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Molecular Imaging in Synthetic Biology, and Synthetic Biology in Molecular Imaging

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Abstract

Biomedical synthetic biology is an emerging field in which cells are engineered at the genetic level to carry out novel functions with relevance to biomedical and industrial applications. This approach promises new treatments, imaging tools and diagnostics for diseases ranging from gastrointestinal inflammatory syndromes to cancer, diabetes and neurodegeneration. As these cellular technologies undergo pre-clinical and clinical development, it is becoming essential to monitor their location and function *in vivo*, necessitating appropriate molecular imaging strategies, and therefore we have created an Interest Group within the World Molecular Imaging Society focusing on synthetic biology and reporter gene technologies. Here, we highlight recent advances in biomedical synthetic biology, including bacterial therapy, immunotherapy and regenerative medicine. We then discuss emerging molecular imaging approaches to facilitate *in vivo* applications, focusing on reporter genes for noninvasive modalities such as magnetic resonance, ultrasound, photoacoustic imaging, bioluminescence and radionuclear imaging. Because reporter genes can be incorporated directly into engineered genetic circuits, they are particularly well suited to imaging synthetic biological constructs, and developing them provides opportunities for creative molecular and genetic engineering.

MAIN TEXT

Role of imaging in biomedical synthetic biology

Synthetic biology is defined by the development of modified genetic elements, circuits and cells to perform new functions that are not part of their normal functional repertoire. Since

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its initial development starting around 2000, this discipline has impacted diverse fields ranging from industrial chemical synthesis to human health[1]. Advances in synthetic biology are accelerating in part due to the exponentially decreasing costs of DNA sequencing, synthesis and assembly[2], providing a rich catalog of natural genetic elements to use in engineered constructs and enabling rapid and inexpensive assembly and testing of genetic circuits. The potential of synthetic biology to make an impact in biomedicine[3, 4] is exemplified by recent developments in cellular diagnostics, therapeutics and genome editing, and imaging has been critical in the development and use of these now tools. In principle, cells are capable of more sophisticated functionality than molecular or nanoparticle-based therapeutic platforms. Cells can migrate, proliferate, detect signals in their environment, perform logic operations and produce outputs such as the secretion or display of biomolecules, targeted cell killing and suicide[5]. Similarly, they can carry indicator lights, molecular tags or molecular antennae for sensing and imaging. These capabilities have led, for example, to the development of genetically programmed microbial cells for gastrointestinal and tumor-targeted therapies and diagnostics, engineered immune cells for cancer immunotherapy, and other modified cell types for regenerative medicine. In addition to cellular therapeutics, gene therapy and genome editing – designed to modify the DNA of endogenous cells – are also rapidly emerging as a viable approach to treating a wide range of diseases. As discussed below, each of these synthetic biological systems is designed to operate at specific anatomical locations *in vivo*, making it important to monitor its distribution and function with molecular imaging technology (Figure 1).

Synthetic biology was first developed in prokaryotes, which provided a convenient platform for genetic engineering and industrial applications. In parallel, studies of the mammalian microbiome uncovered important roles for bacteria in health and disease, including infection, immunity, nervous system function and metabolic homeostasis[6–10]. The convergence of these research areas in now enabling the development of engineered microbial therapeutics and diagnostics. These approaches take advantage of bacterial species' natural abilities to occupy certain biological niches, such as stretches of the GI tract or hypoxic regions of tumors, sense their environment and release therapies such as cytolysins and cytokines, or diagnostic indicators such as β-galactosidase[11–16]. Logic gates, genetic memory devices and kill switches further broaden the capabilities of these bacteria[17].

In eukaryotic synthetic biology[18], immunotherapy has recently emerged as a new class of cancer therapy with promising results in hematological malignancies and some solid tumors[19, 20]. This approach takes advantage of immune cells' ability to eliminate tumors based on the recognition of tumor-specific antigens. Cellular immunotherapy works by genetically modifying patient T-cells to express novel, engineered receptors for tumor antigen recognition and re-introducing them into the body[19]. In addition to engineered receptors, these cells can be designed with cellular logic (*e.g.*, AND gates requiring two tumor-specific signals for activation)[21] and self-inactivating safety mechanisms[22].

