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Genetically encodable materials for non-invasive biological imaging

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Abstract

Many questions in basic biology and medicine require the ability to visualize the function of specific cells and molecules inside living organisms. In this context, technologies such as ultrasound, optoacoustics, and magnetic resonance provide non-invasive imaging access to deep-tissue regions, as used in many laboratories and clinics to visualize anatomy and physiology. In addition, recent work has enabled these technologies to image the location and function of specific cells and molecules inside the body by coupling the physics of sound waves, nuclear spins, and light absorption to unique protein-based materials. These materials, which include air-filled GVs, capsid-like nanocompartments, pigment-producing enzymes, and transmembrane transporters, enable new forms of biomolecular and cellular contrast. The ability of these protein-based contrast agents to be genetically encoded and produced by cells creates opportunities for unprecedented *in vivo* studies of cellular function, while their amenability to genetic engineering enables atomic-level design of their physical, chemical, and biological properties.

Many advances in biology arise from new abilities to observe previously invisible biological processes. Few technologies have had as significant an impact in this regard as the green fluorescent protein (GFP) – an imaging agent that can be genetically encoded inside a cell, providing an intimate connection to its internal life cycle and molecular signals. However, due to the strong scattering of light in biological tissue, fluorescence imaging has limited utility beyond approximately one mm in depth[1] – restricting these studies to transparent animals, small model organisms, and surgically accessed regions. Vast demand exists to go deeper, driven by the need to study cellular function within the context of intact organisms, the development of cell-based diagnostic and therapeutic agents, and the engineering of complex living materials.

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Unlike fluorescent imaging, magnetic resonance imaging (MRI) and ultrasound penetrate deep inside intact tissue, resulting in their widespread use in clinical medicine. Over the past 20 years, substantial effort has been devoted to developing genetically encodable reporters for these non-invasive imaging modalities: a “GFP for MRI” and a “GFP for ultrasound”, resulting in important conceptual and practical advances.

In this Perspective, we review these advances, with a focus on the recent development of richer, more complex biological materials using the tools of protein engineering and synthetic biology. In addition to ultrasound and MRI, we cover optoacoustic (or photoacoustic) imaging – a more recent technology that combines the capability of light to interact with biomolecules with the deep penetration of ultrasound.

Each imaging modality covered in this review operates on its own set of physical principles, which determine its spatiotemporal resolution and specify the requirements of the corresponding biomolecular reporters. The resolution needed for biological research varies from the single-cell level (e.g., individual neuron firing) to the tissue level (e.g., immune cell infiltration to tumors) and organism level (e.g., the distribution of an infectious agent)[1,2]. The techniques covered in this review are best suited to applications on the scale of tissues and above, with deep-tissue spatial resolution in the order of 100 μm, and temporal resolution on the order of 1–100 ms. The exact spatiotemporal resolution depends on the specific implementation of each modality, with approximate ranges listed in Table 1.

The goal of biomolecular reporters is to take advantage of the resolution provided by these modalities by connecting their signals to cellular function. Optoacoustic imaging requires materials that can absorb photons and dissipate the resulting energy as heat[3]. Ultrasound calls for materials that can scatter sound waves due to a mechanical mismatch with their surroundings[4]. MRI requires materials that can interact with nearby nuclear spins[5]. Despite these disparate specifications, the reporter genes developed for these modalities often have a lot in common.

For this reason, we have chosen to organize this article not by imaging modalities, but along different classes of genetically encodable materials, emphasizing their evolution from relatively simple individual proteins to more complex and multifunctional self-assembling complexes. Because our focus is on materials that can be genetically encoded, we say little about the vast and exciting body of work on synthetic contrast agents, which have been the subject of other excellent reviews[6,7]. This focus also does not include reporter genes for radiation-based imaging modalities such as positron emission tomography, which always require exogenously administered radionuclides. These very useful and important reporter types are covered in excellent recent reviews[8,9].

Small proteins: enzymes, peptides, and transporters

The earliest genetically encoded reporter used for MRI was an enzyme working in conjunction with a synthetic organometallic contrast agent (Fig. 1a). In 2000, Louie *et al.* synthesized a Gd^{3+} chelator named EgadMe that incorporated a sugar as part of its organic structure[14]. The coordination of Gd^{3+} by water, which leads to T_1-weighted MRI contrast,

was completely blocked by EgadMe's sugar moiety. The enzyme β-galactosidase, then commonly used as an optical reporter of gene expression visualized with sugar-containing chromogens, cleaved off the sugar on EgadMe and thereby increased MRI contrast. While this reporter has not been used beyond its initial demonstration in frog embryos due to its modest contrast change and challenging biodistribution, it inspired the development of other genetically encodable MRI reporters.

The first protein to produce MRI contrast in the absence of external reagents was the iron storage protein ferritin, which accumulates bioavailable paramagnetic iron inside an 8-nm protein shell (inner diameter) and produces T_2-weighted MRI contrast. In 2005, two groups independently showed that overexpression of this protein could result in contrast detectable *in vivo*[15,16]. To date, ferritin has been used in more MRI reporter gene studies than any other protein. However, it leaves much to be desired in terms of its performance, and significant efforts have been made to engineer improved ferritins and alternative protein nanocompartments, as described in the next section.

Besides ferritin, other iron-containing proteins used to generate MRI contrast include methemoglobin[17], transferrin, and the cytochrome P450-BM3. A variant of the latter protein was the first MRI reagent engineered with the help of directed evolution and served as a dynamic molecular sensor of dopamine[18] (Fig. 1b). While versions of this sensor have been used to map neurotransmitter release in the brain, it has so far been employed as an injectable contrast agent rather than one expressed locally in the tissue. Besides iron, proteins have been engineered to produce MRI contrast by binding other paramagnetic metal ions such as Gd^{3+}[19]. In addition, transporters such as the transferrin receptor and OATP1 have been used to selectively accumulate externally administered iron and Gd^{3+} chelates, respectively[20,21].

Metals are not the only way to achieve MRI contrast. Other early work on protein-based MRI contrast agents focused on proteins with large numbers of exchangeable protons that can be imaged with chemical exchange saturation transfer (CEST) MRI[22] (Fig. 1c). One of the main advantages of CEST-based reporter genes is that they do not require metal cofactors, which may have limited *in situ* availability. On the other hand, they must typically be expressed at relatively high concentrations and imaged at high field strengths to be detected above the background of endogenous cellular proteins. More recently, reporter genes for CEST MRI have also been developed based on enzymes that catalyze the intracellular accumulation of synthetic CEST-active compounds[23].

While these pioneering approaches demonstrated the feasibility of protein-based MRI contrast, they have not been widely adopted by the broader biological community. The primary reasons include the requirement for relatively high concentrations, the need for metal cofactors, and competition from background tissue contrast[5].

