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REVIEW

Click chemistry-driven tumor theranostics: recent advances, challenges, and future perspectives

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ABSTRACT Tumor theranostics, which integrates accurate diagnosis and precise therapy, has emerged as a pivotal approach in modern oncology for improving treatment efficacy and reducing off-target toxicity. Click chemistry, which is characterized by high efficiency, selectivity, biocompatibility, and modularity, has become an indispensable tool for constructing versatile theranostic systems. This review systematically summarizes the recent progress in click chemistry-based tumor theranostic systems, starting with an overview of core click reactions and the unique features in biomedical applications. We then focus on the application of click chemistry within a diagnosis-therapy-theranostics framework to three key aspects of tumor theranostics: (i) tumor diagnosis (molecular imaging probes and circulating tumor cell detection); (ii) tumor therapy (chemotherapy, phototherapy, immunotherapy, and gene therapy); and (iii) integrated theranostics (multimodal imaging-guided combinatorial therapy). Furthermore, the current challenges, such as the biocompatibility of catalysts and *in vivo* reaction efficiency, are critically discussed. Finally, we highlight promising directions, including stimuli-responsive click reactions, AI-assisted probe design, and personalized theranostic systems. This review not only serves as a comprehensive reference for researchers but also highlights how click chemistry uniquely bridges molecular design with clinical functionality, distinguishing click chemistry from conventional conjugation or labeling methods by enabling spatiotemporal control, modular integration, and bioorthogonal precision in complex biological settings.

KEYWORDS Click chemistry; tumor theranostics; molecular imaging; precision therapy; multimodal integration; clinical translation

Introduction

Tumors remain one of the leading causes of global mortality. In 2022 there were an estimated 20 million new tumor cases and 9.7 million tumor-related deaths worldwide[1]. Consequently, the high incidence and mortality rates of tumors remain difficult challenges for diagnostic and therapeutic approaches[2,3]. Conventional diagnostic techniques, such as computed tomography (CT) and biopsy, are often invasive and unsuitable for real-time dynamic monitoring[4-6]. Traditional treatments, such as chemotherapy and radiotherapy, have limitations, including poor targeting, significant systemic toxicity, low delivery efficiency, and a tendency to induce drug resistance, which often results in suboptimal efficacy and reduced quality of life[7-9]. Third, a unified theranostic platform integrating diagnosis and therapy to enable real-time monitoring and dynamically adjustable personalized treatment regimens has yet to be successfully developed[10,11].

The construction of efficient theranostic systems requires a flexible and reliable chemical tool to conjugate diverse functional moieties (e.g., targeting ligands, imaging agents, and therapeutic payloads) with high efficiency and biocompatibility[12,13]. Click chemistry has emerged as a powerful platform for innovative cancer diagnosis and therapy[14-16]. These reactions are characterized by high efficiency, exceptional selectivity, mild reaction conditions, minimal by-products, and modularity, making the reactions particularly suitable for complex biological systems[17]. The core value of click chemistry lies in the ability to efficiently and covalently conjugate functional modules, such as targeting ligands, imaging agents, and therapeutic molecules[18-20]. Click chemistry significantly streamlines the synthesis and modification of theranostic agents *in vitro*, improving preparation efficiency and purity. The excellent bio-orthogonality of click chemistry enables precise molecular assembly *in vivo* and activation within the tumor microenvironment (TME), offering a powerful route for synthesizing

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probes and drug carriers in precision medicine. Over the past two decades click chemistry has evolved from a concept to a practical technology for tumor theranostics[21,22]. Early studies focused on the classic Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction for labeling biomolecules[23], while recent advances have expanded to Cu-free click reactions [e.g., strain-promoted azide-alkyne cycloaddition (SPAAC)] and reversible click reactions [e.g., Diels–Alder (DA) cycloaddition][24], which further enhance biocompatibility and responsiveness[25-27]. Despite numerous reviews on click chemistry and tumor theranostics, a comprehensive and up-to-date summary of the intersection between click chemistry and tumor theranostics, particularly focusing on design principles, application scenarios, and clinical translation potential, is lacking.

Recent reviews have made valuable contributions by exploring specific aspects of click chemistry in oncology, such as use in nanomaterial-based theranostics[28,29], tumor-targeting strategies, or clinical advances in bio-orthogonal chemistry[30-32]. However, an integrated, systematic framework that connects molecular design principles directly to the full range of applications in diagnosis, therapy, and theranostics is lacking. Current discussions tend to focus on individual reaction types, disease models, or material classes, rather than offering a unified perspective that aligns the versatile click toolkit with the continuum of cancer care (from detection and diagnosis-to-treatment and monitoring). This review aims to fill that knowledge gap by introducing a functional objective-driven framework that integrates diagnosis, therapy, and theranostics through click chemistry. Rather than treating these areas separately, we bring together design principles, representative applications, and translational challenges across all three components of the framework, offering a clear and structured pathway forward. Building on established concepts in molecular targeting and delivery, and incorporating the latest developments, we show how click chemistry not only advances diagnostic and therapeutic tools individually but also unites diagnostic and therapeutic tools into cohesive, image-guided platforms. In so doing, this review seeks to clarify the expanding role of click chemistry in precision oncology and provide a practical roadmap for its continued development.

