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Click chemistry: A transformative technology in nuclear medicine

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Abstract

The 2022 Nobel Prize in Chemistry was awarded to Professors K. Barry Sharpless, Morten Meldal, and Carolyn Bertozzi for their pioneering roles in the advent of click chemistry. Sharpless and Meldal worked to develop the canonical click reaction — the copper-catalyzed azide-alkyne cycloaddition — while Bertozzi opened new frontiers with the creation of the bioorthogonal strain-promoted azide-alkyne cycloaddition. These two reactions have revolutionized chemical and biological science by facilitating ligations that are selective, high-yielding, rapid, and clean and by providing unprecedented ways to manipulate living systems. Click chemistry has affected every aspect of chemistry and chemical biology, but few disciplines have been impacted as much as radiopharmaceutical chemistry. The importance of speed and selectivity in radiochemistry make it an almost tailor-made application of click chemistry. In this Perspective, we discuss the ways in which the copper-catalyzed azide-alkyne cycloaddition, the strain-promoted azide-alkyne cycloaddition, and a handful of ‘next generation’ click reactions have transformed radiopharmaceutical chemistry, both as tools for more efficient radiosyntheses and as linchpins of technologies that have the potential to improve nuclear medicine.

Keywords

Click chemistry; azide-alkyne cycloaddition; strain-promoted azide-alkyne cycloaddition; inverse electron-demand Diels-Alder reaction; radiopharmaceutical chemistry; radiochemistry; PET; SPECT; targeted radionuclide therapy

Competing Interests: BMZ and JSL hold intellectual property related to the application of click chemistry to radiopharmaceutical chemistry. DB and SMS declare no competing interests.

INTRODUCTION

The 2022 Nobel Prize in Chemistry was awarded to Professors K. Barry Sharpless, Morten Meldal, and Carolyn Bertozzi for their seminal work on the development of a now ubiquitous suite of chemical transformations known as 'click chemistry'[1]. Designed to facilitate the facile and selective construction of molecules, click chemistry reactions are by design efficient, rapid, modular, clean, and water-compatible. Sharpless and Meldal independently developed what is considered the classical click ligation: the copper-catalyzed 3+2 cycloaddition between an azide and a terminal alkyne (Figure 1A)[2]. This reaction — known as the copper-catalyzed azide-alkyne cycloaddition (CuAAC) — represents a significant improvement to the non-catalyzed but temperature-driven 1,3-diploar Huisgen cycloaddition between the same two moieties.[3] Although hailed as a breakthrough technology, the ternary nature of the CuAAC reaction and the intrinsic toxicity of copper limited its applications in biological systems. Bertozzi circumvented these issues via the creation of a two-component click ligation in which the ring strain of a cyclic octyne — rather than a catalyst — provides the driving force for a 3+2 cycloaddition with an azide (Figure 1B)[4]. This development initiated the era of 'bioorthogonal' click chemistry (*i.e.* the use of click ligations that are compatible with living biological systems). Excitingly, innovations in the field continue to arm chemists and biologists with an arsenal of novel click reactions — *e.g.* the inverse electron-demand Diels-Alder (IEDDA) reaction and the Staudinger ligation — with properties suitable for a wide variety of applications[5,6].

Click chemistry has had a transformative effect on nearly every aspect of chemical science, but radiopharmaceutical chemistry has been impacted particularly profoundly[7]. Indeed, the exigencies of radiochemistry are uniquely well served by speed (a priority due to the decay of radionuclides), selectivity (critical to retain the biological activity of probes), and cleanliness (imperative due to the *in vivo* use of radiopharmaceuticals). As a result, radiopharmaceutical chemists have enthusiastically turned to click chemistry to solve problems in the field[8–11]. In this *Perspective*, we will discuss the practical advantages that the copper-catalyzed azide-alkyne cycloaddition (CuAAC), the strain-promoted azide-alkyne cycloaddition (SPAAC), and a host of emergent click ligations provide in radiopharmaceutical chemistry.

The Copper-Catalyzed Azide-Alkyne Cycloaddition

Surprisingly, the reaction that would later become *the* canonical click ligation — the copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction — is not mentioned in the original 2001 article, which coins the term 'click chemistry'[12]. The CuAAC ligation relies on a Cu(I) catalyst to facilitate the reaction of an azide and a terminal alkyne to form a 1,4-disubstituted 1,2,3-triazole. One of the first applications of the CuAAC reaction to radiopharmaceutical chemistry harnessed the ligation for the synthesis of peptides labeled with the positron-emitting radiohalogen fluorine-18 (^{18}F, $t_{1/2}$ ~ 109 min)[13]. This use has proven enduring, as ^{18}F-labeled synthons like 2-[^{18}F]fluoroethylazide continue to be employed for this purpose over a decade later[14]. The CuAAC reaction has since been used to create radiopharmaceuticals using vectors ranging from small molecules to nanoparticles

and radionuclides spanning from ^{11}C to ^{225}Ac. Here we highlight a selection of studies that best convey the utility of the reaction in the hand of radiochemists.

The most common use of the CuAAC ligation in radiochemistry is the assembly of radiopharmaceuticals. In one particularly elegant example, three different CuAAC reactions were used to create a bifunctional probe for positron emission tomography (PET) and near-infrared fluorescence imaging (NIRF) (Figure 2A)[15]. More specifically, a pair of CuAAC reactions was first leveraged to append two cyclic integrin $\alpha_v\beta_3$-targeting peptides to a central near-infrared fluorophore. Next, following the modification of the fluorophore with yet another azide, a third CuAAC ligation was used to attach a ^{18}F-bearing synthon. Remarkably, this seemingly complicated synthesis was accomplished in up to 90% yield in one-pot in under an hour. Furthermore, the completed imaging agent — dubbed '[^{18}F]F-NIR-cRGD$_2$' — effectively delineated U-87 MG human glioblastoma xenografts in nude mice, suggesting that it may have potential as a tool for both PET and intraoperative NIRF imaging in patients with integrin $\alpha_v\beta_3$-expressing malignancies.