Recently another class of non-metallic MRI reporter genes was introduced that overcomes some of these limitations. These reporters produce contrast in diffusion-weighted imaging (DWI) by altering the apparent diffusivity of water in tissue (Fig. 1d). Recognizing that the cell membrane is a dominant barrier to water diffusion, Mukherjee *et al.* showed that

the overexpression of aquaporin, a simple transmembrane channel that exclusively conducts water, could increase the apparent diffusivity of model tissues by up to 200%, resulting in a dramatic change in DWI contrast[24]. An experiment in mice showed that intracranial tumors triggered to express aquaporin could be distinguished by DWI. In a similar study published at nearly the same time, Schilling *et al.* overexpressed the urea transporter UT-B, which co-transports water with urea and also acts as a passive water channel[25].

Another innovative mechanism for genetically encodable MRI contrast is based on vasoactive peptides, whose expression leads to local vasodilation, resulting in fMRI-like contrast[26] (Fig. 1e). This allows a modest concentration of peptide to produce a relatively large signal. Reliance on hemodynamic signals complicates imaging procedures but may be extendable beyond MRI to other hemodynamic modalities. In addition, vasoactive probes can be engineered as sensors with activity conditioned on other molecules[27].

Unlike in the case of MRI, in which reporter genes had to be developed from scratch, the task of generating optoacoustic contrast with proteins was, in some ways, more straightforward. Optoacoustic imaging is a fast, volumetric technique that can map the distribution of photoabsorbers at deeper tissue layers than accessible by conventional optical microscopy by converting light absorption into sound waves via thermoelastic expansion[28,29]. Any photoabsorbing molecule that dissipates at least some of the absorbed energy non-radiatively can, in principle, produce optoacoustic contrast.

Genetically expressed chromoproteins can provide sufficient optoacoustic contrast, especially if they possess a high extinction coefficient in the near-infrared range in which absorbance from endogenous molecules such as hemoglobin is relatively low (Fig. 1f). In addition, low quantum yield is desired to maximize the conversion of photoexcitation into heat. These conditions can be fulfilled in bacteriophytochromes in which biliverdin serves as a chromophore[30–32].

Particularly attractive for increasing the signal-to-noise ratio are reversibly photoswitchable chromoproteins whose signal time course can be differentiated from static background signals even if the latter have higher amplitude[34]. Multiplexing of several reversibly switchable chromoproteins can be achieved by temporal unmixing of the respective signal time courses. In addition, the concentration-independent switching kinetics can be used to correct signal degradation due to spatially varying intensities of the illumination[35,36]. This strategy to suppress static background was particularly effective using chromoproteins with absorbance spectra in the near-infrared window[37]. Furthermore, molecules that change their absorbance spectrum as a function of surrounding analytes can be used as dynamic optoacoustic sensors. This mechanism was showcased by adapting GCaMP for optoacoustic imaging of calcium transients in zebrafish[38]. The tissue depth and sensitivity with which optoacoustic reporters and sensors can be visualized can be improved by using chromoproteins with absorption spectra further toward near-infrared wavelengths[39,40].

Protein nanocompartments as multimodal contrast materials

As briefly discussed above, the primary mammalian iron-storage compartment ferritin has been overexpressed to generate T_2-weighted MRI contrast. However, its performance is limited by the relatively small size of its iron core (7–8 nm[41]) and its weak magnetism (mostly anti-ferromagnetic[42] with paramagnetic surface spins). Several groups have attempted to improve the properties of mammalian ferritin. A fusion of the heavy and light chain was, for instance, proposed to improve performance as a one-component system[43]. Two prokaryotic one-component ferritins were also subjected to a mutational screen, yielding variants with improved iron occupancy[44,45]. However, the highly conserved iron transport and ferroxidase functionalities in ferritins seem to limit the improvement possible via protein engineering.

Could larger genetically controlled nanocompartments be generated that are more modular than ferritins and provide larger effective storage capacity? Nanostructures that self-assemble from proteinaceous building blocks are widespread in nature and have long been explored as miniature reaction vessels in semi-synthetic approaches. Douglas *et al.* showed in 2002 that the interior of the capsid encoded by the Cowpea chlorotic mottle virus (CCMV) could be subjected to electrostatic engineering to facilitate iron-mineralization *in vitro*[46].

Recently it was shown that members of a large family of prokaryotic nanocompartments called encapsulins[47–49] could be heterologously expressed at high levels in mammalian cells (Fig. 2). There they self-assemble, auto-encapsulate ferritin-like cargo proteins and lead to non-toxic iron biomineralization of up to an order of magnitude more iron per protein complex compared to ferritin[50]. Heterologous expression of encapsulin variants enabled T_2* contrast enhancement in mammalian cells in culture and upon xenografting into rat brains[50] (Fig. 2a,b). Thanks to the electron-dense iron-oxide core, encapsulins can also be detectable as fiducial markers in cryo-electron tomograms[50]. Different variants of encapsulins can also serve as multiplexable reporter genes for conventional transmission electron microscopy (TEM) to, for instance, label neuronal types or states in model organisms based on distinct geometrical features[51] (Fig. 2b,d). The genetically controlled iron biomineralization thus enables multimodal molecular imaging that can be cross-registered across vast scales ranging from MRI to electron microscopy. The two-component encapsulin-shell:ferroxidase system furthermore enables functionalization of the inner surface with proteins that can modify the crystallization process and redox state of iron, such as peptides derived from magnetotactic bacteria[50]. Notably, overexpression of iron-filled encapsulins from *Myxococcus xanthus* allowed for magnetically actuated cell sorting (MACS) using standard commercial columns, whereas expressing iron-loaded ferritin did not enable this feature[50]. Similarly, substantial MRI contrast and MACS separation were also enabled in *E. coli* expressing a fusion protein mediating iron oxidation and accumulation into a disordered ferrogel[52].

While the preceding results demonstrate that high levels of paramagnetic iron can be effective, even stronger MRI contrast and magnetic manipulation could be achieved with the formation of superparamagnetic or ferromagnetic magnetite or maghemite crystals, such as

those found in the magnetosomes of magnetotactic bacteria[53]. To date, magnetosomes have only been heterologously expressed in a close genetic relative of magnetotactic bacteria[54]. This remarkable feat has, however, not yet been achieved in common prokaryotes applied in biotechnology or eukaryotic cells.

Encapsulins can also be engineered to form nanomaterials with other material properties, such as strong photoabsorbance. This feature can, for instance, be achieved by selective targeting of enzymatic activity to the encapsulin lumen by either complementing split enzymes inside the compartment or by attaching a degradation signal that ablates all copies of the enzyme that are not encapsulated. In this way, robust contrast can be obtained in optoacoustic images by encapsulating a soluble bacterial tyrosinase, which converts tyrosine molecules entering through the shell's pores into polymeric melanin that becomes trapped in the lumen (Fig. 2a). Melanin has a broad absorbance spectrum reaching into the near-infrared range and generates strong signals in optoacoustic imaging[55] (Fig. 2b). However, melanin, in its natural form, *i.e.*, in human skin, is sequestered in membrane-enclosed melanosomes expressed by specialized melanophore cells because it tends to be toxic when freely available in cells. Compartmentalizing melanin formation into encapsulin-based "designer melanosomes" thus successfully emulates detoxification by sequestration. Given that multiple enzymes can be arrayed inside encapsulins, biosynthetic pathways for pigments such as violacein[56], with sharper absorption spectra than melanin, could be produced inside encapsulins to optimize multiplexing via multispectral optoacoustic tomography (MSOT). Such use of biosynthetic pigments can be superior to chromoproteins, which have a comparably lower photostability, presenting challenges, especially for optoacoustic microscopy techniques that apply relatively high energy densities to the sample.