The modular nature of click chemistry facilitates the flexible construction of various high-performance imaging probes that are needed for accurate tumor diagnosis. Whether conjugating fluorescent dyes, radionuclides, or magnetic resonance imaging (MRI) contrast agents with targeting molecules (e.g., antibodies and peptides), click chemistry significantly enhances probe synthesis efficiency, purity, and *in vivo* stability, leading to diagnostic images with high signal-to-noise ratios. Moreover, probes exhibit unique advantages for the efficient capture and identification of circulating tumor cells (CTCs). Click chemistry has broad applications in the field of cancer therapy. Click chemistry can be used to construct targeted drug delivery systems that enhance the accumulation and release precision of chemotherapeutic agents in tumor tissues, design stimulus-activated prodrugs, photosensitizers, and immunomodulating agents for precise activation at the disease site, and provide novel assembly strategies for protein degradation therapies [e.g., proteolysis-targeting chimeras (PROTACs)] and gene therapies, which significantly advance innovative treatment paradigms. Click chemistry also enables the convergence of diagnosis and therapy, thereby driving the development of cancer theranostics. Imaging and therapeutic functions can be integrated into a single system *via in vivo* click reactions, achieving synchronous treatment and monitoring, enabling real-time imaging feedback and therapeutic assessment during intervention, and facilitating image-guided therapeutic interventions in precision medicine. However, despite the prospects, the clinical translation of click chemistry in oncology faces challenges involving *in vivo* reaction efficiency, long-term biosafety, and scalable production.

This review is structured to cover the latest progress in click chemistry-driven tumor theranostics (**Figure 1**), as follows: (i) core click reactions and the click reaction characteristics in biomedical contexts are introduced; (ii) applications of click chemistry in tumor diagnosis, therapy, and diagnosis-therapy integration are presented; (iii) the current challenges and technical bottlenecks are discussed; and (iv) future directions are proposed. By synthesizing recent studies, this review highlights the critical role of click chemistry in advancing tumor theranostics and provides insights for future research. This review systematically outlines the applications of click chemistry in tumor diagnosis, therapy, and theranostic integration, analyzes current key bottlenecks, and discusses future trends in convergence with cutting-edge fields, such as artificial intelligence (AI) and nanotechnology, with the aim of providing a reference for promoting clinical advances and application in precision medicine. Notably, this review systematically classifies click-based theranostic systems based on the *in vivo* assembly strategies (exogenous, endogenous, and hybrid) with a dedicated focus on emerging trends in AI-assisted molecular design and stimuli-responsive, activatable click systems, areas that are not comprehensively covered in previous summaries.

Core click reactions for tumor theranostics

Coined by K. Barry Sharpless in the early 2000s, click reactions refer to a class of highly efficient and selective chemical reactions for molecular assembly. In this section key click chemistry terms are briefly defined upon first mention to aid interdisciplinary readers. Representative reactions include CuAAC, catalyst-free SPAAC, and inverse electron demand Diels–Alder (IEDDA). Under mild conditions with minimal byproducts, click reactions exhibit strong biocompatibility and solvent tolerance. Therefore, click reactions are widely

