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# Proteins, air and water: reporter genes for ultrasound and magnetic resonance imaging

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A long-standing goal of molecular imaging is to visualize cellular function within the context of living animals, necessitating the development of reporter genes compatible with deeply penetrant imaging modalities such as ultrasound and magnetic resonance imaging (MRI). Until recently, no reporter genes for ultrasound were available, and most genetically encoded reporters for MRI were limited by metal availability or relatively low sensitivity. Here we review how these limitations are being addressed by recently introduced reporter genes based on air-filled and water-transporting biomolecules. We focus on gas-filled protein nanostructures adapted from buoyant microbes, which scatter sound waves, perturb magnetic fields and interact with hyperpolarized nuclei, as well as transmembrane water channels that alter the effective diffusivity of water in tissue.

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## Introduction

Molecular imaging seeks to visualize the location and function of cells and molecules within a variety of biological settings, including deep inside intact animals. Within this context, much of the powerful repertoire of genetically encoded reporters and sensors based on green fluorescent protein (GFP) and its analogues have limited utility due to the strong scattering and absorption of light by tissue. In his influential 2003 perspective titled 'Imagining Imaging's Future' Roger Tsien recognized this limitation and predicted that 'the prevalence and success of GFP indicate that comparable revolutions might result from genetic sequences that robustly encode image contrast for other methods' [1]. Indeed, reporter genes for noninvasive deep-tissue imaging modalities such as ultrasound and magnetic resonance imaging (MRI) could have great value in both basic biomedical research and the development of cellular diagnostics and therapeutics. However, no such reporter genes have so far achieved the prevalence of GFP, and new ideas are therefore needed to spark the envisioned revolutions.

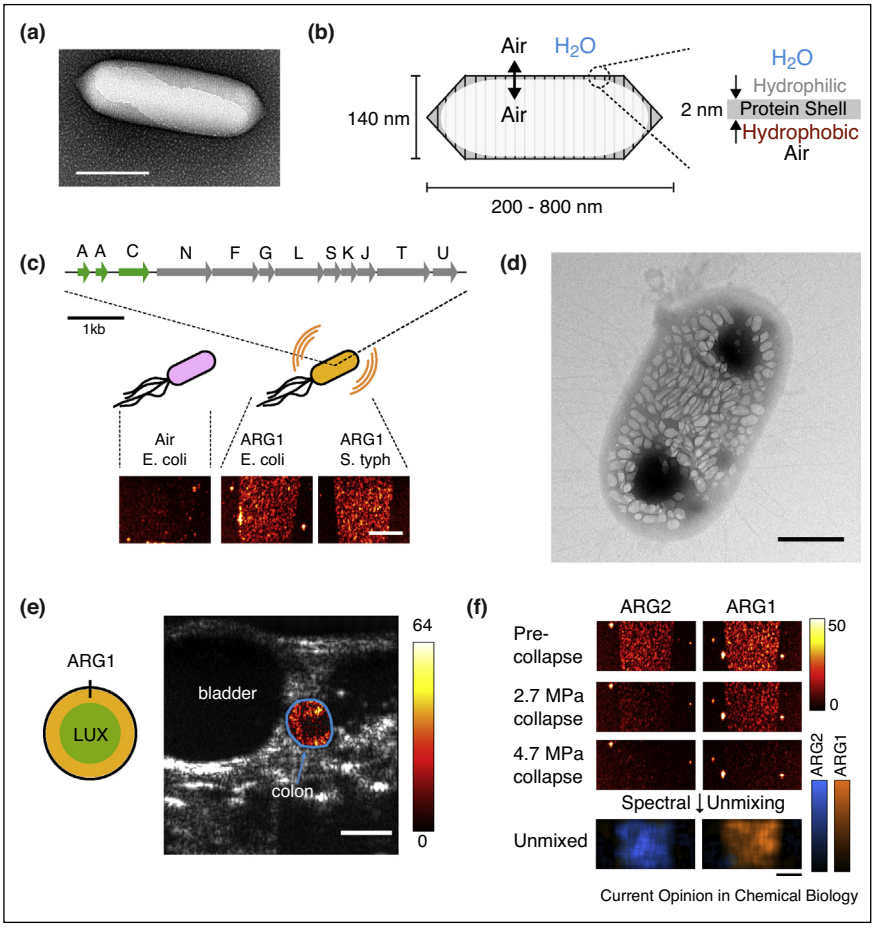
This review summarizes progress on two recently introduced classes of genetically encoded contrast agents for ultrasound and MRI that operate via new biophysical principles. One class is based on gas-filled proteins derived from buoyant microbes, which serve as the first reporter genes for ultrasound and produce contrast in susceptibility-based MRI and hyperpolarized xenon MRI. The second class is based on water channels such as aquaporin, whose overexpression in mammalian cells is brightly detectable with diffusion-based MRI. These reporter genes use fundamental properties of air and water to introduce new forms of contrast to the field of molecular imaging, providing unique capabilities for visualizing cellular function *in vivo*.

