Ultrasound imaging of gene expression in mammalian cells

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Background, Motivation and Objective

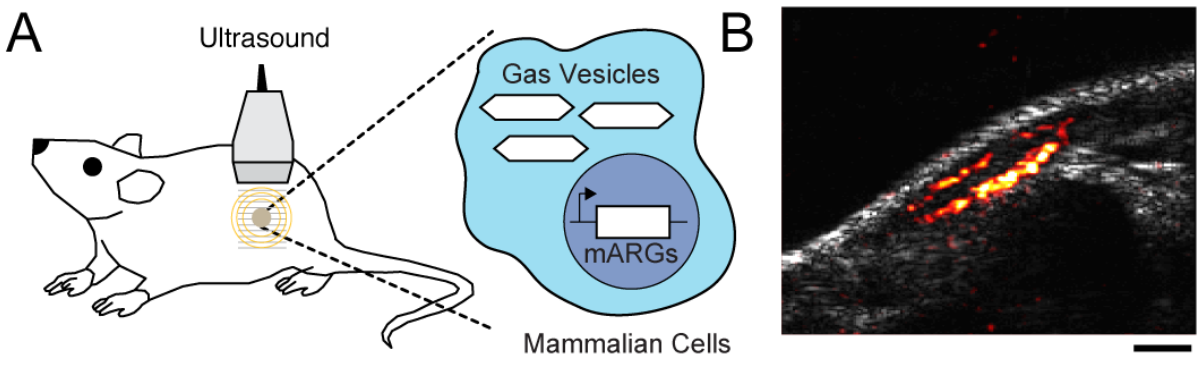
The study of cellular function within the context of intact living organisms is a grand challenge in biological research. Addressing this challenge requires imaging tools that can visualize cells inside the body from brain development to tumorigenesis, to monitor cell-based therapeutics. Today, most common methods for imaging cellular processes such as gene expression rely on fluorescent or luminescent proteins, which have limited performance in intact animals due to the poor penetration of light in biological tissue. Conversely, ultrasound is able to image deep tissues with high spatial and temporal resolution, but lacks genetically encoded molecular reporters analogous to green fluorescent protein (GFP).

Statement of Contribution/Methods

To address this limitation, we recently introduced a unique class of air-filled protein nanostructures, called gas vesicles, as biomolecular reporters for ultrasound, using them to image gene expression in bacteria (Bourdeau et al, *Nature* 553:86, 2018). The ability for these genes to be expressed in mammalian cells has not been demonstrated, and presents a major challenge in synthetic biology due to the large number of genes involved in gas vesicle expression and the differences in transcription and translation between prokaryotes and eukaryotes.

Results/Discussion

Here, we introduce the first mammalian acoustic reporter genes (Farhadi et al. *bioRxiv*, 580647, 2019). Starting with an eleven-gene polycistronic gene cluster derived from bacteria, we engineered a eukaryotic genetic program whose introduction into mammalian cells results in the expression of gas vesicles, as visualized by ultrasound and confirmed with electron microscopy. Using the unique physical properties of gas vesicles, we developed a novel ultrasound pulse sequence that drastically increases the sensitivity of detecting these nanostructures. Using this new technique, mammalian acoustic reporter genes could be visualized at volumetric densities below 0.5% and permitted high-resolution imaging of gene expression in living animals. These mammalian acoustic reporter genes will enable previously impossible approaches to monitoring the location, viability and function of mammalian cells *in vivo*.



Mammalian acoustic reporter genes enable ultrasound imaging of gene expression. (A) Mammalian acoustic reporter genes (mARGs) encode a set of proteins whose expression results in the formation of cytoplasmic gas vesicles – air-filled protein nanostructures which scatter ultrasound waves and thereby produce contrast in ultrasound images. (B) This technology allows ultrasound to image mammalian gene expression non-invasively in intact animals.