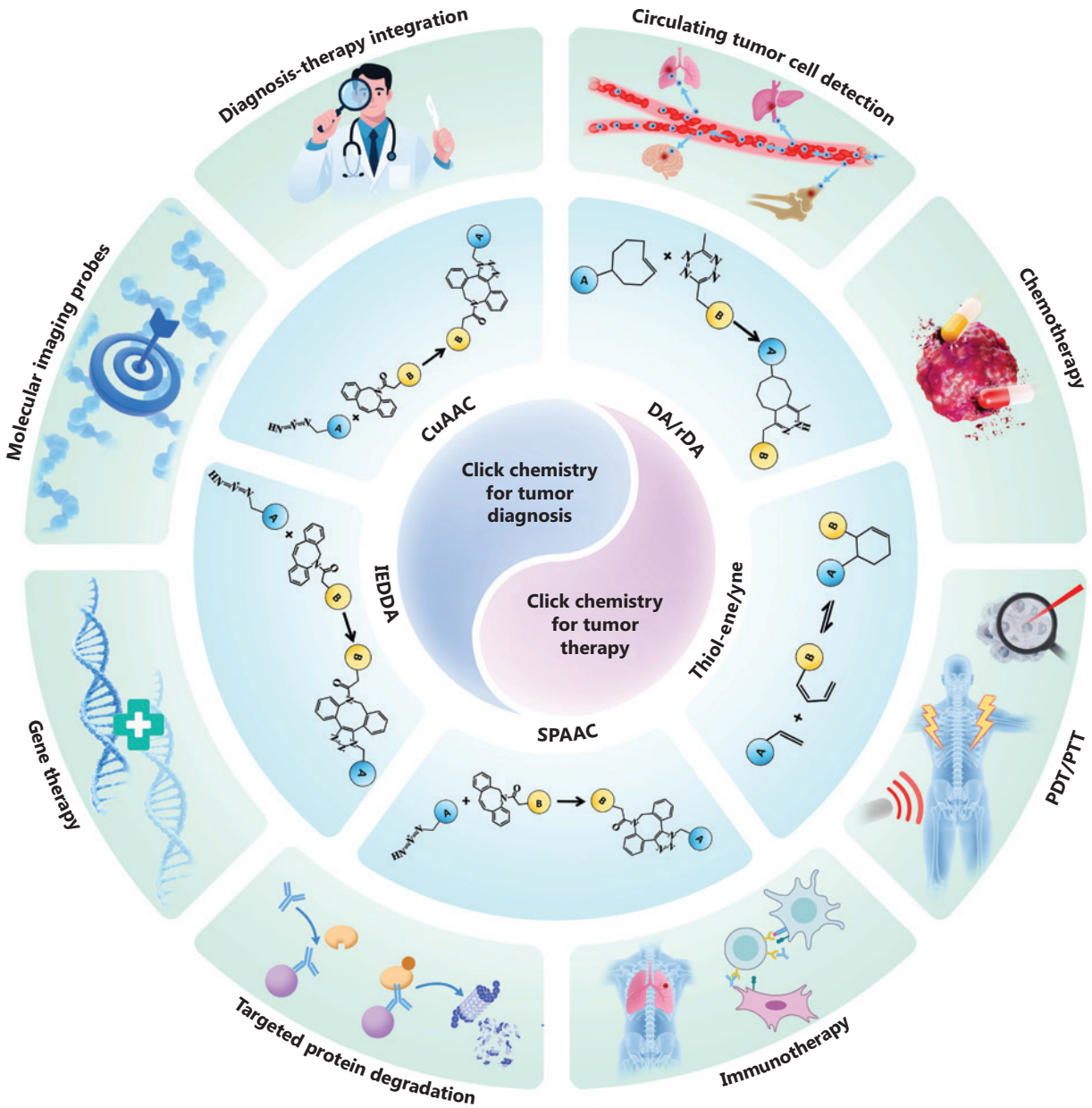


Figure 1 Schematic overview of click chemistry-driven tumor theranostics, illustrating the integration of diagnostic imaging and therapeutic modalities through bio-orthogonal conjugation strategies in oncology.

applied in drug development, chemical biology, and materials science and are indispensable tools for efficient molecular synthesis[33-35]. The selection of click reactions for tumor theranostics depends on biocompatibility (no toxic catalysts or byproducts), *in vivo* reactivity (fast kinetics under physiologic conditions), selectivity (no cross-reaction with endogenous biomolecules), and modularity (easy conjugation of diverse functional groups). The defining characteristics of click reactions (bio-orthogonality, rapid kinetics, and mild reaction conditions) form the basis of utility in precision nanomedicine. Bio-orthogonality minimizes interference with native biological processes, enabling precise molecular tagging and assembly within living systems. The fast reaction kinetics facilitate real-time or near real-time processes, which are critical for dynamic imaging and on-demand drug activation. The mild conditions maintain the integrity and function of sensitive

biomolecules and therapeutic payloads during conjugation. Together, these properties enable the design of sophisticated theranostic systems for controlled drug release, targeted delivery, and reduced off-target effects. **Table 1** summarizes the most widely used click reactions in tumor theranostics along with the mechanisms, advantages, and limitations.

Copper(I)-catalyzed azide-alkyne cycloaddition

CuAAC is the "gold standard" of click chemistry. CuAAC involves [3 + 2] cycloaddition between an azide ($-N_3$) and a terminal alkyne ($-C\equiv CH$) catalyzed by Cu(I) [e.g., $CuSO_4$/ascorbic acid as a reducing agent] to form a stable 1,2,3-triazole ring. CuAAC is widely used for labeling antibodies, peptides, and nanoparticles with imaging agents (e.g., fluorescent dyes and radionuclides) or therapeutic drugs (**Figure 2A**)[36]. For example, Quigley et al.[37] achieved trimerization of the $\alpha_v\beta_6$ integrin-targeting peptide, SDM17, through a CuAAC reaction, constructing a novel PET probe [^{68}Ga-TRAP(SDM17)$_3$], which has significantly enhanced affinity for the $\alpha_v\beta_6$ integrin (IC_{50} of 0.26 nM) and tumor uptake (2.1% ID/g). This process resulted in the high-contrast PET imaging of a mouse model of human lung adenocarcinoma with a tumor-to-background ratio (TBR) up to 29.7.

Diels–Alder cycloaddition and retro-Diels–Alder

The DA reaction is a [4 + 2] cycloaddition reaction between a diene (electron-rich) and a dienophile (electron-poor) to form a six-membered cyclohexene ring[38]. The reverse retro-Diels–Alder (rDA) reaction occurs under stimuli [e.g., heat, pH, and reactive oxygen species (ROS)], making DA/rDA a reversible click reaction. DA/rDA is widely used to construct stimuli-responsive theranostic systems (**Figure 2B**)[39]. For example, Park et al.[40] designed and assembled drugs through DA/rDA orthogonal reactions, introducing rDA reactions as a triggering mechanism in self-assembly systems for the first time. Utilizing the steric hindrance released from the rDA reaction, two previously suppressed diynyl lipid monomers (furan and maleimide) spontaneously assembled into supramolecular polymers on a solid surface, forming poly(diyne) [PDA] under ultraviolet (UV) light with a visible blue signal. This method achieves a triple enhancement of assembly sites, response signals, and post-modification functionalities.