Inspired by the capability of animals such as the cuttlefish to change their skin color by relocalizing pigment-filled organelles inside dedicated chromatophore cells, it was recently furthermore demonstrated that melanin-filled melanophores can be turned into optoacoustic sensors for imaging the activation of the important class of G-protein-coupled receptors (GPCRs)[57]. GPCR-ligand-induced agglomeration of the melanin-filled cellular organelles inside the reporter cells could not only be detected via an increase in the optoacoustic signal amplitude but also via a shift in the optoacoustic signal frequency, providing an orthogonal means of observing dynamically changing molecular contrast[57].

Proteins with gas

The ability of gas to produce contrast is well-established for both ultrasound and MRI. Synthetic ultrasound contrast agents include microbubbles, which obtain their ability to scatter sound waves from their differential density and compressibility relative to aqueous tissue[6]. Meanwhile, the differential magnetic susceptibility of air-filled body cavities (such as lungs and nasal passages) relative to tissue distorts MRI images. Can the unique properties of gas be harnessed in the context of genetically encodable materials?

In 2014, Shapiro *et al.* described the use of a unique class of air-filled protein nanostructures, called gas vesicles (GVs), as acoustic biomolecules for ultrasound imaging[61]. GVs are made

of a 2-nm thick protein shell that assembles into a hollow nanostructure with dimensions on the order of 100 nm (Fig. 3a). GVs are natively expressed as flotation devices in a number of waterborne microbes, where they are encoded by operons of 8–14 genes, including structural proteins and assembly factors essential for GV formation. The large acoustic impedance mismatch between the GVs' gaseous interior and surrounding aqueous media allows these nanoparticles to produce ultrasound contrast *in vitro* and *in vivo*. In addition, the ability of certain natural and engineered GV genotypes to undergo buckling mechanical deformations under ultrasound results in nonlinear contrast, facilitating their detection against background tissue[62–65]. Since GVs are genetically encodable, their mechanics can be tuned using protein engineering techniques, and they can be functionalized with new surface properties and targeting moieties[64,66].

To turn GVs into acoustic reporter genes (ARGs) for ultrasound, the polycistronic gene clusters encoding GV assembly must be adapted from their native organisms into new species. This was first accomplished in bacteria. By combining GV genes from two organisms, *Anabaena flos-aquae* and *Bacillus megaterium*, a hybrid cluster was developed (Fig. 3b) that encodes the expression of GVs in *E. coli* and *Salmonella typhimurium*, two commensal microbes and common chassis for synthetic biology[67]. This approach enabled the imaging of bacterial gene expression inside the GI tract of mice. Bacterial ARGs open the possibility of studying and tracking microbial interactions inside mammalian hosts with ultrasound and can be a powerful tool in the development of microbial diagnostics and therapeutics[68]. Significant scope exists to optimize the expression and acoustic properties of bacterial ARGs and to deploy them in a greater number of species and *in vivo* scenarios. In each application, it will be critical to verify that ARG expression does not present an unacceptable metabolic burden or change in cell phenotype.

After bacterial expression, the next major milestone was to develop ARGs for mammalian cells. Transferring a large polycistronic program for self-assembly from prokaryotes to eukaryotes is a major challenge in synthetic biology due to the differential handling of transcription and translation between these kingdoms and the need to ensure proper folding, stoichiometry, and assembly of the constituent proteins. Farhadi *et al.* overcame this challenge by constructing mammalian ARG operons based on 9 genes from *B. megaterium*, stringing groups of these genes together using viral 2A self-cleavage peptides and controlling stoichiometry through copy number[58] (Fig. 3, c–d). ARG expression could then be imaged in human cells *in vitro* at volumetric densities below 0.5% and in cells expressing just a few GVs per cell. *In vivo*, ARG expression was imaged in a mouse tumor xenograft, revealing localized gene expression with a spatial resolution of 100 μm (Fig. 3e). ARG imaging in mammalian cells was enabled by a highly sensitive ultrasound imaging paradigm taking advantage of strong acoustic emissions from GVs as they collapse in response to acoustic pressure. Further optimization is needed to turn mammalian ARGs into a commonly used reporter gene. For example, expression in primary cells such as neurons and immune cells will benefit from the packaging of ARGs into viral vectors, which typically requires a smaller genetic footprint. Additional study of the immune response to GVs and GV-expression is also needed to enable clinical translation of this technology in the context of cell-based diagnostics and therapeutics.

Very recently, GVs were engineered to function as acoustic biosensors that dynamically change their ultrasound contrast in response to the activity of proteases[69]. This was accomplished by modifying a surface protein of the GV shell to be recognized and cleaved by specific enzymes, resulting in decreased shell stiffness and increased nonlinear ultrasound contrast. The functionality of these biosensors was demonstrated in intracellular sensing of protease activity in bacteria located in the mouse GI tract.

Can GVs also serve as reporter genes for MRI? This possibility was realized by Lu *et al.* by demonstrating that the presence of GVs leads to dephasing of proton nuclear spins, yielding T_2/T_2*-weighted MRI contrast. This phenomenon is based on the magnetic susceptibility difference between the air-filled interior of GVs (slightly paramagnetic) and surrounding aqueous media (diamagnetic) (Fig. 3a)[59]. Furthermore, the collapse of GVs with ultrasound during MRI acquisition allowed acoustic modulation of the GVs' MRI contrast and the acquisition of background-subtracted images. This allowed their molecular contrast to be easily distinguished from potentially confounding endogenous contrast sources, as demonstrated *in vitro* and in several mouse organs (Fig. 3f). In addition to conventional proton MRI, GVs are also able to serve as contrast agents for hyperpolarized ^{129}Xe[70]. In this application, the protein shell of GVs allows xenon dissolved in the surrounding solution to partition in and out of the GV, enabling the production of CEST contrast. Because hyperpolarization greatly boosts the signal obtained from each nucleus, this scheme increases the sensitivity of GV detection, reaching sub-nM levels. Since the introduction of GVs as the first reporter gene for ^{129}Xe-MRI, other proteins have also been shown to bind xenon and produce CEST contrast[71].

Besides ultrasound and MRI, the gaseous core of GVs provides an opportunity for their use as genetically encodable contrast agents for optical imaging techniques sensitive to refractive index, which differs substantially between air and water (Fig. 3a). For example, it was recently shown that GVs can serve as contrast agents for optical coherence tomography (OCT), a modality widely used in biomedical imaging due to its ability to provide single-μm spatial resolution at tissue depths of several mm. In this application, GVs play a role directly analogous to ultrasound by backscattering photons, as shown *in vitro* and the mouse eye[60] (Fig. 3g). In a separate study, it was shown that the propagation of light waves through GVs distorts their phase, allowing GVs and GV-expressing cells to be visualized using digital holographic microscopy (DHM), a volumetric imaging technique with unique advantages for *in vitro* microscopy[72].

