Introduction to Concepts and Strategies for Molecular Imaging

Samuel Achilefu is Professor of Radiology, Biomedical Engineering, Biochemistry and Molecular Biophysics, and the Director of Optical Radiology Lab at Washington University in St. Louis, Missouri, U.S.A. His research interests are in the design, development, and biochemical evaluation of molecular imaging agents and drugs in cells and living organisms. He utilizes optical and multimodal imaging platforms that include nuclear, ultrasound, and magnetic resonance imaging to study the molecular basis of diseases and monitor early response to treatment. He obtained a Ph.D. in Chemistry in 1991 at the University of Nancy, France, and completed his postdoctoral training in oxygen transport mechanisms and blood substitutes at Oxford University, England, between 1991 and 1993. Before joining Washington University School of Medicine in 2001, he served as Principal Investigator in the Discovery Research Department at Mallinckrodt Medical, Inc., where he led the liquid ventilation project and championed the optical molecular imaging program. He is the lead or coinventor on more than 40 U.S. patents covering his diverse interests in molecular imaging. He can be contacted at achilefus@mir.wustl.edu, (314) 362-8599 (Tel.), or (314) 747-5191 (Fax).

Molecular imaging is a branch of medical imaging science that aims to detect, localize, and monitor critical molecular processes in cells, tissue, and living organisms using highly sensitive instrumentation and contrast mechanisms. This area of medical imaging has developed alongside the emergence of molecular medicine, in which the genetic makeup of each patient factors into treatment planning. Through the use of gene analysis, it is possible today to identify patients that are prone to specific diseases, and consequently, novel chemotherapeutics have been developed to target specific disease biomarkers. This treatment strategy spares healthy tissue and maximizes therapeutic efficacy of the drugs.

Critical to the success of molecular medicine is molecular imaging, which is not confined to the traditional silos of imaging specialties such as computed tomography (CT), magnetic resonance imaging (MRI), single photon emission tomography (SPECT), positron emission tomography (PET), ultrasound imaging, and optical imaging methods. Instead, molecular imaging is a multidisciplinary endeavor that harnesses the expertise of diverse scientists, engineers, and clinicians. Chemists, in particular, play a critical role in this effort. They are continuously challenged to use innovative chemical strategies to develop smart imaging agents that can produce detectable signals that arise from low concentrations of target biomolecules in cells and tissue. The past 10 years have witnessed the evolution of molecular design to address the ever-changing and challenging needs of molecular imaging research. Most of the initial studies focused on cell studies, in which molecular imaging has unraveled the dynamics and functions of many biomolecules and molecular interactions using advanced microscopy techniques and diverse molecular probes. Translation of findings in cells to living organisms is the focus of many preclinical and clinical molecular imaging studies. There are several objectives of molecular imaging. These include early detection of disease, monitoring of drug efficacy and treatment response, patient stratification, and identification of disease susceptibility.

In this issue of *Chemical Reviews*, leading experts in the field of molecular imaging examine the recent advances in their various fields of study, identify critical issues limiting progress, and suggest strategies to further advance the field. The articles focus on major imaging technologies that are used in molecular imaging. Those that are predominantly used to produce anatomical information such as CT are excluded. Extensive reviews related to bioluminescent and fluorescent proteins are available elsewhere and appear in some articles as part of a larger discussion on molecular imaging probes for optical imaging.

Each imaging modality, as they are frequently called by imaging scientists, brings unique information to molecular imaging. Optical and nuclear imaging methods are the workhorse of in vivo molecular imaging because of their high detection sensitivity. Currently, only nuclear imaging is used in clinical settings. Although MRI is largely a functional and anatomical imaging method, novel contrast mechanisms and the heightened interest in magnetic resonance spectroscopy (MRS) are making it possible to extract molecular information from this modality. Other molecular sensing methods such as mass spectral imaging and electron paramagnetic resonance (EPR) methods are included with the hope that molecular imaging scientists will incorporate these technologies into their arsenal of imaging tools. The reviews are organized into different themes, starting with traditional optical, nuclear, and MRI/MRS methods, followed by multimodal and cross-modality strategies, concluding with EPR oximetry and mass spectrometry methods.