Thiol-ene/yne click reactions

Thiol-ene reactions involve the addition of a thiol (–SH) to an alkene (–C=C–) *via* a radical mechanism catalyzed by UV light or a thermal initiator, while thiol-yne reactions involve the addition of two thiols to a terminal alkyne ($-C\equiv CH$). Thiol-ene reactions are used for the surface modification of biomaterials and drug delivery systems (**Figure 2C**)[41]. For example, Liang et al.[42] used the condensation reaction of 2-cyanobenzothiazole-1,2-aminothiol as a tool to design monomers based on thiol-ne/yne click reactions with three types of switches for pH/reduction/proteolysis, which rapidly polymerize into circular oligomers in cells and self-assemble into nanofibers or hydrogels to accurately localize the reaction near the Golgi apparatus. This procedure produced a 5–7× signal enhancement for furin protease activity imaging.

Table 1 Core click reactions for tumor theranostics

Click reaction	Reactants	Catalyst/stimulus	Rate constant (k_2, M^{-1} s^{-1})	Advantages	Limitations	Applications
CuAAC	Azide + terminal alkyne	Cu(I) (e.g., $CuSO_4$/AA)	10^3–10^4	High selectivity, fast, stable product	Cu(I) toxicity	Drug-peptide conjugation, *in vitro* labeling[36,37]
DA/rDA	Diene + dienophile	Heat/pH/ROS (for rDA)	10^{-3}–10^{-1}	Reversible, stimuli-responsive	Slow forward reaction	Stimuli-responsive drug delivery[38-40]
Thiol-Ene/Yne	Thiol + alkene/alkyne	UV/visible light/AIBN	10^1–10^2	Versatile, mild conditions	UV light toxicity	Biomaterial surface modification[41,42]
SPAAC	Azide + strained cyclooctyne	None	10^{-1}–10^1	Copper-free, high biocompatibility	Slower than CuAAC	*In vivo* imaging, theranostic nanoparticles[43]
IEDDA	TCO + Tz	None	10^2–10^3	Copper-free, high biocompatibility	Slower than CuAAC	*In vivo* imaging, theranostic nanoparticles[44,45]

AA, ascorbic acid; ROS, reactive oxygen species.

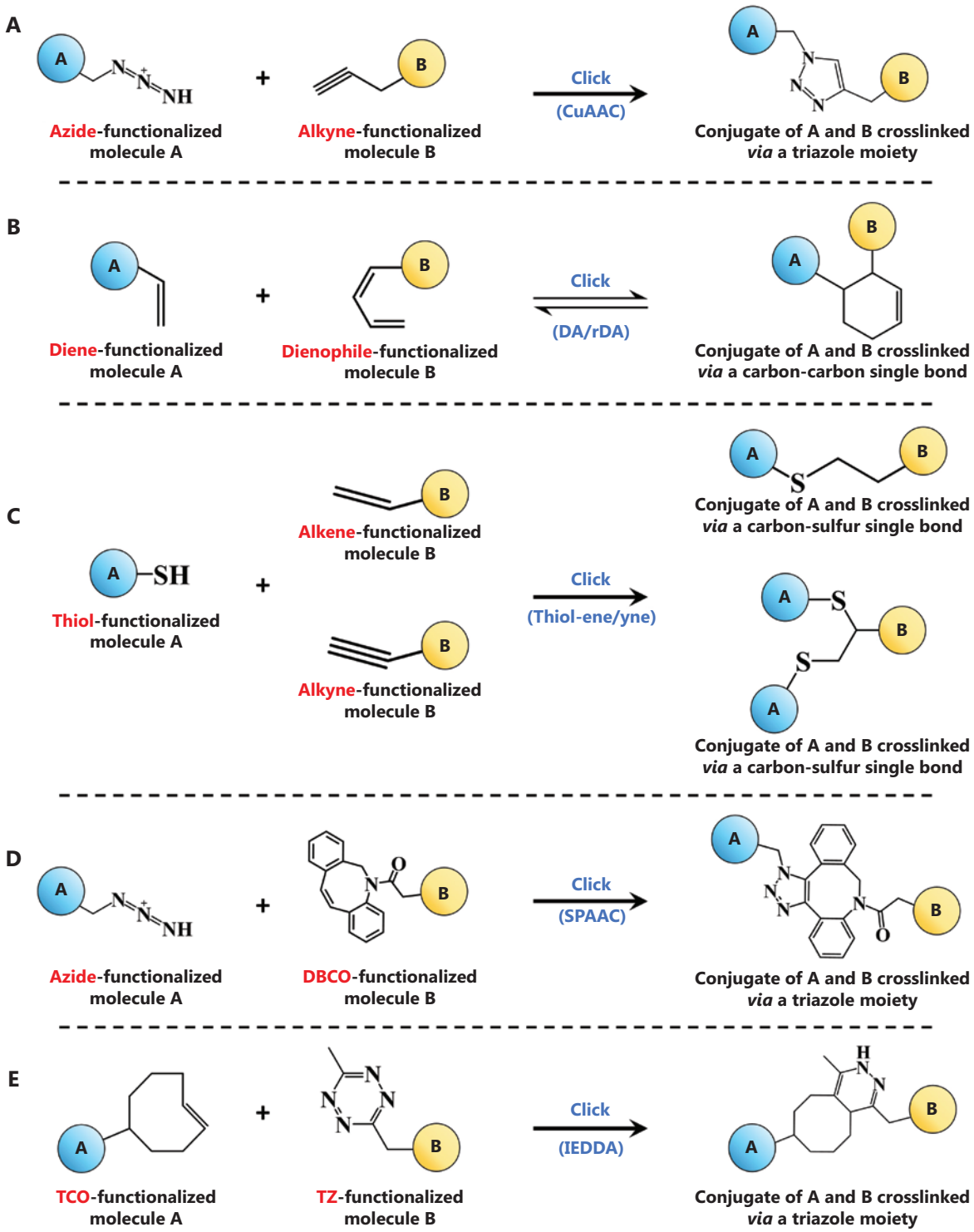


Figure 2 Representative click reaction mechanisms applied in tumor theranostics. (A) Copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC). (B) Diels–Alder (DA) cycloaddition and retro-Diels–Alder (rDA) reaction. (C) Thiol-ene and thiol-yne click reactions. (D) Strain-promoted azide–alkyne cycloaddition (SPAAC). (E) Inverse electron demand Diels–Alder (IEDDA) reaction.

