Title: Ultrasound imaging of gene expression in mammalian cells

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Abstract: The study of cellular function within the context of intact living organisms is a grand challenge in synthetic biology and 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 GFP. 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.

Here, we introduce the first mammalian acoustic reporter genes. 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. The scattering of ultrasound by these nanostructures allows cells to be visualized at volumetric densities below 0.5%, enables the monitoring of dynamic circuit-driven gene expression, and permits 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.