Alongside their uses in imaging, GVs can transduce ultrasound into mechanical force[73] and inertial bubble cavitation[74], allowing GVs and GV-expressing cells to be manipulated with acoustic fields and serve as therapeutic agents for targeted cell killing and drug release. These additional capabilities enable new possibilities in cellular actuation, engineered living materials[75], and theranostics that are beyond the scope of this Perspective.

Outlook

The new materials and approaches described above have the potential to provide unprecedented access to visualizing cellular states and functions *in vivo.* However, many

challenges and opportunities remain for improved performance and broader applications. While most of the materials used as genetically encoded contrast agents have been derived from naturally evolved genes, it should be possible to access a wider range of physical properties through *de novo* protein design, taking advantage of rapid progress in the engineering of proteins with a new structure, self-assembly, and function[76]. As with natural proteins, *de novo* constructs for imaging could be improved with directed evolution[18] and machine learning[77], and new properties could be added by employing non-canonical amino acids and bio-orthogonal chemistry[78]. Going beyond proteins, new ways to generate complex structures with nucleic acids, sugars, and other cellular polymers may enable new functionality. In parallel, natural genomes containing the Earth's collective evolutionary diversity will doubtless continue to offer unexpected new materials and inspiration for biomimetic designs.

This Perspective has emphasized the advantages of leveraging more complex, self-assembling biomaterials. Continuing to engineer such materials and harness even more complex structures such as magnetosomes will require operating at the limits of synthetic biology, including not just improved ways of combining and delivering genes, but gaining control over cellular phases, compartments, and specialized organelles. Besides, modifications of the host cell's genome may also be needed to enable the expression of new materials or minimize the impact on host cell viability and function. In addition, it may be possible to leverage the dynamic behavior of synthetic biological circuits to produce time-varying signals to enhance the sensitivity and specificity of imaging. Achieving these goals is likely to advance not just biological imaging, but synthetic biology itself[79]. In addition, recent discoveries of new cellular compartments and phases may enable the development of entirely new genetically encodable reporter classes that have not yet been explored[80,81].

Another relatively unexplored frontier in biological imaging *in vivo* is the development of dynamic sensors for cellular signals ranging from extracellular neurotransmitters and proteases to intracellular ions such as calcium. A wide array of dynamic biosensors is available for fluorescence microscopy[82], while relatively few examples have been put forward for ultrasound, MRI, or optoacoustic imaging. Beyond imaging, there is ample opportunity for engineering genetically encoded materials, with penetrant forms of energy, to serve as molecular actuators for controlling molecular signals and cellular function non-invasively *in vivo*[4,83].

Considerable effort must be devoted to refining the safety, ease-of-use, and robustness of genetically encodable materials to enable their widespread use in diverse laboratories and clinical scenarios. Ideally, these reporters should minimally affect the cell's endogenous processes and precious metabolic resources. With these improvements in performance and capabilities, genetically encodable reporters for non-invasive imaging will play a more significant role in basic biology, cell-based diagnostics, therapeutics, and engineered living materials. It may one day be possible for biologists to order a GV-expressing or encapsulin-expressing transgenic mouse to study the function of a certain cell type *in vivo* as easily as is standard today with mouse lines expressing GFP or to select from a catalog of viral vectors expressing these reporters for convenient labeling of cells or tissues. In engineered living materials[75], these same reporters are likely to play an increasing role as cell-based

and cell-made structures continue to scale in dimensions beyond the reach of optical microscopy. Finally, genetically encoded reporters have the opportunity to help address the need to track and monitor the performance of genetic and cellular therapeutics during both preclinical development and deployment in patients. The possibility that some of the materials discussed in this Perspective can also serve as agents for cellular manipulation and therapy will help propel them deeper into each of these application areas.

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REFERENCES

1. Piraner DI et al. Going Deeper: Biomolecular Tools for Acoustic and Magnetic Imaging and Control of Cellular Function. Biochemistry 56, 5202–5209 (2017). [PubMed: 28782927]
2. Marblestone AH et al. Physical principles for scalable neural recording. Front. Comput. Neurosci 7, 137 (2013). [PubMed: 24187539]
3. Wang LV & Yao J A practical guide to photoacoustic tomography in the life sciences. Nat. Methods 13, 627–638 (2016). [PubMed: 27467726]
4. Maresca D et al. Biomolecular Ultrasound and Sonogenetics. Annu. Rev. Chem. Biomol. Eng 9, 229–252 (2018). [PubMed: 29579400]
5. Mukherjee A, Davis HC, Ramesh P, Lu GJ & Shapiro MG Biomolecular MRI reporters: Evolution of new mechanisms. Prog. Nucl. Magn. Reson. Spectrosc 102–103, 32–42 (2017).
6. Paefgen V, Doleschel D & Kiessling F Evolution of contrast agents for ultrasound imaging and ultrasound-mediated drug delivery. Front. Pharmacol 6, 197 (2015). [PubMed: 26441654]
7. Wahsner J, Gale EM, Rodríguez-Rodríguez A & Caravan P Chemistry of MRI Contrast Agents: Current Challenges and New Frontiers. Chem. Rev 119, 957–1057 (2019). [PubMed: 30350585]
8. Chung J-K Sodium iodide symporter: its role in nuclear medicine. J. Nucl. Med 43, 1188–1200 (2002). [PubMed: 12215558]
9. Kircher MF, Gambhir SS & Grimm J Noninvasive cell-tracking methods. Nat. Rev. Clin. Oncol 8, 677–688 (2011). [PubMed: 21946842]
10. Errico C et al. Ultrafast ultrasound localization microscopy for deep super-resolution vascular imaging. Nature 527, 499–502 (2015). [PubMed: 26607546]
11. Luís Dean-Ben X & Razansky D Localization optoacoustic tomography. Light Sci Appl 7, 18004 (2018). [PubMed: 30839533]
12. Seeger M et al. Pushing the boundaries of optoacoustic microscopy by total impulse response characterization. Nat. Commun 11, 2910 (2020). [PubMed: 32518250]
13. Suetens P Fundamentals of Medical Imaging. (Cambridge University Press, 2017).
14. Louie AY et al. In vivo visualization of gene expression using magnetic resonance imaging. Nat. Biotechnol 18, 321–325 (2000). [PubMed: 10700150]
15. Genove G, DeMarco U, Xu H, Goins WF & Ahrens ET A new transgene reporter for in vivo magnetic resonance imaging. Nat. Med 11, 450–454 (2005). [PubMed: 15778721]
16. Cohen B, Dafni H, Meir G, Harmelin A & Neeman M Ferritin as an endogenous MRI reporter for noninvasive imaging of gene expression in C6 glioma tumors. Neoplasia 7, 109–117 (2005). [PubMed: 15802016]