Another way in which the CuAAC ligation can be harnessed for radiopharmaceutical chemistry relies on a critical yet often underappreciated property of radiotracers: their molar activity, *i.e.* the amount of radioactivity per mole of radiopharmaceutical. Molar activity is important because it denotes the fraction of the total molecules in a formulation of radiopharmaceutical that is radiolabeled. If the molar activity of a given radiotracer is too low (*i.e.* only a very small fraction of the molecules is radiolabeled), unlabeled molecules will outcompete radiolabeled ones for binding sites, lowering target uptake and target-to-background signal ratios. Molar activity is especially important in the context of low abundance targets, as the risk of being outcompeted by unlabeled molecules is particularly high. Against this backdrop, the CuAAC reaction was used to quickly and easily label a molar excess of alkyne-modified HER2-targeting peptides with [^{18}F]fluoroethylazide (Figure 2B)[16]. Subsequently, more than 98% of the unreacted, excess alkyne-bearing peptides was removed via the CuAAC ligation using an inexpensive azide-modified resin. In this manner, the ^{18}F-labeled peptides with molar activities greater than 200 GBq/μmol were isolated, a 200-fold increase compared to the peptides that had not been 'purified via click'. Importantly, this boost in molar activity provided practical benefits, as the radiopeptides with high molar activity produced better PET images than their counterparts with lower molar activity in murine models of ovarian adenocarcinoma and triple-negative breast cancer.

In the first two examples, the radionuclides in question simply acted as cargoes, small parts of synthons to be clicked to other components within the tracer. Yet this need not be the case. Indeed, in the next two applications of the CuAAC reaction, the radionuclides themselves interact far more intimately with the ligation's 1,2,3-triazole product. The "click-to-chelate" strategy represents a way to radiolabel molecules with technetium- and rhenium-tricarbonyl cores[17]. In this approach, a one-pot-two-step reaction creates a triazole that not only forms a linkage to the targeting vector but also becomes an integral part of the coordination environment of the $^{99m}Tc/Re(CO)_3$ core via its electron-rich N3 nitrogen (Figure 2C). Given ^{99m}Tc's status as a workhorse of single photon emission computed tomography (SPECT), this synthetic strategy can be used to streamline the development

of novel ^{99m}Tc-labeled radiopharmaceuticals as well as radiotherapeutics labeled with its radiocongeners ^{186}Re and ^{188}Re. More recently, the CuAAC ligation has been harnessed to create a straightforward strategy for radiolabeling with ^{211}At, a short-lived α-emitting radiohalogen that holds great potential for the targeted radionuclide therapy (TRT) of metastatic lesions but remains underutilized due to its mercurial labeling chemistry and supply chain challenges (Figure 2D). In this approach — which is based on prior work with ^{125}I[18] — the CuAAC ligation is run in the presence of the anionic radiohalogen as well as excess Cu(II).[19] In addition to catalyzing the click reaction, Cu(II) oxidizes the radiohalogen to ^{211}At^{+}, which subsequently undergoes an electrophilic substitution with an intermediate of the cycloaddition, ultimately producing a 5-[^{211}At]astatotriazole. Preliminary results suggest that this strategy may yield more stable ^{211}At-labeled radiopharmaceuticals than other synthetic approaches, an important development given the radionuclide's propensity for *in vivo* dehalogenation.

The radiopharmaceuticals synthesized using the CuAAC reaction have been successfully translated to the clinic. In 2011, the first-in-human evaluation of an integrin-targeted radiotracer synthesized via the CuAAC ligation — [^{18}F]F-RGD-K5 — was reported in healthy volunteers[20]. The first-in-human trial of a click-based somatostatin receptor-targeting probe — [^{18}F]fluoroethyltriazole-Tyr^{3}-octreotate ([^{18}F]FET-βAG-TOCA) — in patients with neuroendocrine tumors was published in 2016[21]. Recently, the CuAAC ligation was used to synthesize [^{68}Ga]Ga-Trivehexin, a novel imaging agent composed of the Ga-chelator TRAP linked via triazoles to a trio of cyclic $\alpha_v\beta_6$-targeting peptides (Figure 3A)[22]. This multimeric construct boasts increased target avidity compared to its monomeric cousins and has been used for the PET imaging of both pancreatic adenocarcinoma and head and neck cancer. Indeed, first-in-human data suggests that [^{68}Ga]Ga-Trivehexin is capable of effectively mapping the expression of $\alpha_v\beta_6$-integrin in a clinical setting (Figure 3B)[22].

The Strain-Promoted Azide-Alkyne Cycloaddition

While the CuAAC ligation has had a tremendous impact on radiopharmaceutical chemistry, it nonetheless has limitations. Most notably, the Cu(I) catalyst can interfere with the use of radiometals, outcompeting the radionuclides — which are often used at only nanomolar levels — for chelators. Furthermore, the presence of high concentrations of Cu(I) can threaten the structural integrity of more sensitive biomolecules such as proteins and immunoglobulins. Consequently, many radiopharmaceutical chemists have turned to Bertozzi's copper-free SPAAC ligation[23].