Strain-promoted azide-alkyne cycloaddition

Bertozzi et al. developed SPAAC to eliminate the issue of Cu toxicity, which uses strained cyclooctynes [e.g., dibenzocyclooctyne – (DBCO) and bicyclo (6.1.0nonyne) – (BCN)] instead of terminal alkynes. The ring strain (≈20–30 kcal/mol) of cyclooctynes drives the [3 + 2] cycloaddition with azides under Cu-free conditions, forming a triazole ring. SPAAC is the preferred click reaction for *in vivo* theranostic system construction (**Figure 2D**).[46] For example, Koo et al.[43] utilized SPAAC to combine metabolic glycoengineering with bio-orthogonal Cu-free click chemistry. Koo et al.[43] first injected Ac_4ManNAz *in situ* in tumors to induce the presentation of azide-containing abnormal sialic acid on the surface of cancer cells, then intravenously administered surface-modified DBCO PEGylated liposomes to achieve tumor-specific binding through copper-free click reactions. This method overcomes limitations, such as natural receptor saturation and non-specific tissue distribution, demonstrating dose-dependent enhanced accumulation of Ac_4ManNAz in cellular and tumor-bearing mouse models with tumor uptake increased by approximately 14-fold. The *in vivo* application of SPAAC relies on a two-step, pre-targeting strategy. First, cells are metabolically labeled with azide-modified monosaccharides, such as Ac_4ManNAz, which are incorporated into surface glycans to provide artificial chemical handles. Subsequently, DBCO- or BCN-conjugated probes or drug carriers are administered, enabling a specific, catalyst-free click reaction exclusively on the pre-labeled target cells. This approach separates the metabolic labeling step from the delivery step, minimizing potential toxicity and improving the specificity and safety of *in vivo* targeting and drug release.

The inverse electron demand Diels–Alder

Electron-deficient tetrazine (diene) acts as a dienophile to accept electrons, whereas electron-rich trans-cyclooctene (TCO) or BCN acts as a diene to provide electrons. Both react through reverse electronic demand in the [4 + 2] cycloaddition involving $LUMO_{41}$ and HOMO(dienophile), instantaneously forming a stable dihydrotriazine or triazine ring in a catalyst-free, second-level process that is bio-orthogonal and often used for the surface modification of biomaterials and drug delivery systems (**Figure 2E**)[44]. Advanced individuals form supramolecular nanofibers containing tetrakistriazole (Tz) within tumor cells using the IEDDA click reaction. Hu et al.[45] utilized bio-orthogonal reactions coupled with a TCO-modified E3 ligase ligand (TP2T) to form the SA-PROTAC structure, which enables the selective capture and degradation of wild-type H2B and Cofilin-2 proteins. Hu et al.[45] achieved excellent selectivity and biocompatibility by leveraging tumor-specific enzymes (ENTK) or the high expression environment of ROS to trigger assembly, which significantly enhanced sensitivity to chemotherapy drugs and induced cell apoptosis. The exceptional kinetics and bio-orthogonality of IEDDA are ideal for pre-targeting strategies in complex biological environments. In one approach, a TCO-modified antibody or small molecule ligand is first administered and allowed to accumulate at the tumor site. After clearance of unbound agents, a tetrazine (Tz)-labeled radionuclide, drug, or imaging agent is introduced. The rapid cycloaddition between TCO and Tz leads to immediate and stable capture of the secondary agent specifically at the tumor, dramatically improving TBRs for imaging and reducing systemic exposure in therapy.

Comparative analysis of key click reactions

CuAAC, SPAAC, and IEDDA each present distinct trade-offs in theranostic applications. CuAAC demonstrates the fastest kinetics (~10^3–10^4 $M^{-1}s^{-1}$), yet the inherent toxicity of Cu(I) limits use *in vivo*. SPAAC avoids metal catalysts but proceeds at slower rates (~10^{-1}–10^1 $M^{-1}s^{-1}$), potentially affecting applications that require real-time imaging or rapid drug release. IEDDA offers a practical balance with relatively fast kinetics (~10^2–10^3 $M^{-1}s^{-1}$) and good biocompatibility, holding strong promise for clinical translation. The dependence on synthetic tetrazine/TCO pairs can complicate scalable production. In clinical practice SPAAC and IEDDA are moving forward in imaging probes, whereas CuAAC remains largely restricted to *ex vivo* conjugation. Indeed, new reaction designs will need to carefully weigh the interplay between speed, safety, and synthetic accessibility.