Another area of cellular therapy benefiting from synthetic biology is regenerative medicine, which offers hope for patients by introducing progenitor cells *in situ* to induce tissue repair and reverse deficits in conditions including diabetes, heart failure and neurodegeneration.

Treatment with stem cells is a powerful approach on three levels: (i) stem cells secrete cytokines and protective factors that provide trophic support, prevent cell death, and help in recovery of the tissue; (ii) stem cells can be used as a vehicle for continuous delivery of therapeutic agents locally; (iii) stem cells can differentiate and integrate into a tissue, replacing the function of diseased cells. Synthetic biology circuits can be used as switches for reprograming of cells, either to push the cells in the direction of pluripotency or in the reverse direction toward differentiation into a specific type of cells. For example, by synthetically activating the Yamanaka factors[23], generation of induced pluripotent stem cells (iPSCs) can be achieved[3]. This fit the model described in figure 2b of an “off”/”on” function by activating specific transcription factor under very specific conditions. Additionally, communities of cells that associate with each other to form tissues can be generated using engineered receptors and ligands[24], and indicator switches can be built into cells that reveal their proximity to one another through imaging[25]. Another direction is the construction of cellular feedback circuits or oscillators that can allow cyclic production of cytokines, metabolites, neurotropic factors or drugs that is built into the stem cells and use the stem cells as a delivery vehicle. Examples of this approach include rewiring of optogenetics controlled blood glucose levels[26] and control of blood levels of uric acid associated with gout[27].

A major challenge in applying engineered microbial, immune and regenerative cell therapies is that the fate of the injected or transplanted cells is largely unknown. After introduction into the body, the cells may or may not survive, reach their anatomical target, proliferate, differentiate or otherwise carry out their intended function. These factors will profoundly influence long-term patient outcomes. While the molecular imaging field has devoted considerable attention to *in vivo* imaging and tracking of cells, it has mostly done so with synthetic labels, which are difficult to connect to long-term viability and function, and become diluted through proliferation. However, the integration of molecular imaging in to the field of synthetic biology is increasing for the purpose of assessing locales and functions of cells *in vivo*; similarly the use of synthetic biology to create novel imaging tools is also advancing at a dramatic rate. Therefore, we have created an interest group within the World Molecular Imaging Society (WMIS) called Synthetic Biology and Reporter Gene (SyBRG) to address this rapidly advancing intersection of technologies. Here we review this technological interface and point to future directions where the combination of tools addresses critical unmet needs in biomedicine.

In addition to enabling new cellular therapies, synthetic biology provides new methods to alter the genetic contents of existing cells. Breakthroughs in genome editing such as zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and clustered regulatory interspaced short palindromic repeat (CRISPR)/Cas systems[28], have reinvigorated the field of gene therapy by making it possible to fix errant genes and precisely introduce synthetic circuits into mammalian cells. Using a single vector, a specific gene can be targeted and suppressed with an efficiency and accuracy that were not possible previously[29]. Like cell therapy, *in vivo* gene therapy and genome editing are usually targeted to specific cell types and anatomical locations, making it critical to image their fate in the body. By designing appropriate molecular imaging tools it may be possible to fine tune and help translate many of these technologies to the clinic.

Development of reporter genes for noninvasive imaging of engineered cells and genetic vectors

A natural imaging approach for synthetic biology makes use of reporter genes, whose products are proteins that produce signals detectable with imaging modalities, wherein the gene is either fused to the gene of interest or, most commonly, cloned under its cognate promoter (Figure 2). The main applications for these reporters include: (i) monitoring gene expression levels; (ii) investigating dynamic molecular signaling; (iii) studying cellular interactions and (iv) tracking cells in normal and abnormal development or cellular therapy. The first reporter genes (Figure 3) were designed to catalyze chemical reactions that produce a light-absorbing pigment, and later to generate photons following fluorescent[30, 31] or chemiluminescent excitation[32–35]. This pioneering work created for the first time a connection between molecular biology and imaging. In the early nineties, this field was expended to nuclear imaging[36, 37] and MRI[38].