17. Duewell S, Kasserra CE, Jezzard P & Balaban RS Evaluation of methemoglobin as an autologous intravascular MRI contrast agent. Magn. Reson. Med 35, 787–789 (1996). [PubMed: 8722831]
18. Shapiro MG et al. Directed evolution of a magnetic resonance imaging contrast agent for noninvasive imaging of dopamine. Nat. Biotechnol 28, 264–270 (2010). [PubMed: 20190737]
19. Yang JJ et al. Rational design of protein-based MRI contrast agents. J. Am. Chem. Soc 130, 9260–9267 (2008). [PubMed: 18576649]
20. Deans AE et al. Cellular MRI contrast via coexpression of transferrin receptor and ferritin. Magn. Reson. Med 56, 51–59 (2006). [PubMed: 16724301]
21. Patrick PS et al. Dual-modality gene reporter for in vivo imaging. Proceedings of the National Academy of Sciences 111, 415–420 (2014).
22. Gilad AA et al. Artificial reporter gene providing MRI contrast based on proton exchange. Nat. Biotechnol 25, 217–219 (2007). [PubMed: 17259977]
23. Yuan Y et al. Furin-mediated intracellular self-assembly of olsalazine nanoparticles for enhanced magnetic resonance imaging and tumour therapy. Nat. Mater 18, 1376–1383 (2019). [PubMed: 31636420]
24. Mukherjee A, Wu D, Davis HC & Shapiro MG Non-invasive imaging using reporter genes altering cellular water permeability. Nat. Commun 7, 13891 (2016). [PubMed: 28008959]
25. Schilling F et al. MRI measurements of reporter-mediated increases in transmembrane water exchange enable detection of a gene reporter. Nat. Biotechnol 35, 75–80 (2017). [PubMed: 27918546]
26. Desai M, Slusarczyk AL, Chapin A, Barch M & Jasanoff A Molecular imaging with engineered physiology. Nat. Commun 7, 13607 (2016). [PubMed: 27910951]
27. Ohlendorf R et al. Target-responsive vasoactive probes for ultrasensitive molecular imaging. Nature Communications 11, 2399 (2020).
28. Ntziachristos V Going deeper than microscopy: the optical imaging frontier in biology. Nat. Methods 7, 603–614 (2010). [PubMed: 20676081]
29. Wang LV & Hu S Photoacoustic tomography: in vivo imaging from organelles to organs. Science 335, 1458–1462 (2012). [PubMed: 22442475]
30. Shu X et al. Mammalian Expression of Infrared Fluorescent Proteins Engineered from a Bacterial Phytochrome. Science 324, 804–807 (2009). [PubMed: 19423828]
31. Filonov GS et al. Bright and stable near-infrared fluorescent protein for in vivo imaging. Nat. Biotechnol 29, 757–761 (2011). [PubMed: 21765402]
32. Fuenzalida Werner JP et al. Structure-Based Mutagenesis of Phycobiliprotein smURFP for Optoacoustic Imaging. ACS Chem. Biol 14, 1896–1903 (2019). [PubMed: 31389680]
33. Goddard TD et al. UCSF ChimeraX: Meeting modern challenges in visualization and analysis. Protein Sci. 27, 14–25 (2018). [PubMed: 28710774]
34. Stiel AC et al. High-contrast imaging of reversibly switchable fluorescent proteins via temporally unmixed multispectral optoacoustic tomography. Opt. Lett 40, 367–370 (2015). [PubMed: 25680049]
35. Deán-Ben XL et al. Light fluence normalization in turbid tissues via temporally unmixed multispectral optoacoustic tomography. Opt. Lett 40, 4691–4694 (2015). [PubMed: 26469596]
36. Luís Deán-Ben X et al. Light fluence estimation by imaging photoswitchable probes with temporally unmixed multispectral optoacoustic tomography. in Biomedical Optics 2016 OTu2A.6 (Optical Society of America, 2016).
37. Yao J et al. Multiscale photoacoustic tomography using reversibly switchable bacterial phytochrome as a near-infrared photochromic probe. Nat. Methods 13, 67–73 (2016). [PubMed: 26550774]
38. Deán-Ben XL et al. Functional optoacoustic neuro-tomography for scalable whole-brain monitoring of calcium indicators. Light Sci Appl 5, e16201 (2016). [PubMed: 30167137]
39. Qian Y et al. A genetically encoded near-infrared fluorescent calcium ion indicator. Nat. Methods 16, 171–174 (2019). [PubMed: 30664778]
40. Li L et al. Small near-infrared photochromic protein for photoacoustic multi-contrast imaging and detection of protein interactions in vivo. Nat. Commun 9, 2734 (2018). [PubMed: 30013153]

41. Jutz G, van Rijn P, Santos Miranda B & Böker A Ferritin: a versatile building block for bionanotechnology. Chem. Rev 115, 1653–1701 (2015). [PubMed: 25683244]
42. Gossuin Y, Gillis P, Hocq A, Vuong QL & Roch A Magnetic resonance relaxation properties of superparamagnetic particles. Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol 1, 299–310 (2009). [PubMed: 20049798]
43. Iordanova B, Robison CS & Ahrens ET Design and characterization of a chimeric ferritin with enhanced iron loading and transverse NMR relaxation rate. J. Biol. Inorg. Chem 15, 957–965 (2010). [PubMed: 20401622]
44. Matsumoto Y, Chen R, Anikeeva P & Jasanoff A Engineering intracellular biomineralization and biosensing by a magnetic protein. Nat. Commun 6, 8721 (2015). [PubMed: 26522873]
45. Liu X et al. Engineering Genetically-Encoded Mineralization and Magnetism via Directed Evolution. Sci. Rep 6, 38019 (2016). [PubMed: 27897245]
46. Douglas T et al. Protein engineering of a viral cage for constrained nanomaterials synthesis. Adv. Mater 14, 415 (2002).
47. McHugh CA et al. A virus capsid-like nanocompartment that stores iron and protects bacteria from oxidative stress. EMBO J. 33, 1896–1911 (2014). [PubMed: 25024436]
48. He D et al. Structural characterization of encapsulated ferritin provides insight into iron storage in bacterial nanocompartments. Elife 5, (2016).
49. Giessen TW & Silver PA Widespread distribution of encapsulin nanocompartments reveals functional diversity. Nat Microbiol 2, 17029 (2017). [PubMed: 28263314]
50. Sigmund F et al. Bacterial encapsulins as orthogonal compartments for mammalian cell engineering. Nat. Commun 9, 1990 (2018). [PubMed: 29777103]
51. Sigmund F et al. Iron-Sequestering Nanocompartments as Multiplexed Electron Microscopy Gene Reporters. ACS Nano 13, 8114–8123 (2019). [PubMed: 31194509]
52. Ramesh P et al. Ultraparamagnetic Cells Formed through Intracellular Oxidation and Chelation of Paramagnetic Iron. Angew. Chem. Int. Ed Engl 57, 12385–12389 (2018). [PubMed: 30089191]
53. Komeili A Molecular mechanisms of compartmentalization and biomineralization in magnetotactic bacteria. FEMS Microbiol. Rev 36, 232–255 (2012). [PubMed: 22092030]
54. Kolinko I et al. Biosynthesis of magnetic nanostructures in a foreign organism by transfer of bacterial magnetosome gene clusters. Nat. Nanotechnol 9, 193–197 (2014). [PubMed: 24561353]
55. Stritzker J et al. Vaccinia virus-mediated melanin production allows MR and optoacoustic deep tissue imaging and laser-induced thermotherapy of cancer. Proc. Natl. Acad. Sci. U. S. A 110, 3316–3320 (2013). [PubMed: 23401518]
56. Jiang Y et al. Violacein as a genetically-controlled, enzymatically amplified and photobleaching-resistant chromophore for optoacoustic bacterial imaging. Sci. Rep 5, 11048 (2015). [PubMed: 26091543]
57. Lauri A et al. Whole-Cell Photoacoustic Sensor Based on Pigment Relocalization. ACS Sens 4, 603–612 (2019). [PubMed: 30663315]
58. Farhadi A, Ho GH, Sawyer DP, Bourdeau RW & Shapiro MG Ultrasound imaging of gene expression in mammalian cells. Science 365, 1469–1475 (2019). [PubMed: 31604277]
59. Lu GJ et al. Acoustically modulated magnetic resonance imaging of gas-filled protein nanostructures. Nat. Mater 17, 456–463 (2018). [PubMed: 29483636]
60. Lu GJ et al. Genetically Encodable Contrast Agents for Optical Coherence Tomography. ACS Nano 14, 7823–7831 (2020). [PubMed: 32023037]
61. Shapiro MG et al. Biogenic gas nanostructures as ultrasonic molecular reporters. Nat. Nanotechnol 9, 311–316 (2014). [PubMed: 24633522]
62. Maresca D et al. Nonlinear ultrasound imaging of nanoscale acoustic biomolecules. Appl. Phys. Lett 110, (2017).
63. Maresca D, Sawyer DP, Renaud G, Lee-Gosselin A & Shapiro MG Nonlinear X-Wave Ultrasound Imaging of Acoustic Biomolecules. Phys. Rev. X 8, 041002 (2018). [PubMed: 34040818]
64. Lakshmanan A et al. Molecular Engineering of Acoustic Protein Nanostructures. ACS Nano 10, 7314–7322 (2016). [PubMed: 27351374]