## Proteins with air: gas vesicles as acoustic reporter genes

Until very recently, no reporter genes were available for ultrasound, a versatile modality capable of imaging centimeters-deep into soft tissue with spatial and temporal resolution on the order of 100 μm and 1 ms. In addition to being one of the most widely used modalities in medicine, ultrasound scales to smaller model organisms to enable basic and translational research. Recent advances in equipment and signal processing have provided ultrasound with the ability to image faster (down to tens of μs) and more precisely (below 10 μm with super-localization techniques) [2•,3,4]. The classic contrast agents used in ultrasound are micron-sized bubbles of gas stabilized by a lipid or protein shell, which scatter sound waves due to their differential density and compressibility relative to water [5]. Could similar physical principles be embodied in a genetic sequence?

In 2014, it was discovered that a unique class of gas-filled protein nanostructures known as gas vesicles (GVs),

**Figure 1**




Acoustic reporter genes. **(a)** Transmission electron micrograph (TEM) of an individual gas vesicle from *A. flos-aquae*. **(b)** Diagram of the structure and composition of a gas vesicle. **(c)** Engineered genetic construct, ARG1, comprising genes from *A. flos-aquae* (green) and genes from *B. megaterium* (gray) to produce ultrasound-detectable gas vesicles in heterologous bacteria. (Bottom) Ultrasound images of *E. coli* and *S. typhimurium* expressing ARG1 or the luminescent LUX operon. **(d)** TEM of an *E. coli* Nissle 1917 cell expressing ARG1. **(e)** Ultrasound image of live mouse with ARG1-expressing *E. coli* arranged in the colon as indicated in diagram. Color map represents collapse-subtracted contrast within the colon region of interest (outlined in blue), overlaid on grayscale anatomical image. **(f)** Ultrasound images of *E. coli* expressing ARG1 or ARG2 before and after the application of two different collapse pressures. (Bottom) Unmixed contrast maps corresponding to each type of bacteria. Scale bars represent 150 nm (a), 2 mm (c), 500 nm (d), 2.5 mm (e) and 2 mm (f).
Data adapted with permission from [14••,21••].

which evolved in certain photosynthetic microbes as a means to achieve buoyancy in water, could produce ultrasound contrast [6•]. GVs are cylindrical or spindle-shaped gas-filled compartments with dimensions on the order of 200 nm, surrounded by a 2-nm thick protein shell [7] (Figure 1a,b). This shell allows gases dissolved in the surrounding media to exchange freely in and out, while preventing water from forming a liquid inside the GV due to the strong hydrophobicity of the shell's interior face. GVs are encoded in diverse organisms by operons of 8–14 genes, comprising a mixture of structural proteins and assembly factors [8]. Because they contain gas, it was hypothesized that GVs could scatter sound waves and produce ultrasound contrast. Indeed, this ability was demonstrated using GVs isolated from cyanobacteria and haloarchaea [6•]. Building on this initial discovery, several other studies have been undertaken to understand the acoustic properties of GVs [9], to engineer them through genetic and biochemical modifications [10•,11], to devise ultrasound imaging techniques tailored to distinguish their signal from background [12], and to characterize their *in vivo* biodistribution as purified, injectable agents [13]. These studies revealed remarkable non-linear acoustic properties and engineering versatility, enabling selective detection, multiplexed imaging and molecular targeting.

In parallel, a major effort was undertaken to express GVs heterologously as acoustic reporter genes, initially in commensal and pathogenic bacteria being developed as