MRI reporters have been developed to use a variety of mechanisms afforded by spin physics. Pioneering examples include enzymes that alter the relaxivity of gadolinium chelates[39], human iron storage and transport genes such as ferritin[40, 41] and transferrin[42], and natural and engineered proteins with large numbers of chemically labile protons for chemical exchange saturation transfer (CEST) imaging[43–48]. Recent developments have also included reporter genes causing accumulation of MRI-detectable compounds[49], proteins interacting with hyperpolarized molecules[50, 51], channels that alter the diffusion of water across cell membranes[52, 53] and vasodilators altering hemodynamic signals[54]. Several of these reporter genes are covered in detail in previous review articles[55–57]. Nuclear imaging reporter genes, some of which have already been tested in the clinic[58], typically lead to cellular accumulation of radiolabeled nucleotides for imaging with positron emission tomography or single photon emission computed tomography[59].

As a complement to existing reporter gene modalities, ultrasound is inexpensive, non-ionizing, portable, and capable of imaging deep tissues[60] with sub-millisecond temporal resolution and a spatial resolution scalable with penetration depth—see review in this issue on Ultrasound Molecular Imaging and Drug Delivery. For example, in small animal imaging, the spatial resolution of high-frequency ultrasound (> 15 MHz) is routinely below 100 μm[61, 62] and can approach the single-micron level with recently developed super-resolution techniques[63]. Although no ultrasound reporter genes current exist, a unique class of biomolecules called gas vesicles – gas-filled protein nanostructures from buoyant photosynthetic microbes – was recently found to produce ultrasound contrast[64]. Efforts are now underway to engineer these molecules at the genetic level[65] and express them heterologously as reporter genes. In addition, photoacoustic imaging, which combines diffuse optical excitation with acoustic readout for *in vivo* imaging applications[66, 67], has engendered the development of optically absorbing molecules as reporter genes[68–70].

Challenges and opportunities

With the emergence of the synthetic biology as a field, it is possible to engineer microorganisms and mammalian cells and use them as diagnostic tools. Harnessing the power of molecular imaging can be a game changer for synthetic biology by improving the

ability to look closely into processes in live organisms. On the other hand, we can use synthetic biology to manufacture more robust imaging probes. For example, one of the challenges of the traditional molecular imaging reporter genes is that the reporters are not switchable i.e., the reporters are constantly activated. Using synthetic biology tools this hurdle can be overcome. Building a switch can ensure that the reporter can provide a signal only at the right place and the right time. Circuit designs such as oscillators can provide a unique temporal aspect to reporter gene signals, helping distinguish them from background. Another emerging frontier of reporter gene engineering relates to genetically encoded sensors of dynamic cellular signals such as calcium, phosphorylation and neurotransmission. Such sensors based on fluorescent proteins are already widely used in optically accessible preparations[71], and recent efforts have focused on developing such sensors for MRI[72] and photoacoustic imaging.

Another potential direction is to augment the visualization capabilities of molecular imaging with the ability to intervene non-invasively in the function of cells in the target tissue. For example if we are already delivering into the tissue energy in the form of, light, ultrasound or electromagnetic fields, we could also use it, either directly or via conversion to heat[73–75], to activate intracellular molecules, proteins or cells.

In summary, as synthetic biology moves toward *in vivo* biomedical applications, it is becoming critical to monitor the functionality of genetically engineered devices in intact model organisms and patients. Molecular imaging technologies such as reporter genes are primed to address this challenge, and we feature these advances at the annual meeting of the WMIS and endeavor to advance this field though fostering interaction and collaborations between scientists using imaging to reveal spatiotemporal functions of engineered cells, and those using synthetic biology to advance imaging tools for biomedical applications.

