ± 20% increase in the R-GECO fluorescence of engineered prestin transfected cells, suggesting it possible to evoke cellular calcium responses by US. Strikingly, heterologous expression of engineered prestin endows transfected cells with ~11 folds better US sensitivity compared to wild-type prestin. The optimal frequency for evoked a cellular calcium response was 0.5 MHz, indicating its frequency-dependent property. This technology provides a new strategy for as new strategies to precisely manipulate cellular activities for various therapeutic applications. Future works include non-invasive control of neural activity in deep tissues *in vivo*.