Optical imaging can furnish images of cells and tissues at shallow depths with exceptionally high spatial resolution, but this resolution decreases rapidly as a function of the imaging depth. However, there is a clear path for the use of optical molecular imaging in humans. For example, oral, skin, cervical, and endoscope-accessible tissues are amenable to optical molecular imaging. Although many reports of the use of optical methods in human subjects are available, few such reports on *molecular* optical imaging have been documented. Nearly all the optical imaging methods reported in this thematic issue use a fluorescence signal for molecular imaging because of the high detection sensitivity of this imaging technique.

A cell is the fundamental unit of living organisms, and we can apply information gleaned from molecular processes in cells to intact living organisms. It is therefore appropriate that Sinkeldam et al. lead off the issue by taking us on a journey through the cell. Starting with carbohydrates on the cell surface, through cell membranes and into the cytoplasm, the authors describe approaches and challenges of developing

10.1021/cr1001113
Published on Web 04/26/2010

fluorescent analogues of key molecular constituents of the cell. By using fluorescent analogues of natural biomolecules, molecular processes and the fate of the fluorescent biomolecules can be tracked by fluorescence imaging. The findings of these studies form the basis for translating some of the materials from cells to living organisms. A major challenge in this research is to design fluorescent compounds that maintain the biological functions of the natural biomolecules. Strategies to accomplish this objective are discussed in the review.

The paper by Kobayashi et al. focuses on the strategies to develop elegant fluorescent probes for imaging molecular processes. A notable component of the review is the concept of using activatable fluorescent probes to image molecular processes. Unlike other imaging methods, the molecular probes can be designed to switch on or off their fluorescence in response to a specific molecular event or environment. This approach reduces nonspecific signal to give sensitive detection of lowly expressed target diagnostic molecules. Typical applications of activatable probes include the imaging of diagnostic enzyme activity in cells and living organisms. It is occasionally desirable to image the expression of multiple targets in the same animal to determine the expression levels and dynamics of different molecular targets. Thus, a variety of molecular events can be investigated simultaneously in a single animal model. Excellent illustrations of in vivo multicolor imaging are provided in this article.

Sometimes fluorescence intensity based imaging is not adequate for molecular imaging due to fluctuations in the intensity as a function of the probe concentration and many other factors. These hardly predictable factors complicate quantitative analysis of the measured fluorescence, especially in tissue. In this case, an alternative fluorescence contrast mechanism is needed. Berezin and Achilefu describe the history of fluorescence lifetime techniques and their evolution into a powerful method for molecular optical imaging. A unique advantage of lifetime measurement is that the parameter is less dependent on the probe concentration but can be affected by its environment or sudden changes in the structural features of the dye. Different molecular designs are used to induce changes in fluorescence lifetime in response to a target molecular process. An interesting application uses molecular probes with different lifetimes but similar spectral properties to report multiple molecular events. This is akin to multicolor fluorescence intensity imaging. The authors provide ample illustrations of the application of lifetime imaging.

Fluorescence polarization is another imaging technique to visualize or assess the milieu of fluorescent molecular probes. Originally used to study biochemical events and limited to specialized laboratories, polarization studies have found their way into biological imaging because molecular interactions can be determined in a mixture of bound and unbound molecular probes with highly reproducible results. Recent studies have demonstrated the miniaturization and automation of the detector and data acquisition systems for fluorescence polarization imaging. Jameson and Ross provide a compelling historical background, the theoretical basis, and practical considerations in the use of polarization techniques for molecular imaging. Although most of the applications are currently in clinical chemistry and in vitro biological assays, there are efforts by researchers to incorporate this fluorescence technique into molecular imaging of living systems. In fact, some reports on the use of fluorescence polarization for imaging cancer have appeared in the literature.

