Modular Strategies for PET Imaging Agents

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Modular Strategies for PET Imaging Agents

Jacob M Hooker
Brookhaven National Laboratory, Medical Department, Building 555, Upton, NY 11973-5000

Summary of Recent Advances

In recent years, modular and simplified chemical and biological strategies have been developed for the synthesis and implementation of positron emission tomography (PET) radiotracers. New developments in bioconjugation and synthetic methodologies, in combination with advances in macromolecular delivery systems and gene-expression imaging, reflect a need to reduce radiosynthesis burden in order to accelerate imaging agent development. These new approaches, which are often mindful of existing infrastructure and available resources, are anticipated to provide a more approachable entry point for researchers interested in using PET to translate in vitro research to in vivo imaging.

Introduction

The development of tools to image biological systems with molecular precision has been a primary goal of chemical biology over the past decade [1]. Increased adaptation of in vitro techniques and concepts to in vivo imaging will have a huge impact on our understanding of complex biological processes in human physiology. In this context, positron emission tomography (PET) provides a powerful non-invasive means of translating molecular tools from the bench to human imaging in order to evaluate and optimize novel treatment strategies such as gene therapy, stem cell implantation, advanced drug delivery systems, and new small molecule pharmaceuticals. The resolution of PET in terms of space and time may not be as impressive as other imaging modalities, but its chemical specificity and sensitivity are exquisite —allowing for the direct observation of molecular interactions at picomolar concentrations with properly designed radiotracers.

Several recent reviews outline the synthetic challenges of incorporating short-lived radioactive isotopes and developing imaging probes for PET [2••,3-4], and others detail the concepts and considerations involved in quantifying PET image data [5-6]. Therefore to avoid redundancy, this review will highlight recent technology and conceptual advances in PET imaging with a focus on techniques that may allow chemical biologists to rapidly translate in vitro research to in vivo imaging, thus bridging the gap between basic and clinical research. The review emphasizes new strategies in radionuclide bioconjugation, such as modular imaging-agent delivery systems, advancements in PET-based gene expression imaging, and developments in PET radiotracer synthesis.

hooker@bnl.gov.

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Availability of PET (exploiting the infrastructure that exists)

The use of PET in clinical diagnosis, treatment monitoring, and academic research is in a stage of exponential growth (Figure 1) that has spawned an infrastructure of isotope production, distribution, and imaging centers across the United States. In 2005, it was estimated that 97% of the people in the U.S. live within a 75 mile radius of a PET imaging location [7]. Competition to supply these imaging centers has driven down the cost of common isotopes and imaging agents, including $2-[^{18}\text{F}]\text{fluoro-2-deoxy glucose (^{18}FDG)}$ and while there are still limitations to accessing PET infrastructure, chemical biologists should recognize that short-lived radioactive isotopes used in PET are available both commercially and through collaboration to test ideas in their full complexity (i.e. in vivo).

Two recent examples highlight ways of exploiting the existing PET infrastructure for in vivo imaging. In both cases, $^{18}\text{FDG}$ was used as a synthon for peptide labeling, in one case via direct condensation of $^{18}\text{FDG}$ with an aminooxy-functionalized peptide [8], and in the other by indirect means via a maleimide intermediate [9]. Bear in mind, 20 mCi of $^{18}\text{FDG}$ can be delivered to a research site from a commercial vendor for under $300. With proper planning, this amount of radioactivity can be used for dozens of animal imaging/biodistribution experiments. Unfortunately, there are a few drawbacks to these methods that may prevent their immediate use in high specific activity applications, since $^{18}\text{FDG}$ is not separated from glucose during commercial synthesis. Undoubtedly, as additional refinements are made, employing $^{18}\text{FDG}$ as a synthon will enable researchers not formally trained as radiochemists to utilize PET imaging.