The efficacy of click reactions *in vivo* is profoundly influenced by the biological microenvironment, extending beyond the intrinsic kinetics. For example, the efficiency of CuAAC may be compromised in reducing environments that destabilize Cu(I) ions. However, these variables can also be leveraged for rational design. Acid-labile linkers can protect azide groups until activation within the slightly acidic TME and reductase-sensitive alkynes can be engineered for triggered activation. A deep understanding and strategic manipulation of these parameters are essential for optimizing click reactions to ensure reliable performance under both physiologic and pathologic conditions.

Furthermore, from the perspective of clinical translation readiness, CuAAC, SPAAC, and IEDDA exhibit distinct stages of development. CuAAC, with fast kinetics (~10^3–10^4 $M^{-1}s^{-1}$) and well-established protocols, is widely used for *ex vivo* labeling and conjugate synthesis but copper-associated toxicity limits direct *in vivo* applications. SPAAC avoids metal toxicity and shows good biocompatibility, yet slower rates (~10^{-1}–10^1 $M^{-1}s^{-1}$) may constrain real-time imaging or rapid drug-release applications. SPAAC has shown promise in preclinical imaging and targeted delivery, especially in metabolic labeling and pre-targeting strategies. IEDDA strikes a balance between speed (~10^2–10^3 $M^{-1}s^{-1}$) and biocompatibility.

The catalyst-free, rapid nature makes IEDDA attractive for *in vivo* pre-targeted imaging (e.g., antibody-guided radiotheranostics) with several approaches advancing to late-stage preclinical studies **Table 2**. However, the reliance on synthetic tetrazine/TCO pairs poses challenges in scalable production and cost. In summary, the choice of click reaction must weigh kinetics, safety, selectivity, and translational maturity. Ongoing optimization in reaction design and manufacturing processes will further broaden the clinical utility of click chemistry in tumor theranostics.

Application of click chemistry in tumor theranostics

Click chemistry enables the precise conjugation of targeting moieties, imaging agents, and therapeutic payloads into integrated theranostic systems[47,48]. The two parts that undergo a click reaction are connected by two groups capable of undergoing the click reaction. In recent years researchers have often combined click reactions with *in vivo* self-assembly strategies. **Scheme 1** involves using click reactions to combine two imaging components or two therapeutic components, then achieves therapeutic or imaging effects through *in situ* self-assembly in the body, thereby enhancing cellular retention and accumulation. The applications in tumor diagnosis, tumor therapy, and diagnosis-therapy integration are categorized below, highlighting design strategies and recent case studies.

Click chemistry for tumor diagnosis

Tumor diagnosis using click chemistry primarily focuses on molecular imaging to visualize tumor location and heterogeneity and CTC detection to monitor metastasis. The core principle is to use click reactions to conjugate targeting ligands (e.g., antibodies, peptides, and aptamers) with imaging agents (e.g., fluorescent dyes, radionuclides, and MRI contrast agents) to improve probe specificity and imaging sensitivity.

Molecular imaging probes

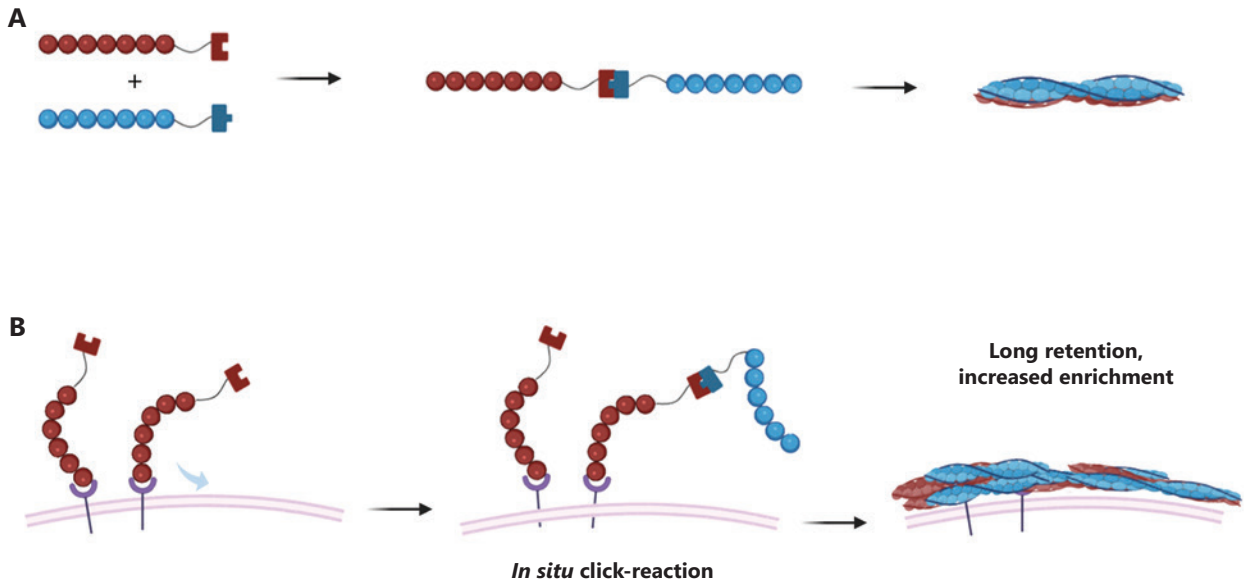
Molecular imaging is a non-invasive technique that detects molecular changes in tumors and provides critical information for early diagnosis and treatment planning. Click chemistry facilitates the modular design of imaging probes, facilitating the replacement of targeting ligands or imaging agents to adapt to different tumor types[49-51].