The exciting article by Han and Burgess focuses on the design of molecular probes that can sense intracellular pH. The excitement about monitoring intracellular pH arises from findings that diseased cells can have significant changes in intracellular pH. Similarly, the tissue that surrounds proliferating tumors can also have abnormal pH. By designing fluorescent molecular probes that use a ratiometric measurement method, obtaining pH values by this method overcomes several problems inherent in other pH measurement techniques. The tabulation of fluorescent pH indicators possessing pK_a values that are within the physiologically relevant range is valuable reference information. Recent studies demonstrate the potential for using pH-sensitive probes in the molecular imaging of cancer. The contrast in one instance was generated by receptor-mediated trafficking of a pH-sensitive dye-labeled antibody through acidic lysosomes in cells.

Apart from organic fluorescent dyes, many other luminescent compounds and materials are useful for molecular imaging. Comprehensive reviews about quantum dots and other luminescent nanoparticles are available elsewhere but are briefly covered in some articles in this issue as part of a discussion of imaging probes. An important class of nonorganic luminescent probes is based on lanthanide chelate chemistry. Interest in this class of biological probes increased because their long excited-state lifetimes are amenable to time-resolved luminescent assays using simple detection systems. Moreover, these complexes are generally more photostable than many organic fluorophore systems. Their use for imaging molecular targets in live cells and living organisms presents a new dimension to the arsenal of optical contrast mechanisms available to researchers. Bünzli addresses key issues with the design and use of lanthanide molecular probes and suggests strategies to overcome the limitations of this technique for biological imaging.

To achieve greater depth detection with optical imaging, researchers have developed a new hybrid technology called photoacoustic or optoacoustic tomography (PAT). The basic mechanism of contrast for PAT relies on the thermo-elastic expansion resulting from the absorption of light by target tissue using intrinsic or exogenous chromophores to generate sound waves that are then detected by an ultrasound transducer. In addition to improving imaging depth, PAT also produces high-resolution images. This technology has already been applied to diverse biological systems and diseases. Efforts are underway to apply the technology in functional and molecular imaging of human patients. Kim et al. reviewed studies in the past 7 years covering this burgeoning field of study. A new variant of PAT utilizes a multispectral approach to identify the molecules responsible for light absorption in the tissue of interest. This approach, which is particularly useful for longitudinal studies, is reviewed by Ntziachristos and Razansky.

Some light-absorbing molecules may produce cytotoxic species in addition to dissipating absorbed energy in the form of heat or fluorescence, enabling light-based therapeutic applications. This light-based treatment is known as photodynamic therapy (PDT), and the enabling molecules are known as photosensitizers. PDT is used in many clinical centers to treat diverse human diseases, but its inclusion in this thematic issue is not because of this function. Instead, the two review articles on this subject highlight the dual role of photosensitizers in molecular imaging and therapy. This

feature is made possible because most photosensitizers also produce fluorescence that is useful for molecular imaging. In the first paper on this topic, Celli et al. reviewed the body of literature on how medical imaging can enhance PDT outcome. Of particular interest is the application of dual fluorescence and PDT in oncology. A new breed of targeted molecular photosensitizers appears to improve delivery of the drug to the target tissue. The second paper on photosensitizers by Lovell et al. focuses on the emerging activatable photosensitizer designs based on energy transfer between a fluorophore or quencher dye and the photosensitizer. These molecular constructs can report the presence of a target biomolecule and simultaneously unleash the active form of the phototosensitizer for subsequent PDT. The combined "see and treat" strategy has great potential to enhance the therapeutic efficiency of PDT.

Although optical molecular imaging is widely used in preclinical studies, nuclear molecular imaging methods such as SPECT and PET are widely used in clinics. In addition to the high detection sensitivity of these methods, SPECT and PET do not suffer from rapid signal attenuation in deep tissue, as is the case with optical imaging. Thus, whole-body imaging of humans is readily attainable. Moreover, the molecular imaging probes for this modality are used at tracer level, thereby enabling easier translation to humans than optical or other molecular imaging agents. A series of articles provides a comprehensive review of the field of nuclear molecular imaging, focusing on the fundamental principles, practical considerations, unique applications, and limitations of the technology.