65. Cherin E et al. Acoustic Behavior of Halobacterium salinarum Gas Vesicles in the High-Frequency Range: Experiments and Modeling. Ultrasound Med. Biol 43, 1016–1030 (2017). [PubMed: 28258771]
66. Lakshmanan A et al. Preparation of biogenic gas vesicle nanostructures for use as contrast agents for ultrasound and MRI. Nat. Protoc 12, 2050–2080 (2017). [PubMed: 28880278]
67. Bourdeau RW et al. Acoustic reporter genes for noninvasive imaging of microorganisms in mammalian hosts. Nature 553, 86–90 (2018). [PubMed: 29300010]
68. Riglar DT & Silver PA Engineering bacteria for diagnostic and therapeutic applications. Nat. Rev. Microbiol 16, 214–225 (2018). [PubMed: 29398705]
69. Lakshmanan A et al. Acoustic biosensors for ultrasound imaging of enzyme activity. Nat. Chem. Biol 16, 988–996 (2020). [PubMed: 32661379]
70. Shapiro MG et al. Genetically encoded reporters for hyperpolarized xenon magnetic resonance imaging. Nat. Chem 6, 629–634 (2014). [PubMed: 24950334]
71. Wang Y, Roose BW, Palovcak EJ, Carnevale V & Dmochowski IJ A genetically encoded β-lactamase reporter for ultrasensitive 129Xe NMR in Mammalian Cells. Angew. Chem. Int. Ed 55, 8984–8987 (2016).
72. Farhadi A et al. Genetically encoded phase contrast agents for digital holographic microscopy. bioRxiv 833830 (2019) doi:10.1101/833830.
73. Wu D et al. Genetically encoded nanostructures enable acoustic manipulation of engineered cells. bioRxiv 691105 (2019) doi:10.1101/691105.
74. Bar-Zion A et al. Acoustically Detonated Biomolecules for Genetically Encodable Inertial Cavitation. bioRxiv 620567 (2019) doi:10.1101/620567.
75. Gilbert C & Ellis T Biological Engineered Living Materials: Growing Functional Materials with Genetically Programmable Properties. ACS Synth. Biol 8, 1–15 (2019). [PubMed: 30576101]
76. Huang P-S, Boyken SE & Baker D The coming of age of de novo protein design. Nature 537, 320–327 (2016). [PubMed: 27629638]
77. Yang KK, Wu Z & Arnold FH Machine-learning-guided directed evolution for protein engineering. Nat. Methods 16, 687–694 (2019). [PubMed: 31308553]
78. Lang K & Chin JW Cellular incorporation of unnatural amino acids and bioorthogonal labeling of proteins. Chem. Rev 114, 4764–4806 (2014). [PubMed: 24655057]
79. Gilad AA & Shapiro MG Molecular Imaging in Synthetic Biology, and Synthetic Biology in Molecular Imaging. Molecular Imaging and Biology vol. 19 373–378 (2017). [PubMed: 28213833]
80. Banani SF, Lee HO, Hyman AA & Rosen MK Biomolecular condensates: organizers of cellular biochemistry. Nat. Rev. Mol. Cell Biol 18, 285–298 (2017). [PubMed: 28225081]
81. Bracha D, Walls MT & Brangwynne CP Probing and engineering liquid-phase organelles. Nat. Biotechnol 37, 1435–1445 (2019). [PubMed: 31792412]
82. Palmer AE, Qin Y, Park JG & McCombs JE Design and application of genetically encoded biosensors. Trends Biotechnol. 29, 144–152 (2011). [PubMed: 21251723]
83. Szablowski JO, Bar-Zion A & Shapiro MG Achieving Spatial and Molecular Specificity with Ultrasound-Targeted Biomolecular Nanotherapeutics. Acc. Chem. Res 52, 2427–2434 (2019). [PubMed: 31397992]