In 2011, Campbell-Verduyn, *et al.* reported one of the first uses of the SPAAC reaction in radiopharmaceutical chemistry[24]. Specifically, the investigators successfully labeled a cyclooctyne-bearing variant of the gastrin-releasing peptide receptor (GRPR)-targeting peptide bombesin with various ^{18}F-labeled azide moieties (Figure 4A). This reaction yielded several ^{18}F-labeled peptides with high affinity for GRPR, demonstrating the promise of SPAAC for the reliable production of radiotracers. The SPAAC ligation has also been used to radiolabel azide-containing nanoparticles (SCK-NP) with a [^{64}Cu]Cu-DOTA-labeled variant of dibenzocyclooctyne (DBCO)[25], producing [^{64}Cu]Cu-DOTA-SCK-NP with exceptionally high molar activities of ~36 TBq/μmol (Figure 4B). Yet a handful

of PET imaging and biodistribution experiments have illustrated one potential drawback of the SPAAC ligation as a radiosynthetic tool: its 'footprint'. To wit, ^{64}Cu-labeled somatostatin receptor-targeting peptides synthesized via the SPAAC reaction exhibited significantly slower clearance from healthy tissues than those synthesized using traditional methods, a phenomenon attributed to the hydrophobicity of the bulky, tetracyclic product of the SPAAC ligation (Figure 4C)[26]. The same study also addressed another easily overlooked complication of the SPAAC reaction: unlike the CuAAC ligation — which forms predominantly 1,4-disubstituted triazoles — the SPAAC reaction is not regioselective, forming both 1,5- and 1,4-disubstituted triazole products that must be separated. It is important to note, however, that both obstacles (*i.e.* hydrophobicity and regioselectivity) will exert a much greater impact with small vectors than with larger biomolecules.

Easily one of the most common applications of the SPAAC ligation in radiochemistry is the synthesis of radioimmunoconjugates. Historically, the overwhelming majority of radiolabeled antibodies have been created via the stochastic attachment of chelators and prosthetic groups to the lysines of immunoglobulins. This approach is facile, but it frequently yields poorly defined and heterogenous products with suboptimal *in vitro* and *in vivo* performance[27]. In response, the community has dedicated considerable resources to the development of novel strategies for the attachment of cargoes to discrete sites within antibodies, a process called 'site-specific bioconjugation'. The selectivity and bioorthogonal nature of the SPAAC reaction makes it perfectly suited for the task. For example, a genetic code expansion technique was used to integrate an azide-bearing unnatural amino acid — N^{ε}−2-azideoethyloxycarbonyl-L-lysine (NEAK) — into the anti-CD20 mAb rituximab to yield an immunoconjugate dubbed 'A122NEAK-rituximab' (Figure 5A)[28]. This immunoconjugate was then conjugated with a DBCO-bearing variant of DOTA and labeled with ^{64}Cu, producing a ^{64}Cu-labeled radioimmunoconjugate with excellent *in vivo* behavior in a murine model of B cell lymphoma. In another case, a cell-free expression system was used to create a variant of trastuzumab containing a quartet of *para*-azidomethyl phenylalanine (pAMF) residues[29]. These unnatural amino acids were modified with DBCO-bearing versions of desferrioxamine (DFO) and 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid (DO3A), and the immunoconjugates were labeled with ^{89}Zr and ^{111}In, respectively (Figure 5B). The *in vivo* performance of the ^{89}Zr- and ^{111}In-labeled radioimmunoconjugates was interrogated via PET and SPECT imaging, respectively, in a murine model of ovarian cancer, with both radiotracers yielding excellent tumor uptake and tumor-to-background activity concentration ratios.

The SPAAC ligation can also be used for site-specific bioconjugation without resorting to expensive and complex genetic engineering. For example, a chemoenzymatic strategy featuring a promiscuous galactosyltransferase was employed to incorporate a pair of azide-modified sugars into the heavy chain glycans of the HER2-targeted mAb pertuzumab (Figure 5C)[30]. The azide-modified pertuzumab was then clicked to a DBCO-bearing variant of DFO and labeled with ^{89}Zr, yielding an immunoPET probe that boasted dramatically improved performance compared to a stochastically labeled analog in both NSG and humanized NSG mice bearing HER2-expressing human breast cancer xenografts. This radioimmunoconjugate — [^{89}Zr]Zr-^{ss}DFO-pertuzumab — is currently the subject of a first-in-human clinical trial at Memorial Sloan Kettering Cancer Center (NCT04692831)

designed to evaluate its potential for the PET imaging of patients with metastatic, HER2-positive breast, bladder, and renal cancer. A recent purely chemical approach to SPAAC-mediated site-specific bioconjugation eschews both genetic engineering *and* enzymatic transformations by relying upon the unique selectivity of a branched, azide-bearing perfluorophenyl ester reagent — PFP-bisN_3 — for the K188 sites within the light chains of κIgG_1 antibodies (Figure 5D)[31]. Using this strategy, the HER2-targeting mAb trastuzumab was modified with a quartet of azides that were then conjugated via SPAAC with a DBCO-modified variant of DFO. The subsequent labeling of the immunoconjugate with ^{89}Zr produced a radioimmunoconjugate with high stability, high immunoreactivity, and excellent *in vivo* performance in a murine model of breast cancer. Each of these four SPAAC-mediated methods produces well-defined and homogeneous radioimmunoconjugates. While the first-in-human study of [^{89}Zr]Zr-^{ss}DFO-pertuzumab will certainly prove valuable for the field in demonstrating the clinical viability of site-specifically labeled radioimmunoconjugates, the recently developed PFP-bisN_3 approach may ultimately prove to be the simplest and most readily translatable strategy.