Unlike other imaging modalities that can provide imaging contrast from endogenous sources, SPECT and PET require the administration of exogenous imaging agents. Accordingly, Wadas et al. present a didactic and comprehensive review of studies in the last 10 years related to the production and use of radiometal-based nuclear imaging agents. The inclusion of representative applications of radiometal-based radiopharmaceuticals in the article makes it a stand-alone reference work. The chemistry of ^{99m}Tc is the focus of the article by Bartholomä et al. Arguably, ^{99m}Tc is one of the most widely used SPECT radiopharmaceuticals in the world, but recent developments have led to worldwide shortage of this important imaging agent. The authors describe the challenges this shortage pose to the imaging community and suggest that the use of Ga-based radioisotopes may solve this problem. Consequently, the chemistry of ^{99m}Tc and $^{67/68}$Ga were placed in perspective based on literature reports.

The following five articles deal with the challenges and exciting opportunities of using MRI and MRS in molecular imaging. MRI is well-known for its ability to produce exquisite anatomical and functional images of the body, but it is not a major player in molecular imaging because of its poor sensitivity to contrast agents. However, recent advances in instrumentation and imaging agent design, including T_2 sensitive imaging agents, have enhanced the potential of applying MRI in molecular imaging. The consequence of such accomplishments are enormous, one of which is that MRI can then provide its own structural, functional, and molecular imaging information. This is the holy grail of molecular imaging. The article by Villaraza et al. describes the theory and mechanism of MRI contrast generation and some practical tips for using MRI contrast agents. The authors carefully walk the reader through the intricacies of developing small molecule and macromolecule MRI agents. While the authors clearly document the enhancement of MRI contrast through careful control of rotational correlation times, optimal water residency times, and retention of the macromolecules in blood plasma, it is important to note that adding more paramagnetic agent such as Gd^{3+} into nanomaterials or creating larger biomolecules loaded with Gd^{3+} does not always translate into contrast enhancement.

Despite the large number of successful imaging studies with Gd^{3+}, the need to develop environment-sensitive MRI agents similar to what is available by optical imaging probes has led to the development of alternative MRI contrast mechanism based on chemical exchange saturation transfer (CEST) agents. The use of paramagnetic CEST agents (PARACEST) in recent studies creates a unique opportunity to selectively activate different MRI agents at specific time points. An authoritative review of the subject by Viswanathan et al. provides an important thesis on the theory, design, applications, and limitations of PARACEST agents used to assess the biological environment by MRI. The section on MRI authored by Terreno et al. concludes with a cautionary account of the overall problems faced by researchers who want to use MRI for molecular imaging. It offers potential solutions, which largely rely on using chemistry principles to develop highly sensitive MRI agents.

The recent surge of interest in the use of MRS (commonly known to chemists as nuclear magnetic resonance, NMR) to unravel the molecular basis of diseases will further enhance information derived from MRI. MRS is well-established for the analysis of chemical and biochemical structure of molecules. Applying a similar approach to tissue, MRS reports the chemical composition of a region of interest. It follows that changes in the structure of molecules such as metabolites can be readily detected by MRS. Moreover, the measured signal is quantitative, which enables the use of spectral peak area to determine the concentration of the target compound or biological molecule. To provide a spatial landmark to MRS data, there is a trend to combine MRI with MRS. Excellent reviews of the theory and applications of MRS in oncology and neurology are provided by Glunde et al. and Mountford et al., respectively.

The reviews described thus far have largely focused on the application of molecular imaging in specific imaging modality areas. However, it is difficult to find any single imaging method that can satisfy the ever-increasing need for more diagnostic information. For example, CT and MRI are recognized for their high spatial resolution but are limited by poor sensitivity to contrast agent detection. In contrast, optical and nuclear imaging methods have exceptionally high detection sensitivity but provide poor anatomical information, especially in deep tissues. This dichotomy has led to the development of hybrid instrumentation and imaging agents that combine desirable features of the different imaging methods. This hybrid imaging strategy is termed multimodality imaging. Occasionally, chemists transform imaging agents developed for one modality into another. For example, a peptide can be labeled with fluorescent dyes, radiometals, or paramagnetic metal chelates. Although the inherent properties of the resulting products may change, it is expected that such labeling will not disrupt the recognition of the biomolecule by its receptor or target.