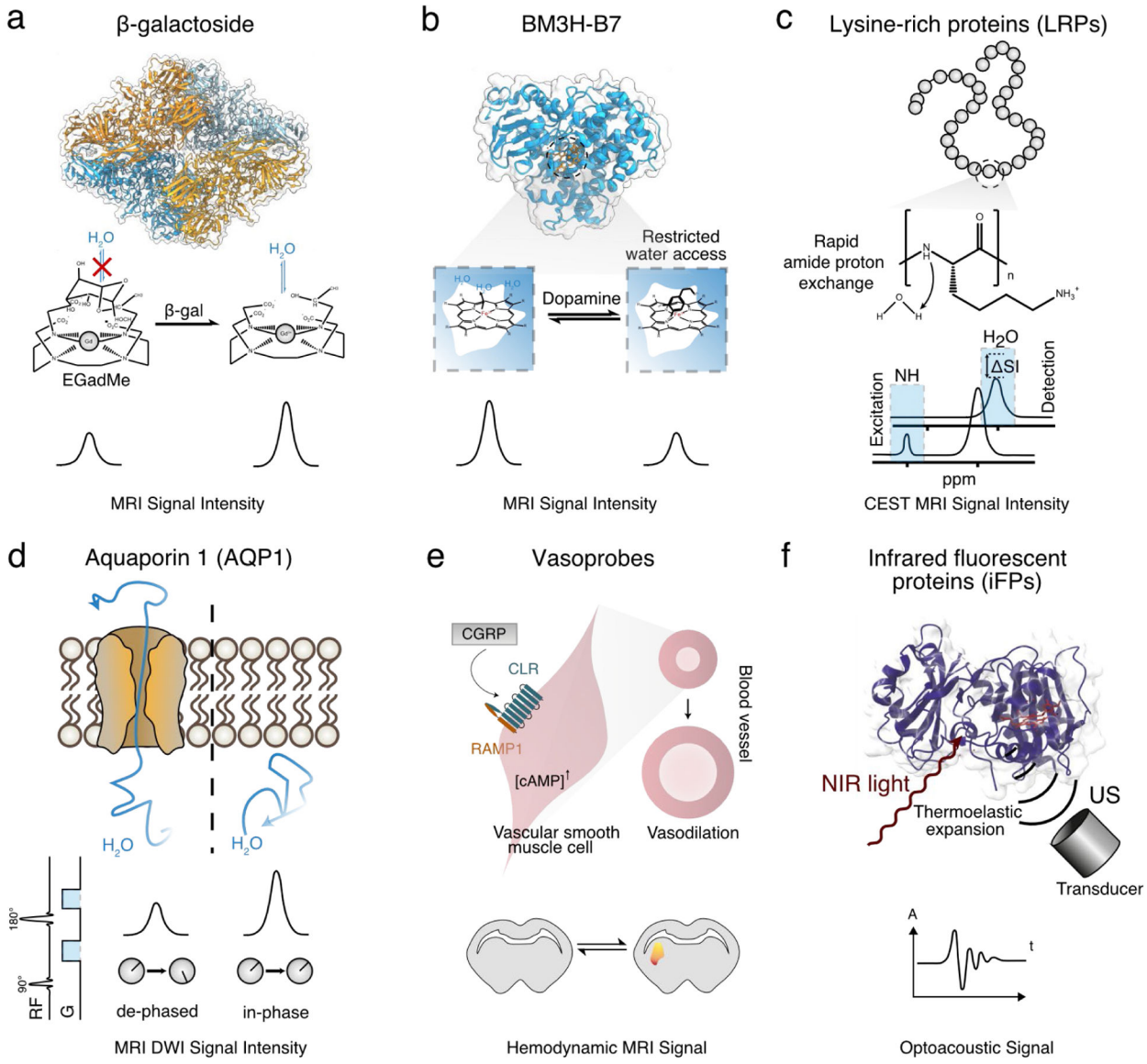

Fig. 1. Small proteins as genetically encoded contrast agents for non-invasive imaging.

(a) The tetrameric enzyme beta-galactosidase cleaves the galactopyranosyl ring on the synthetic Gd^{3+} chelator EgadMe, leading to increased water binding and T_1-weighted MRI contrast. (b) The heme-binding domain of P450-BM3 was evolved to selectively bind the neurotransmitter dopamine to alter water access to the paramagnetic Fe^{3+}, yielding a molecular sensor of dopamine for T_1-weighted MRI. (c) Designed lysine repeat proteins (LRPs) rapidly exchange amide protons with water, thus yielding enhanced contrast in chemical exchange saturation transfer (CEST) MRI. (d) Reporter gene for diffusion-weighted MRI based on increased water diffusion across the cell membrane after overexpression of Aquaporin 1 (AQP1) (e) Hemodynamic contrast mechanism based on local expression and release of vasoactive peptides lead to increased blood flow detectable with fMRI or other imaging techniques sensitive to hemodynamics. (f) Bacterial phytochrome-derived infrared fluorescent proteins (iFPs) can serve as contrast agents

for optoacoustic imaging. When absorbing near-infrared laser pulses, the chromophores transform photons into pressure waves detectable with ultrasound. PDB structures 3J7H (β-galactosidase), 4DU2 (BM3h-B7) and 4CQH (iFP 2.0) were visualized using ChimeraX[33]. Adapted from ref.[26], Springer Nature Ltd. (e).

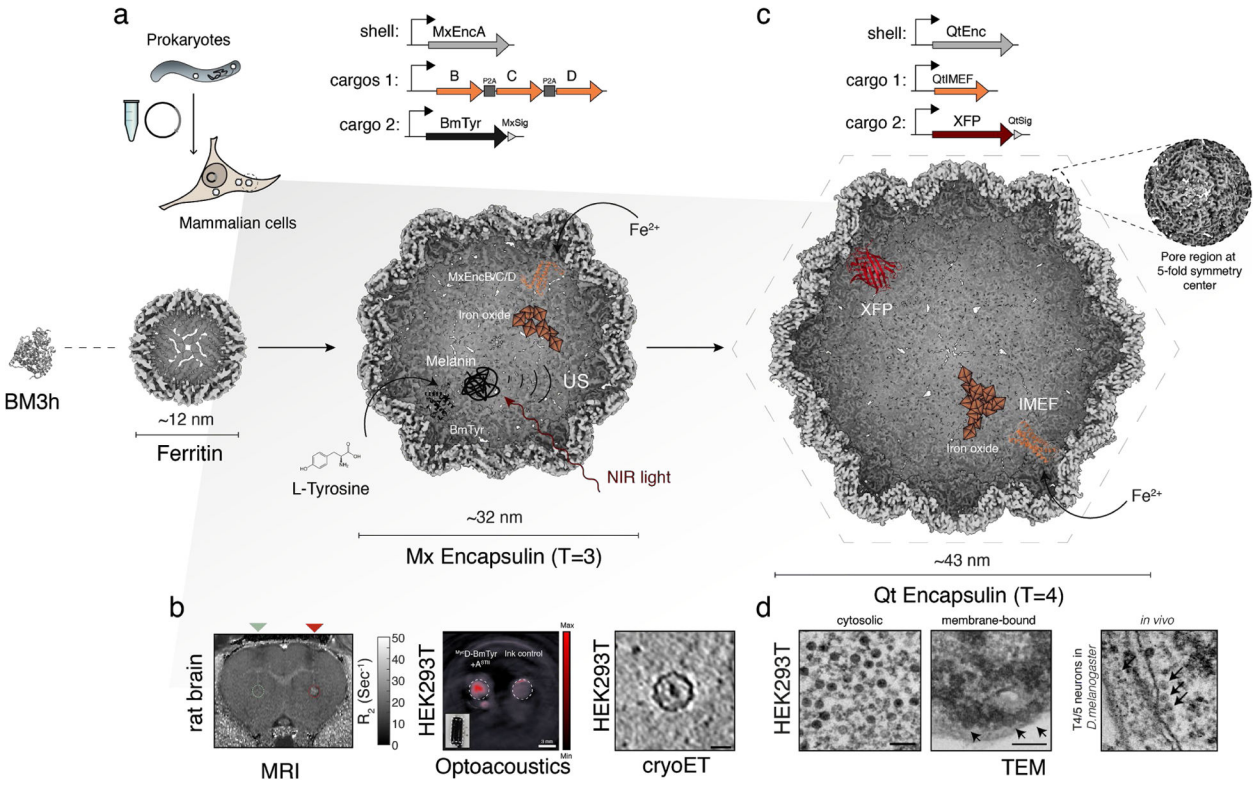

Fig. 2. Proteinaceous nanocompartments as multiscale contrast agents.