“Kit-like” methods for peptide and protein labeling

Along these same lines, new “kit-like” methods have been devised for protein labeling with $[^{18}\text{F}]\text{fluoride}$, which are envisioned to reduce the radiosynthesis burden thus providing PET access to a larger community (Figure 2). Methods specifically designed to address issues in PET radiochemistry include $^{18}\text{F}/^{19}\text{F}$ isotopic exchange of a silicon-fluoride bond [10,11], capture of aqueous $[^{18}\text{F}]\text{fluoride}$ with arylboronic esters [12,13•], and most recently formation a stable $\text{Al}^{18}\text{F}$-chelate bound to a peptide [14••]. The goal of these methods is to simplify protein and peptide labeling by eliminating the somewhat arduous and lengthy synthesis of a protein reactive functional group. Both the boron- and aluminum-based methods use aqueous fluoride, which is clearly advantageous given that $[^{18}\text{F}]\text{fluoride}$ is produced by a nuclear reaction $[^{18}\text{O}\ (p,n)]^{18}\text{F}$ in isotopically enriched water; however, none of the methods operate within the narrow window of pH and temperature conditions necessary to preserve the structure and function of most proteins and the methods require relatively large amounts of substrate to drive the labeling reaction forward.

These issues are not insurmountable and may be overcome in the future by using innovative strategies like enzymatic $^{18}\text{F}$-fluorination to produce protein-reactive $^{18}\text{F}$-molecules or potentially to label proteins with $^{18}\text{F}$ directly. Enzymatic labeling with $^{18}\text{F}$ has already been demonstrated as an elegant technique to produce $^{18}\text{F}$-nucleosides [15,16•]. Other advances in PET radiochemistry for peptide and protein labeling, such as click chemistry, originated as adaptations of general bioconjugation strategies [17-18] will also push the field forward. Simplification of $^{18}\text{F}$-bioconjugation strategies and the development of methods to purify labeled biomolecules from unlabeled reagents will be an area of tremendous growth in the coming years.

Modular Approaches and Advanced Delivery Strategies

Developments in PET imaging agent delivery systems have paralleled advances in drug delivery strategies [19]. Given that each potential biological imaging agent introduces a unique set of synthetic constraints for radionuclide incorporation, modular strategies are being
developed that decouple radionuclide labeling from biomolecule identity. The fundamental goal of this work is to provide a general platform that consists of at least two elements: 1) a targeting group such as a peptide to direct the imaging agent to a particular cellular target or tissue of interest and 2) a chemically independent functional handle for radionuclide incorporation, (Figure 3). The dual component design of these modular imaging agents offers several advantages, including the potential to display multiple copies of a targeting moiety (thus increasing its affinity) and the ability to modify the imaging agent shell to alter its pharmacokinetics and in vivo stability. It is anticipated that these platforms could readily afford imaging agents for a range of biomedical applications while reducing redundancy in chemical optimization.

The flexibility of a modular synthesis allows researchers access to two distinct types of in vivo information provided by PET: 1) the disposition (pharmacokinetics and distribution) of a compound (e.g. drug molecule, protein, delivery scaffold); and 2) the characterization of a physiological parameter (e.g receptor concentration/occupancy, enzyme activity, metabolism, or gene expression). PET imaging can based on a modular platforms can accomplish either or both depending the desired information and the design of the platform.

Although many research groups have developed modular platforms for imaging agents, only a few illustrative examples will be referenced here. The collaborative efforts of the Welch group at Washington University have led to several modular PET imaging agents. With the Wooley group, shell cross-linked knedel-like (SCK) nanoparticles were labeled with $^{64}$Cu using DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) as a chelating ligand, enabling the authors to explore the effect of nanoparticle size, core material, and flexibility on biodistribution [20-21]. The original labeling method placed the radionuclide on the exterior of the surface of the nanoparticle; however, more recently the authors were able to move $^{64}$Cu-chelate to the interior of the SCK nanoparticles by using amphiphilic block copolymers [22••]. Using this more tunable chemical strategy, the surface of the nanoparticle was modified with various densities of methoxy-terminated poly(ethylene glycol) (mPEG). With this system, a mathematical model was developed to describe how mPEG density, and the corresponding hydrodynamic volume, affects circulation half-life in vivo. Analogous studies using a core-shell star copolymer labeled with $^{64}$Cu confirmed that a modular polymer-based system could be used to tune biodistribution [23]. These fundamental studies are important as they provide general guidelines for the development and optimization of PET imaging agent and drug delivery systems.