Three articles in this issue report examples of this multimodality approach. Focusing on peptides and peptide hormones as delivery vehicles of contrast agents to target

tissue, Lee et al. reviewed the development and application of these agents in molecular imaging. Strategies to modify the structural framework of these peptides for labeling with different reporter systems and application of diverse peptide based imaging agents for MRI, optical, and nuclear imaging are discussed. A complementary article by Signore et al. examines the development and application of nuclear and optical molecular probes for imaging inflammation and microbial infections. Animal models for this line of research are described, providing a new researcher the tools needed to work in this exciting field of study. The last article in this category by Louie develops the concept of using multifunctional nanoparticles for imaging molecular processes. The nanoparticles can be used to normalize disparate contrast mechanisms and detection sensitivities. For example, the concentration of Gd^{3+} needed to produce a detectable signal by MRI is several orders of magnitude larger than what is needed for radiopharmaceuticals used in nuclear imaging. The article reviews different strategies to accomplish this goal and illustrates the application of the multifunctional nanoparticles for MRI, nuclear, and optical imaging studies.

As demonstrated in several articles in this issue of *Chemical Reviews*, peptides are an important part of the equation in developing smart contrast agents for molecular imaging applications. However, many researchers rely on published data to select peptides as carriers of imaging agents. There are many strategies to discover new peptides for use as a targeting group for drugs or imaging agents. In this thematic issue, Deutscher examines the application of phage display technology in the discovery of peptides used in molecular imaging. Phage display is a high-throughput method to screen and identify high-affinity peptides for biological applications. The article highlights representative peptides that were discovered through phage display and used in molecular imaging studies.

Determining oxygen levels in tissue with a high degree of accuracy is an interesting topic to scientists in diverse areas of research. In oncology, for example, the oxygenation level of tumors can be used to predict response to radiation therapy. A variety of methods has been developed to measure tissue oxygen concentration, but varying results were reported. Molecular imaging has made significant contributions in this area of research. Efforts to use electron magnetic resonance (EPR) oximetry are beginning to show promise as a viable method to measure oxygen levels in tissue. There are many reasons to persist in advancing this technology, which include the ability to report absolute oxygen partial pressure in tissue. Ahmad and Kuppusamy provide a thorough review of the principles, instrumentation, and practical use of EPR oximetry in biomedical research, with the hope that molecular imaging scientists can adopt this method as a complementary reporting strategy in their work.

This thematic issue concludes with a potentially label-free technology based on mass spectrometric imaging. Mass spectrometry is a destructive method that is not currently amenable to in vivo imaging. However, its ability to rapidly detect and identify biological molecules within a small tissue sample could provide accurate validation of the presence of target biomarkers identified by noninvasive molecular imaging methods. Toward this goal, two articles on mass spectrometric imaging are part of this thematic issue. The first one by Chughtai and Heeren provides a timely and in-depth review of basic principles and applications of the technology in tissue analysis. The second paper by Végvári and Marko-Varga focuses on recent advances in mass spectrometry and its contribution to the understanding of molecular signatures in lung cancer.

Without doubt, the field of molecular imaging is broad and continues to grow. While we have attempted to provide a comprehensive review, some important topics could have been omitted inadvertently in this thematic issue, for which the guest editor apologizes. Others were not included because recent comprehensive reviews on the subject are available. It is hoped that the diverse topics covered in this issue of *Chemical Reviews* will provide valuable information to readers of the journal.

Samuel Achilefu
Department of Radiology, Washington University School of Medicine, 4525 Scott Ave, St. Louis, Missouri 63110

CR1001113