Schematic summarizing work on metalloproteins for molecular imaging applications. (a) Genetic constructs for expression of the *M. xanthus* encapsulin system in mammalian systems consisting of its shell forming monomer MxEncA and a multigene expression cassette for co-expression of its endogenous cargo proteins (MxEncBCD) or engineered cargos such as a soluble bacterial tyrosinase (BmTyr) with a C-terminal encapsulation signal. Cutaway view of the MxEnc nanocompartment (T=3) schematically showing internal cargo proteins either yielding iron oxides for detection in MRI or cryoET or melanin pigments that afford (b) detection by MRI, optoacoustics, and cryo-electron tomography. (c) Genetic constructs for expression of the *Q. thermotolerans* encapsulin system in mammalian systems consisting of its shell forming monomer QtEnc and its iron-mineralizing cargo protein QtIMEF, or other engineered cargos such as fluorescent proteins. Cutaway view of the larger QtEnc nanocompartment (T=4 icosahedral symmetry) showing a zoom-in onto the pore region at the fivefold symmetry center and docked QtIMEF cargo yielding effective iron biomineralization affording contrast in TEM images of (d) HEK293T cells and T4/5 *Drosophila* neurons. Structures of BM3h (PDB: 4DU2), ferritin (EMD-2788), Mx Encapsulin (EMD-5917), BmTyr (PDB: 3NM8), Qt Encapsulin (EMD-4879) and QtIMEF (PDB: 6N63) were visualized using ChimeraX[33]. Adapted from Ref.[50], Springer Nature Ltd (b) and Ref.[51], American Chemical Society (d).

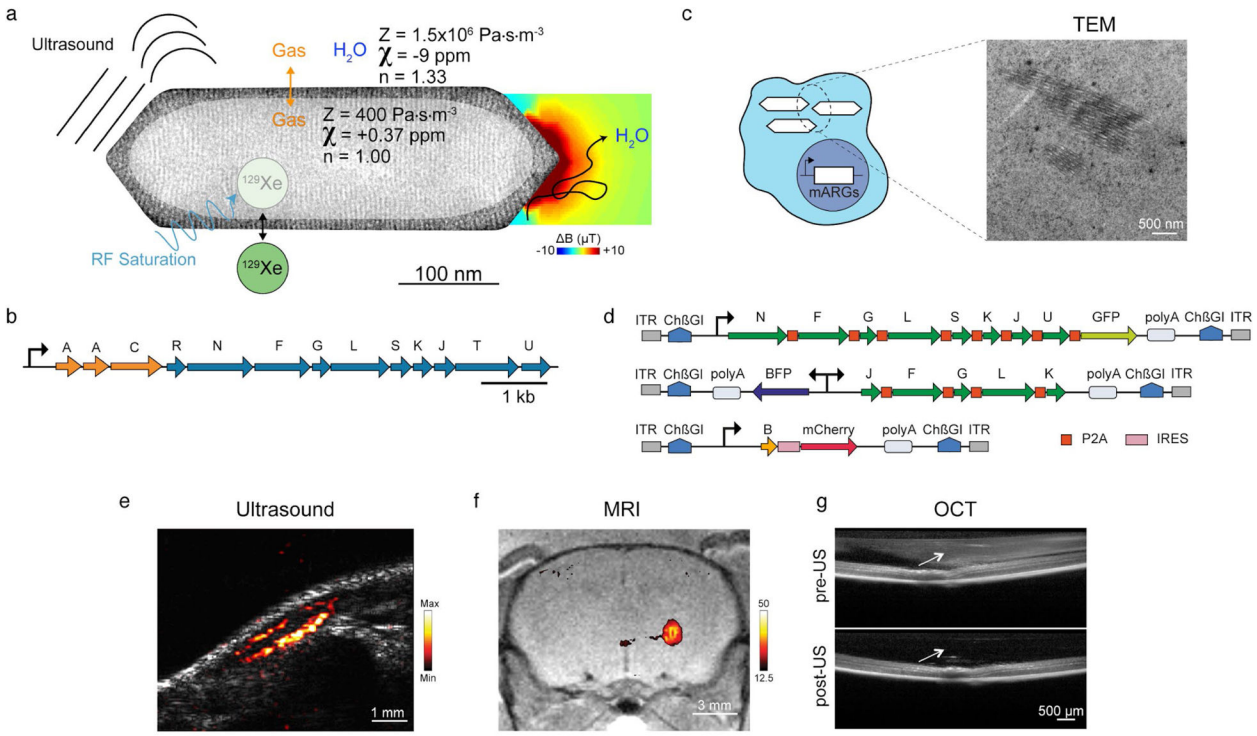

Fig. 3. Genetically encodable air-filled protein nanostructures as multimodality contrast agents.

(a) Transmission electron micrograph of a GV, and a diagram of the various material properties used to produce contrast in imaging modalities. Z, acoustic impedance; χ, magnetic susceptibility; n, index of refraction. (b) Engineered bacterial gene cluster, ARG1, comprising genes from *A. flos-aquea* (orange) and *B. megaterium* (blue) that encode the heterologous expression of GVs in bacteria. (c) Representative electron micrograph of heterologously expressed GVs in the cytosol of mammalian cells. (d) Synthetic mammalian operon, mARG1, comprising 9 genes originating from *B. megaterium* that result in GV expression in mammalian cells. (e-g) GVs as genetically encodable contrast agents and reporter genes for *in vivo* (e) ultrasound imaging[58], (f) MRI[59], and (g) OCT[60]. Adapted from ref.[58], AAAS (c,d,e), ref.[59], Springer Nature Ltd. (f).

Table 1

Key parameters for imaging modalities covered in this article.

Imaging Modality	Material property of contrast agent	Imaging Depth	Spatial Resolution	Temporal Resolution
Optoacoustics[#3]	Photoabsorption and nonradiative decay	~ 2 cm[#]	50–500 μm[#]	1–100 ms
Ultrasound[4]	Acoustic scattering	~ 10 cm	50–500 μm[#]	1–100 ms
MRI[5,13]	Spin relaxation or saturation	~ 50 cm	100 μm–1 mm	100–1000 ms

[#]Acoustically-resolved; localization microscopy techniques can achieve spatial resolution in the μm range[10,11]; optically-resolved optoacoustics can achieve sub-μm resolution below ~ 1 mm imaging depth[12]