Biodegradable core-shell systems, which may minimize scaffold accumulation and toxicity, have been developed for PET radionuclide delivery based on protein assemblies and dendrimers. For example, the 27 nm hollow protein shell of bacteriophage MS2 was modified through a series of chemical reactions resulting in $^{18}$F-incorporation on the interior surface of the virus. By co-modification of the interior surface of the virus with a fluorescent dye, the authors demonstrate that the protected “cargo” on the interior surface does not alter biodistribution [24•]. In a synthetic equivalent using a biodegradable dendritic nanoprobe targeted to αvβ4 integrin and labeled with positron emitting bromine-76, Fréchet and coworkers have demonstrated that multivalent macromolecular delivery systems can address imaging targets, such as angiogenesis, which have not been addressed effectively with small molecule approaches [25].

As evidenced by the work described above with $^{64}$Cu, metal-chelating groups and positron emitting metal isotopes can be employed as facile and reliable components of modular imaging probes [26]. Consequently there is ongoing work to improve the stability of radiometal complexes for peptide, protein, and macromolecule labeling. As an example, a very promising divalent imaging probe was recently constructed using an ethylene cross-bridged tetraamine
ligand that forms a neutral octahedral complex with $^{64}$Cu II [27]. In addition to new chelating ligands, improved methods for generating PET isotopes that are mobile, self-contained, and commercially available will drive advancements in modular imaging approaches. For instance, the use of generator-produced gallium-68, which is independent of cyclotron availability, has rapidly increased over the past few years [28-29].

Imaging Gene-Expression in vivo with PET

Imaging gene-expression with PET is a research area ripe with unrealized potential, as it would confer the benefits of near-perfect target fidelity, wide applicability to proteins, and broad versatility arising from its modularity at the genetic level (Figure 3). PET reporter systems can be thought of as in vivo correlates of what are now conventional reporter systems, such as green fluorescent protein (GFP) and luciferase, which are used routinely in vitro for cellular imaging and increasingly for in vivo imaging [30,31]. Due to its high sensitivity and tissue-penetrant radiation, PET-based gene-expression imaging may have offer advantages over optical imaging methods for human translational research, particularly research related to the brain. Since its conception roughly a decade ago, noninvasive reporter gene imaging with PET has been refined steadily [32-33••] and used recently in many exciting applications [34-35].

The most common reporter systems have been based on the variations of the herpes simplex virus type 1 thymidine kinase (HSV1-tk), which can be introduced to specific cells by retroviral infection. A major limitation to the use of PET imaging for monitoring gene-expression in this case has been the development of radiotracers that act as substrates for HSV1-tk and upon phosphorylation are trapped in the cells expressing the reporter gene. The most promising candidates, such as $[^{18}\text{F}]$FEAU (2′-[$^{18}$F]fluoro-2′deoxy-5-ethyl-1-β-D-arabinofuranosyluracil) and $[^{18}$F]$^9$HBG (9-(4-[18$F$]fluoro-3-hydroxymethylbutyl)guanine), suffer from challenging low yielding multi-step syntheses that in many case require more than 3 h [36]. Despite a growing effort to improve the reporter enzyme [37], the lack of suitable and readily accessible PET radiotracers remains an unsolved problem for HSV1-tk-based systems as well as other enzyme-based reporter systems such as LacZ [38].

Although there have been limited human trials with HSV1-tk PET [39-40], HSV1-tk and mutants derived from it are not endogenously expressed in humans and may present immunogenicity issues. Therefore, there is an ongoing effort to identify gene-reporter systems based on human proteins [41] such as the norepinephrine transporter [42], the sodium iodide symporter [43], and human mitochondrial thymidine kinase 2 [44]. As the challenges in optimizing reporter/radiotracer pairs and radiotracer synthesis are overcome (and perhaps a commercial radiotracer is realized), PET gene-expression imaging may become a transformative tool in biomedical research.