Next Generation Click Reactions

In the years since Sharpless, Meldal, and Bertozzi's seminal discoveries, the field has continued to innovate and has developed a host of novel click ligations (and especially *bioorthogonal* click ligations) that radiopharmaceutical chemists have enthusiastically adopted. For example, Bertozzi's own laboratory successfully used the Staudinger ligation to facilitate the labeling of azide-bearing biomolecules with triarylphosphine-modified fluorophores in live cells (Figure 6A). This reaction has been exploited for radiochemistry — most notably for the development of an arsenal of ^{18}F-labeled phosphines for the mild radiolabeling of azide-bearing constructs — but its widespread adoption has been limited both by its sluggish kinetics ($k_2 \sim 10^{-3}$ $M^{-1}s^{-1}$) as well as the intrinsic instability of triarlyphosphines[32]. In another example, the click reaction between dibenzoazacyclooctyne (DIBAC) groups and sydnones (1,2,3-oxadiazoles) was used for synthesizing ^{18}F-labeled peptides via the ligation between 4-[^{18}F]fluorophenyl sydnone and DIBAC-bearing biomolecules (Figure 6B)[33].

Sulfur fluoride exchange (SuFEx) chemistry, first described in the chemical literature almost 100 years ago, has more recently gained traction as a useful click ligation for radiochemistry[34]. SuFEx chemistry has facilitated the synthesis of ^{18}F-labeled probes via isotope exchange (Figure 6C)[35], as demonstrated by the labeling of micrograms of ^{19}F-bearing precursors with ^{18}F in seconds at room temperature to produce high purity, isotopologous radiotracers in molar activities up to 280 GBq/μmol that did not require purification via HPLC. Silicon fluoride acceptor (SiFA) chemistry is a more recent iteration of this isotopic exchange-based approach, and it offers similar benefits. 'RIKEN' click chemistry — the rapid and mild 6π-azaelectrocyclization between α, β, γ, ∂-unsaturated aldehydes and primary amines — has also been exploited for the creation of PET imaging agents[36]. Specifically, this ligation has been used to append a ^{68}Ga-labeled variant of DOTA bearing an α, β, γ, ∂-unsaturated aldehyde to the lysine residue of a cyclic RGD peptide to create a probe for imaging the expression of $\alpha_v\beta_3$ integrin (Figure 6D).

While each of these ligations has a place in radiopharmaceutical chemistry, the next-generation click reaction that has had the biggest impact on the field is, without question, the inverse electron-demand Diels-Alder (IEDDA) ligation between tetrazine (Tz) and *trans*-cyclooctene (TCO) (Figure 7A). The IEDDA reaction's earliest applications in radiochemistry centered on the labeling of peptides and antibodies[37,38]. Along these lines, one of the more creative uses of the IEDDA ligation was the repurposing of 2-[^{18}F]deoxyfluoroglucose to create ^{18}F-labeled tetrazines for the site-specific labeling of single-domain antibodies bearing C-terminal TCO moieties (Figure 7B)[39]. Yet even from these earliest days, it was clear that the exceptional speed of the IEDDA ligation (k_2 values of more than 100,000 $M^{-1}s^{-1}$) made it nearly perfect for one application in particular: *in vivo* pretargeting.

Briefly, *in vivo* pretargeting is an approach to nuclear imaging and radioimmunotherapy designed to circumvent the high radiation doses to healthy tissues associated with traditional, directly labeled radioimmunoconjugates[40]. In pretargeting, the radionuclide and antibody are decoupled and injected sequentially (antibody first; radioligand second), and a selective ligation facilitates the *in vivo* combination of the two components at the tumor site (Figure 8A). In the ligation's first foray into *in vivo* pretargeting, a colorectal cancer xenograft-bearing mouse was injected with a TCO-modified variant of the TAG72-targeting antibody CC49 (CC49-TCO) and then, 24 hours later, a ^{111}In-labeled dipyridyltetrazine radioligand[41]. SPECT imaging revealed the remarkable efficacy of this approach, as it produced selective tumor uptake and high tumor-to-background image contrast. Following this initial proof-of-concept, the approach has been expanded to different antibody/antigen systems as well as an array of radionuclides — most notably ^{68}Ga, ^{18}F, and ^{64}Cu[42–44]. But the most promising work to date has been in the context of pretargeted radioimmunotherapy (PRIT). The earliest work here was performed with ^{177}Lu, but the ^{225}Ac-PRIT investigations best demonstrate the potential of the strategy[45]. Here, a CA19.9-targeting, TCO-bearing immunoconjugate (5B1-TCO) and an ^{225}Ac-labeled tetrazine radioligand ([^{225}Ac]Ac-DOTA-PEG$_7$-Tz) were used in mice bearing pancreatic ductal adenocarcinoma xenografts (Figure 8B). The strategy delivered high concentrations of the α-emitting radiometal to tumor tissue, ultimately producing higher dose rates to the tumor *and* better therapeutic indices than directly labeled [^{225}Ac]Ac-DOTA-5B1. Furthermore, it has been demonstrated that sequential injections of two tetrazine radioligands labeled positron- and β-emitting isotopologues of copper (^{64}Cu and ^{67}Cu, respectively) can also be used to create a theranostic approach to PRIT in which the PET images acquired with the former can be used to predict the response to therapy with the latter (Figure 8C)[46]. Although IEDDA-based pretargeting has not yet been translated to the clinic, a recent pretargeted PET study in companion dogs with osteodestructive lesions suggests that the approach could work in the larger blood volumes of humans[47]. The first-in-human demonstration of this technology is anticipated in the near future.