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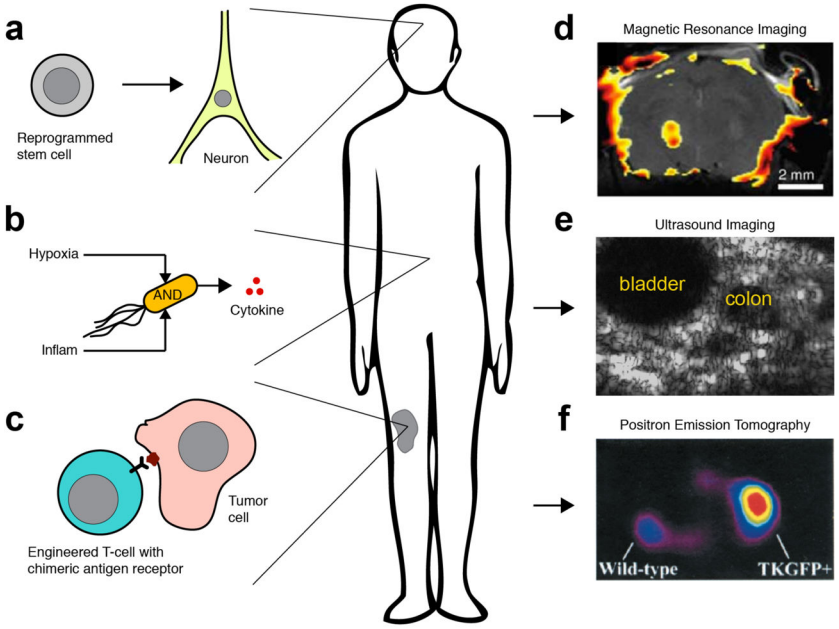

Figure 1. Emerging approached in biomedical synthetic biology and molecular imaging

(a) Illustration of genetically reprogrammed stem cell differentiating into a neuron following implantation into the brain. (b) Illustration of microbial cell in the gastrointestinal tract engineered to release cytokines after a logical AND computation established the presence of hypoxic and inflammatory inputs. (c) T-cell engineered with a chimeric antigen receptor to recognize a specific tumor antigen. (d) MRI image of cells implanted into a mouse brain using CEST imaging of a lysine-rich protein (used with permission from Ref X). (e) Cross-sectional ultrasound image of a mouse torso showing bladder and colon. (f) PET image of T-cells heterologously expressing a T-cell receptor and a reporter gene causing accumulation of a PET tracer, following *in vivo* activation of the cells.

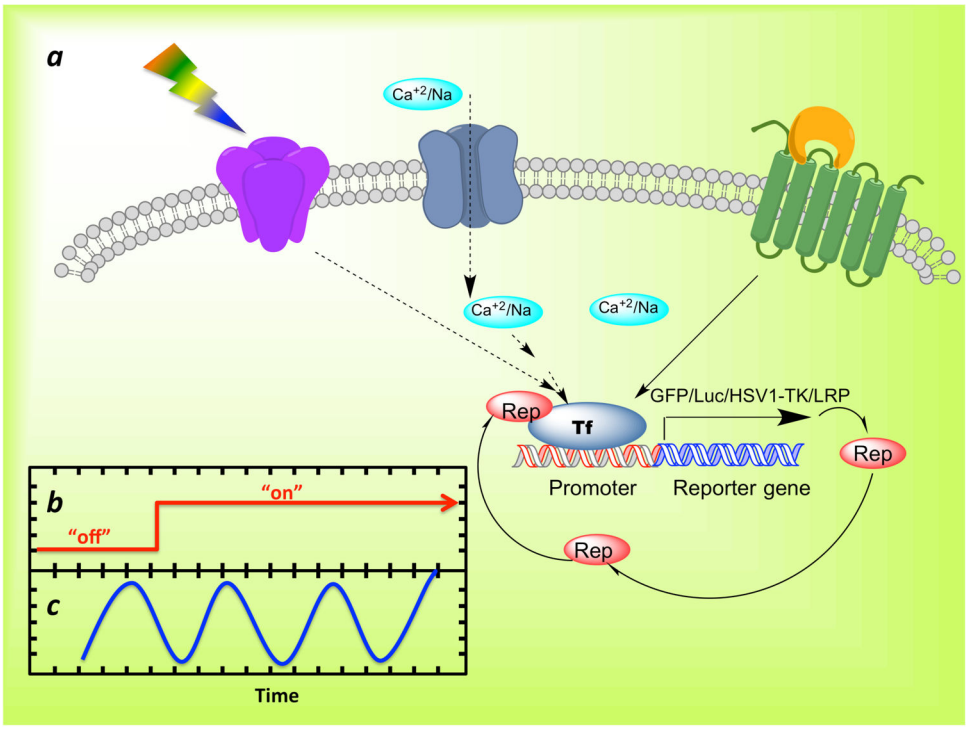

Figure 2. the importance of reporter genes for *in vivo* imaging of synthetic biology