Fluorescence imaging (FI) is widely used for *in vitro* and *in vivo* tumor imaging because of high sensitivity and low cost. Near-infrared (NIR) dyes (e.g., Cy5.5, Cy7, and ICG) are preferred for *in vivo* imaging because of deep tissue penetration (up to 1 cm) and minimal autofluorescence. Click chemistry enables the conjugation of NIR dyes with targeting ligands to reduce non-specific tissue accumulation. For example, Xu et al.[52] integrated drug response groups with fluorescent imaging groups into a dual-lock-type probe (CyNAP-SS-FK) *via* click reactions, triggering intramolecular cyclization and self-assembly under the combined action of tumor-specific GSH and cathepsin B. This integration resulted in the *in situ* activation of NIR fluorescence (NIRF) and prolonged retention time, significantly enhancing the signal-to-noise ratio and sensitivity of FI.

In addition to conjugating NIR dyes with targeting ligands, click chemistry can also be used to establish covalent targets at tumor sites. Wang et al.[53] first used the target peptide TP (CD44v6 ligand) to recognize bladder cancer cells before introducing the reaction-induced aggregating peptide, RAP, through a reagent-free click reaction. This strategy addresses the limitations of intraoperative fluorescent probes in clinical bladder cancer, which are prone to photobleaching and have a short signal duration (**Figure 3A**). Because the click chemistry-formed monomer self-assembles into a β-sheet nanofiber network at the cell membrane surface, the monomer allows for long imaging times (>4 h) with a high signal-to-noise ratio

Table 2 Comparative performance of major click reactions in tumor theranostics

Click reaction	Typical rate constant (k_2, $M^{-1}s^{-1}$)	Biocompatibility	Clinical translation stage	Representative applications in tumor theranostics
CuAAC	10^3–10^4	Low (Cu toxicity)	Preclinical (*ex vivo* mainly)	Radiolabeling of peptides/antibodies; *in vitro* probe synthesis
DA/rDA	10^{-3}–10^{-1}	Moderate-to-high (stimuli-dependent)	Preclinical	*In vivo* pre-targeted imaging; metabolic labeling-based drug delivery
Thiol-Ene/Yne	10^1–10^2	Moderate (UV/initiator concern)	Preclinical (material modification)	Pre-targeted PET imaging; bio-orthogonal prodrug activation; PROTAC assembly
SPAAC	10^{-1}–10^1	High (metal-free)	Preclinical/early clinical	Surface functionalization of nanocarriers; stimuli-responsive hydrogel design
IEDDA	10^2–10^3	High (metal-free, fast kinetics)	Advanced preclinical/early clinical	Stimuli-responsive drug release; thermally/pH-activated theranostic systems



Scheme 1 (A) Schematic of bioorthogonal reactions *in vitro* and self-assembly. (B) Schematic diagram of bio-orthogonal reactions and *in situ* self-assembly *in vivo*.

[S:N, 2.8] (**Figure 3B**) and clearly delineates tumor boundaries in tumor-bearing mice and *in vitro* human bladder cancer tissues [tumor-to-normal (T:N) ratio, 5.6] (**Figure 3C**).

Positron emission tomography (PET) offers high sensitivity (down to picomolar concentrations) and quantitative capabilities, making PET ideal for early tumor detection and staging. Click chemistry is used to efficiently label targeting ligands with PET radionuclides (e.g., ^{18}F, ^{64}Cu, and ^{89}Zr)[54-57]. A representative study by Chen et al.[58] introduced a next-generation PET tracer, [^{18}F]-C-SNAT4, by incorporating 2-pyridylmethyl nitrile with a phenylmethyl linker into the precursor probes. This tracer undergoes intramolecular cyclization in the presence of caspase-3 and glutathione (GSH), resulting in *in situ* self-assembly into nanoparticles, thereby enhancing the radioactive retention and imaging contrast. This probe achieved high uptake [tumor-to-muscle (T:M) ratio, 5.8] in cisplatin-sensitive NCI-H460 tumors and was significantly enriched in CT26 tumors that responded to immunotherapy, effectively distinguishing between treatment-responsive and non-responsive tumors and greatly improving the ability of PET imaging to assess the early efficacy of chemotherapy and immunotherapy.

In addition to applying click reactions to the design of single-modal PET imaging probes, Wang et al.[59] designed a click reaction-activated PET/photoacoustic dual-modal probe [(^{18}F)-IR780-1] based on triazole-IR780 NIR fluorophores. Caspase-3 and GSH undergo an intramolecular click reaction to form a macrocycle and assemble into nanoparticles *in situ*, achieving probe enrichment and signal amplification in apoptotic tumors. This click reaction significantly enhances the PET and photoacoustic imaging signals, successfully enabling the early evaluation of the *in vivo* tumor chemotherapy response.