Outlook—Over the past twenty years, click chemistry has transformed radiopharmaceutical chemistry. These reactions have not only enabled the synthesis of radiotracers with heretofore unattainable levels of precision and efficiency but have also emerged as key components of technologies — such as site-specific bioconjugation and *in vivo* pretargeting

— that have the potential to fundamentally alter the way the field operates. Yet we believe the next decade of work at the intersection of click chemistry and radiochemistry may be the most exciting of all. Along these lines, two nascent trends stand out. First, radiochemists and organic chemists are increasingly collaborating on the development of new click transformations (*e.g.* SuFEx chemistry) with radiochemical applications in mind. Second, and even more remarkably, an ever-expanding array of click-based radiopharmaceuticals are being translated in first-in-human studies, expanding click chemistry's impact on nuclear medicine from the laboratory to the clinic. As we move forward in the wake of last year's Nobel Prize, we are eager to see the ways in which the work of the three laureates and those they have inspired continue to impact our field and drive the development of novel radiopharmaceutical technologies for the patients that depend on them.

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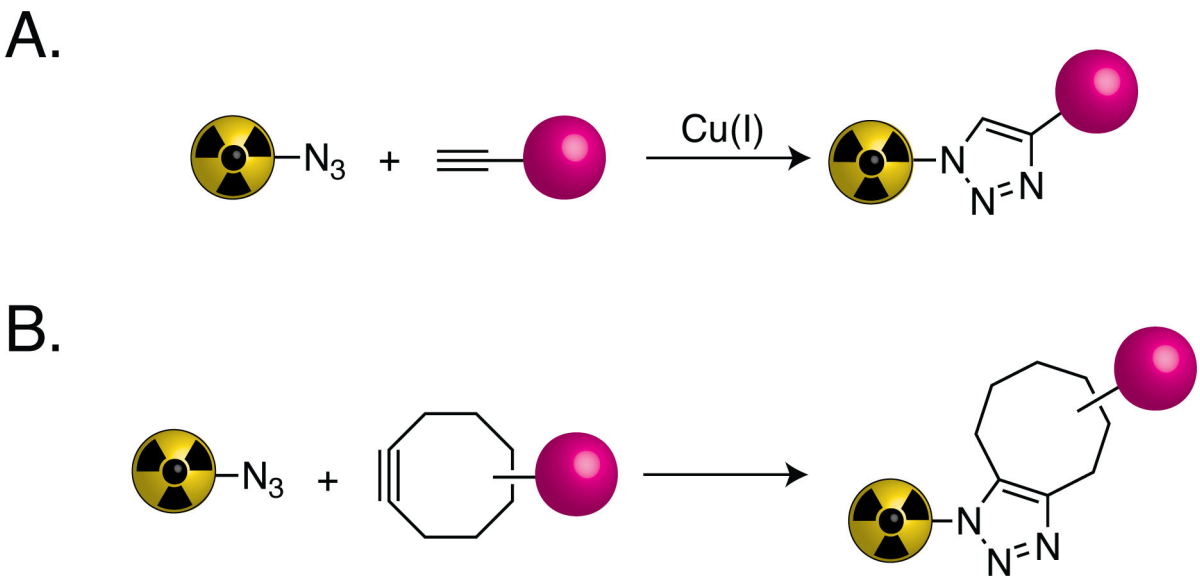

Figure 1.

Schematic of (A) the copper-catalyzed azide-alkyne cycloaddition (CuAAC) and (B) the strain-promoted azide-alkyne cycloaddition (SPAAC). Magenta spheres represent cargoes.