(**a**) There are several "bioparts" and biological circuits that can be used for activation of reporter genes. For example, light activated channels (optogenetics) or other membrane channels and receptors can that activate the transcription factors (Tf) that binds to specific gene promoters and consequently will transcribe reporter genes. This is useful for imaging of "toggle switches" that will result in "on/off" pattern (**b**) and are important for example to report on (stem) cell differentiation or cell fate. Co-expression of the reporter with repressors (Rep) can results in creating "oscillators" that are important for controlled release of metabolites, cytokines, drugs and neurotropic factors (**c**).

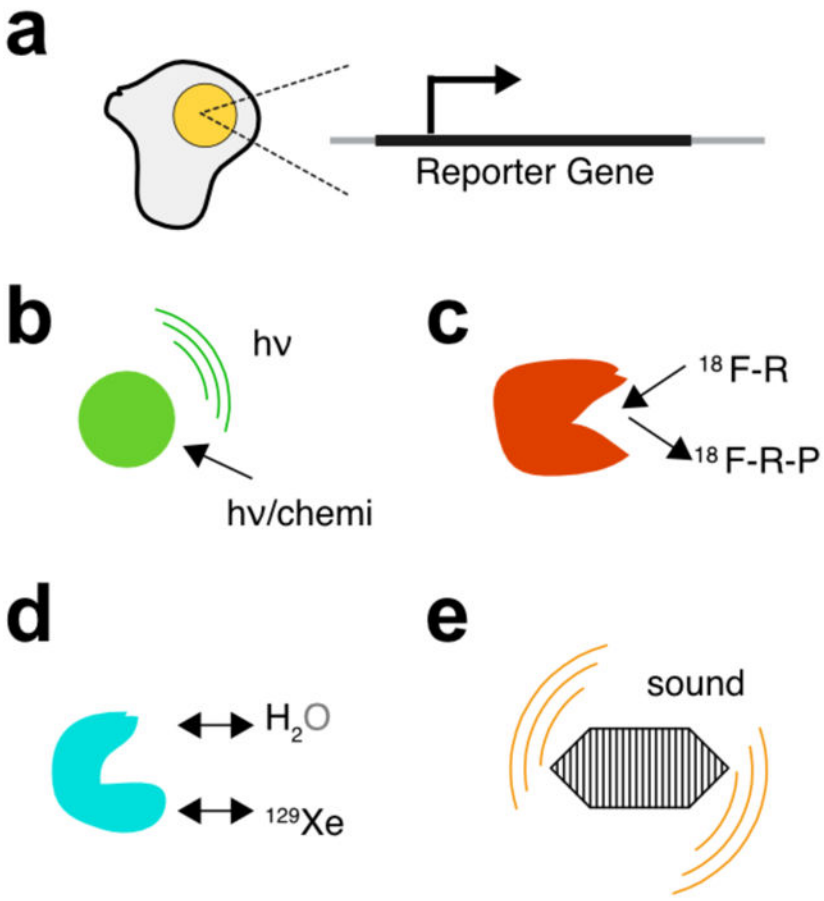

Figure 3.

Reporter genes, Illustration of the operating principles of (**a**) reporter genes for (**b**) optical imaging, (**c**) radionuclear imaging, (**d**) MRI and (**e**) ultrasound. Optical reporter genes convert optical excitation or chemiluminescent substrate bond energy into photons. Radionuclear reporter genes concentrate radioactive substrates in cells, *e.g.* by phosphorylating them. MRI reporters are detected via a variety of interactions with aqueous protons or other nuclei such as hyperpolarized xenon. Ultrasound reporters could be based on proteins capable of scattering sound waves.