New chemistry will accelerate the use of PET

Although the focus of this review has been on modular, largely macromolecular, approaches to PET imaging, we cannot ignore that small-molecule radiotracers will continue to play the dominant role in human imaging. Thus, it is worth noting that the concepts of modularity, process simplification, and expanded use of existing PET infrastructure can and should apply to very fundamental advances in small molecule radiotracer synthesis. Although this is true for chemical methods developed for any short-lived isotope, we will use a few recent examples of new carbon-11 labeling methods to illustrate this point [2,45].

$[^{11}$C]$^9$Carbon monoxide is an incredibly appealing synthon for metal-catalyzed carbonylation reactions that has yet to reach its full potential in radiotracer synthesis because of its limited solubility in organic solvents, which complicates trapping and limits chemical reactivity. However, it was recently shown that copper(I) scorpionate complexes can be used to trap and
release $^{11}$CO at atmospheric pressure with incredible efficiency, and can be used in situ as a $^{11}$CO donor for Pd-mediated reactions [46•]. This process simplification and its future refinements will open the door for many PET radiotracer chemists to use $^{11}$CO and inevitably in new reaction scenarios.

New developments involving other one-carbon synthons have also paved the way for more facile synthesis of carbon-11 radiotracers without the need for new equipment, exotic reagents, or complex processes. In particular, two methodologies with broad applications have been developed by our research group. The first takes advantage of $^{11}$CO$_2$, the product of the nuclear reaction $^{14}$N(p,$\alpha$)$^{11}$C, which is the starting point for nearly all carbon-11 chemistry. After capturing $^{11}$CO$_2$ in solution with DBU (1,8-diaza-bicyclo[5.4.0]undec-7-ene), we demonstrated that $^{11}$C]carbamates can be formed in high radiochemical yields from an amine and alkyl halide. The reaction set-up was extremely simple consisting of one vessel, occurred at relative low temperatures, and had a wide substrate tolerance thus allowing $^{11}$C]carbamates to be “stitched” together in modular fashion [47]. A second new methodology uses $^{11}$CH$_3$I—the predominant labeling reagent in use today - to access to $^{11}$C]formaldehyde through conversion with trimethylamine-N-oxide [48•]. While this route might not seem intuitive, it takes advantage of a highly optimized building block already available ($^{11}$CH$_3$I) rather than starting from scratch and thus immediately provides a new synthon to carbon-11 radiochemists. Recognizing and utilizing available tools is an important design consideration for new methodology that will facilitate the transition of a new method from initial description to routine use.

Conclusions

As the use of PET continues to increase and expand in scope over the next decade, new strategies in radiotracer design and synthesis will be required that decrease the barriers involved in radiochemistry and allow more researchers to utilize PET imaging. As with any technology, the associated costs will go down, PET isotopes and scanners will become more available, and distribution centers will continue to sprout. As this occurs and PET becomes available to a more diverse research community, chemists developing new methods must begin to think carefully about how to place PET radiotracers in the hands of more end users, so that access to radiotracers does not impede PET utilization. While automation and engineering will play a key role in providing access [49-50], exploitation and clever use of existing infrastructure combined with an expansion of modular strategies and chemical transformations like those described in this review will no doubt increase the types of studies that can be performed.

Acknowledgments

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References


Figure 1. Exponential growth of PET
Number of year-limited publication hits for “positron emission tomography” using the Scopus abstract and citation database.
Figure 2.

a. $^{18}$FDG as a synthon for peptide and protein labeling [8-9]

b. Modular, “kit-like” methods for fluoride-18 chemistry [10-14]
Key features of macromolecule imaging agents. Regardless the specific design, macromolecular approaches feature tunable independent surfaces for targeting and radionuclide incorporation. Multivalency can be used to both amplify affinity for a target and increase the overall specific radioactivity of an agent.
Figure 4.
Reporter gene methods used in PET