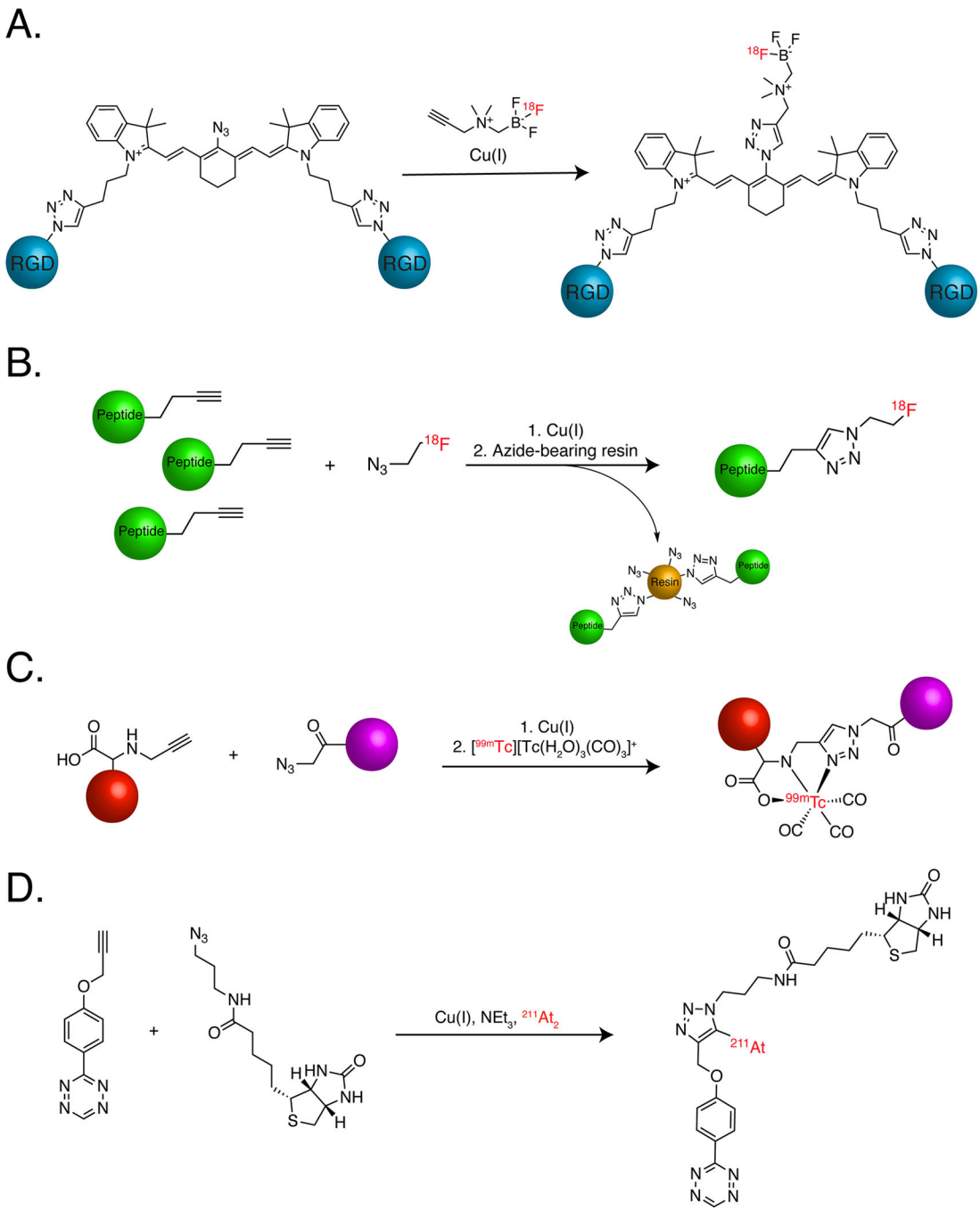

Figure 2.

The use of the CuAAC ligation to (A) synthesize an integrin-targeted multimodal PET/NIRF imaging agent, (B) increase the specific activity of ^{18}F-labeled peptides, (C) radiolabel a vector with a [^{99m}Tc]Tc(CO)$_3$ core via the 'click-to-chelate' strategy, and (D) create an ^{211}At-labeled probe in which the radiohalogen is appended to the triazole moiety. Red and purple spheres represent cargoes.

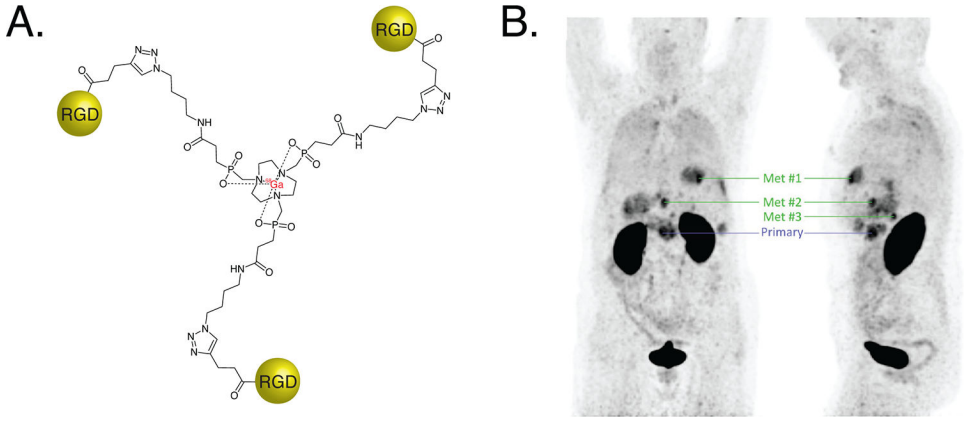

Figure 3.

(A) The structure of [^{68}Ga]Ga-Trivehexin; (B) PET images acquired 120 minutes after the administration of 105 MBq of [^{68}Ga]Ga-Trivhexin to a patient with $\alpha_v\beta_6$-expressing pancreatic ductal adenocarcinoma showing uptake in a primary tumor as well as several metastatic lesions. Figure 3B was reprinted under the Creative Commons Attribution 4.0 International License (CC BY 4.0) terms from reference 22.

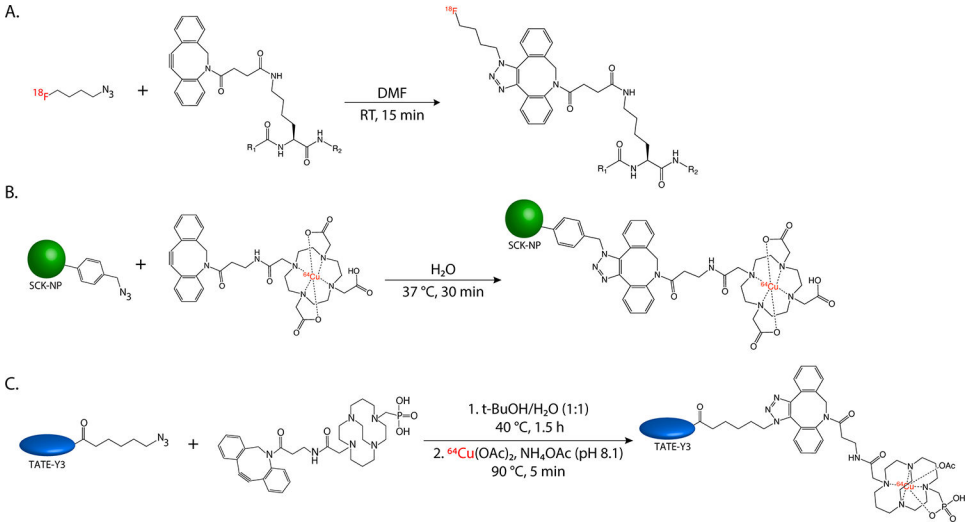

Figure 4.

The use of the SPAAC ligation to create (A) ^{18}F-labeled GRPR-targeting peptides, (B) ^{64}Cu-labeled nanoparticles, and (C) ^{64}Cu-labeled somatostatin receptor-targeting peptides.

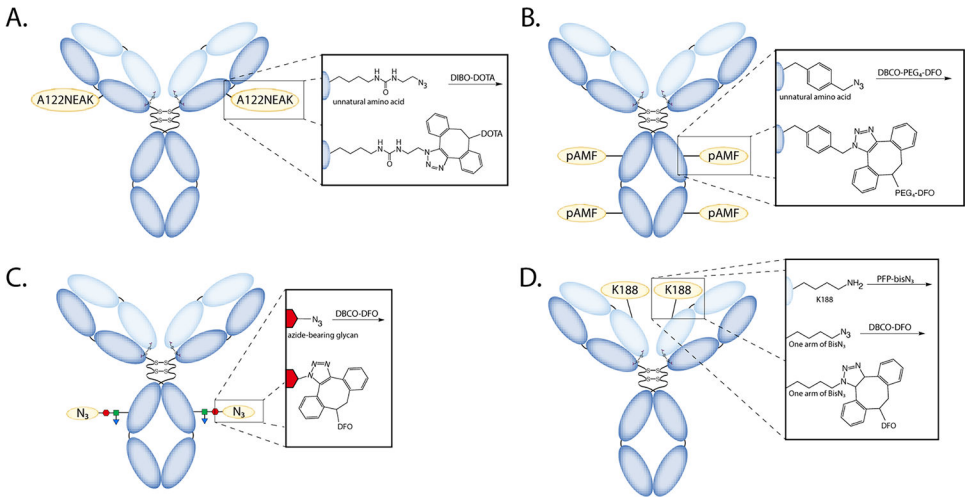

Figure 5.

Four approaches that harness the SPAAC ligation to facilitate the site-specific bioconjugation of antibodies. In each case, the site-specificity is predicated on (A) the incorporation of the unnatural amino acid N^{ε}−2-azideoethyloxycarbonyl-L-lysine, (B) the incorporation of the unnatural amino acid *p*-azidomethyl phenylalanine, (C) the chemoenzymatic manipulation of the heavy chain glycans to append azide-modified sugars, and (D) the selective ligation of a branched, azide-containing perfluorophenyl ester with the K188 residues of the light chain.

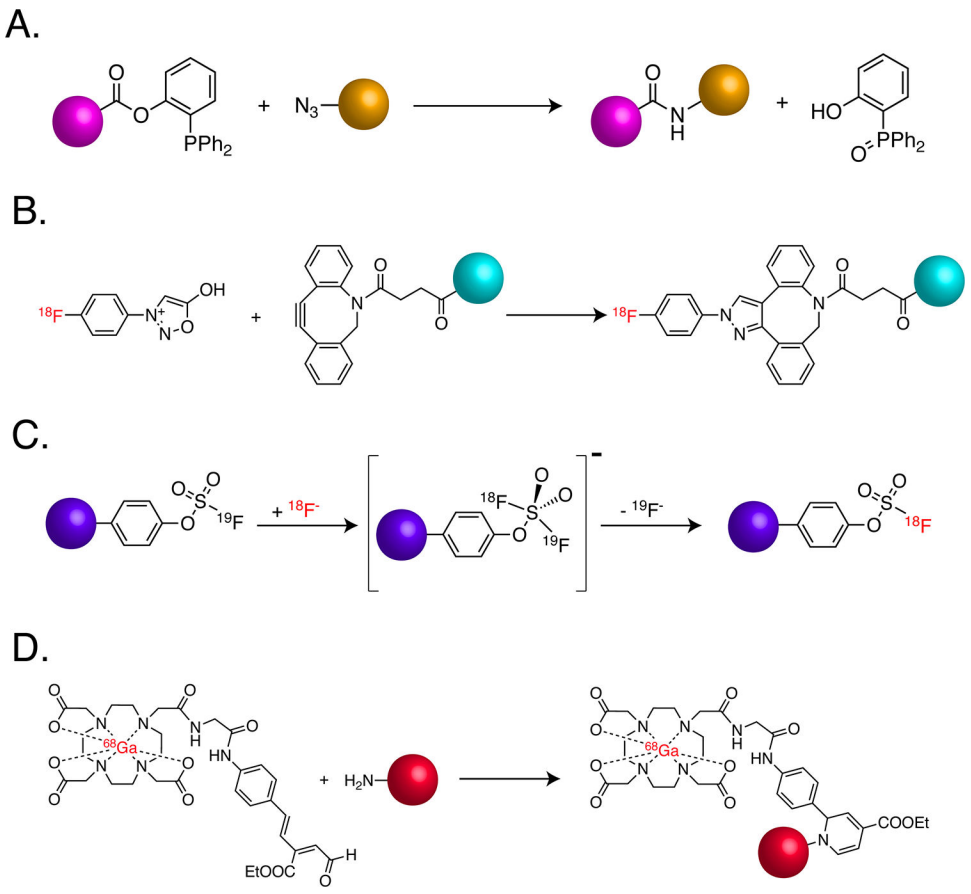

Figure 6.

(A) The traceless Staudinger ligation; (B) ^{18}F-labeling via the sydnone-alkyne cycloaddition; (C) ^{18}F-labeling via sulfur-fluoride exchange (SuFEx) chemistry; (D) the bioconjugation of a [^{68}Ga]Ga-DOTA-moiety via the RIKEN reaction. Colored spheres represent cargoes.

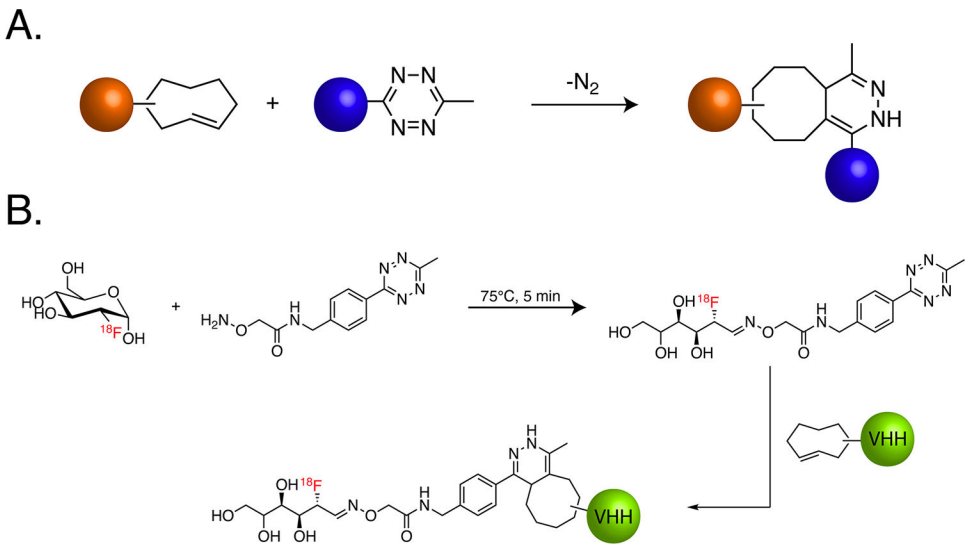

Figure 7.

(A) Schematic of the inverse electron-demand Diels-Alder (IEDDA) ligation; (B) schematic of the method devised by Ploegh and coworkers that harnesses [^{18}F]FDG and the IEDDA ligation for the site-specific radiolabeling of antibody fragments. Orange and blue spheres represent cargoes.

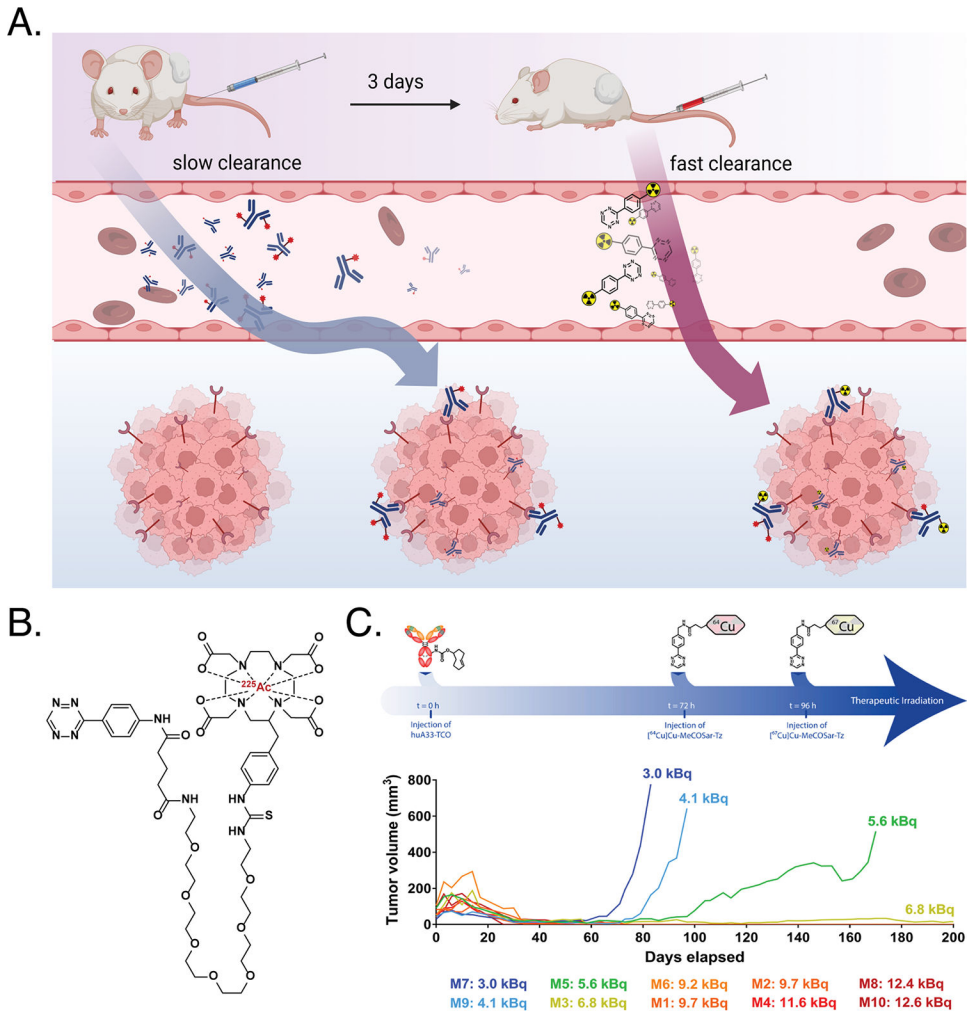

Figure 8.

(A) Schematic of *in vivo* pretargeting based on the IEDDA ligation; (B) structure of [^{225}Ac]Ac-DOTA-PEG$_7$-Tz; (C) timeline of an approach to theranostic pretargeting that leverages a ^{64}Cu-labeled tetrazine for PET imaging and a ^{67}Cu-labeled tetrazine for targeted radionuclide therapy (top) as well as the relationship between the image-derived cumulative activity of the ^{64}Cu-labeled tetrazine in the tumor and the tumor's response to therapy with the ^{67}Cu-labeled tetrazine (bottom). Figure 8A is an original figure created with BioRender.com. Figure 8C was reprinted under the Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND 4.0) terms of reference 46.