MRI provides high spatial resolution (down to 100 μm) and soft tissue contrast. However, MRI sensitivity is relatively low. Click chemistry is used to enhance MRI sensitivity by conjugating MRI contrast agents [e.g., Gd(III) complexes and iron oxide nanoparticles (IONPs)] with targeting ligands to increase tumor accumulation[60-62]. For example, Hai et al.[63] integrated γ-glutamyl transpeptidase (GGT) substrate, a reduction-responsive CBT-Cys clicking unit, and a DOTA-Gd chelating group into the same molecule. After intravenous injection, CBT-Cys clicking condensation was triggered by GGT enzyme cleavage in the tumor, leading to *in situ* self-assembly into Gd nanoparticles. The r_2 value of these nanoparticles increased to 27.8 $mM^{-1}s^{-1}$ and the r_2:r_1 ratio reached 10.6, enhancing T_2-weighted MRI contrast by 28.6% under 9.4 T, which is significantly better than traditional Gd-DTPA, achieving a leap in tumor-specific T_2 imaging effects at high fields.

CTCs detection

CTCs are tumor cells shed from primary or metastatic tumors into the bloodstream and serve as non-invasive biomarkers for tumor staging, prognosis, and treatment response monitoring[64]. Click chemistry improves CTC detection sensitivity by enhancing the binding specificity between capture substrates and CTCs[65-68]. Xiang et al.[69] developed bio-orthogonal click bubbles to combine drug recognition groups with click reactions for the efficient capture of CTCs. This system utilizes DNA-assembled suction cup-style multivalent cell surface

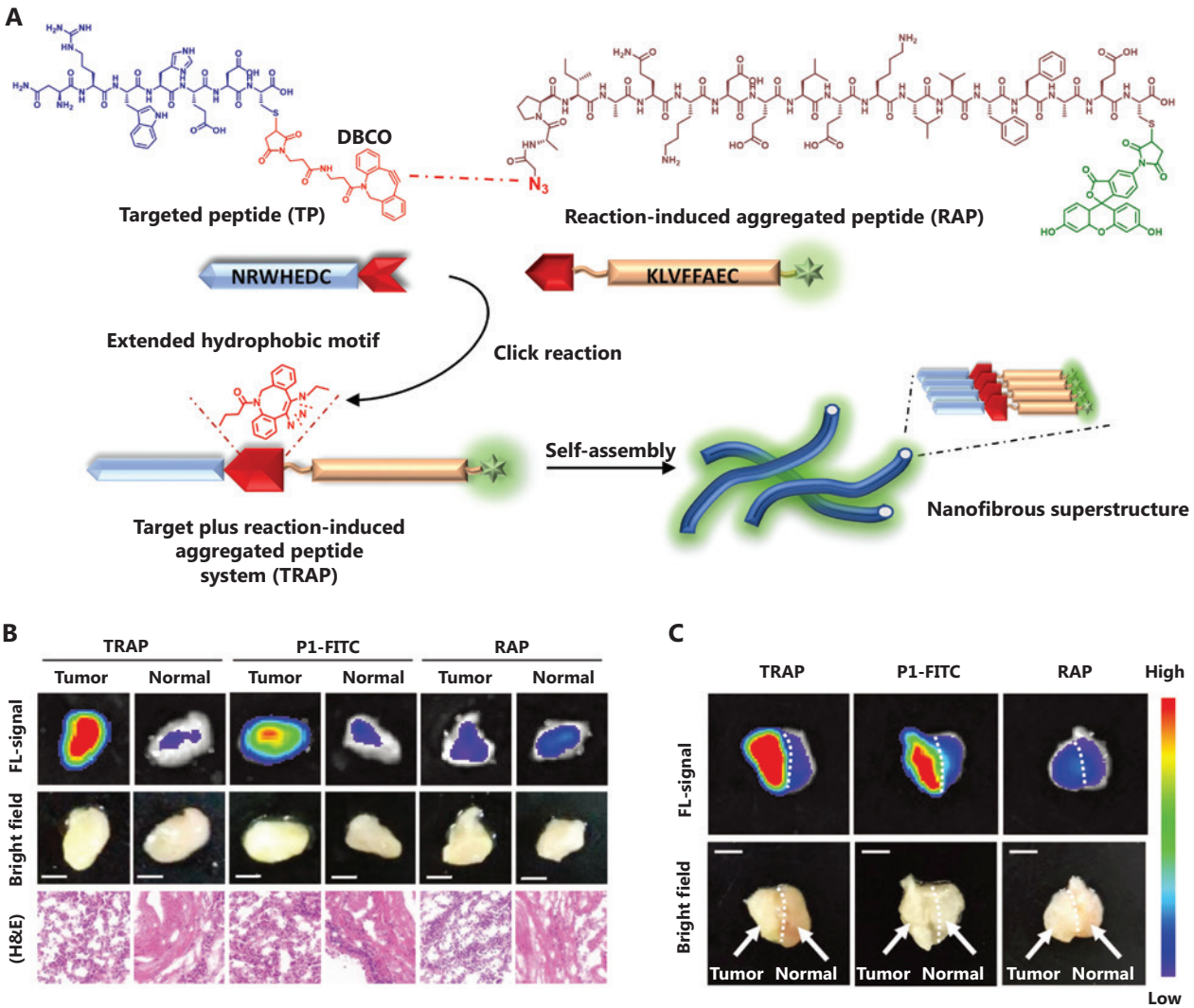


Figure 3 Modular molecular design and targeted fluorescence imaging of bladder cancer. (A) Schematic of the modular molecular design: Targeting peptide (TP) specifically recognizes CD44v6 overexpressed in bladder cancer; RAP (self-assembly peptide) conjugates with TP *via* reagent-free click chemistry to form TRAP; TRAP self-assembles into supramolecular nanofibers driven by hydrophobic extension and imbalanced hydrophilic–hydrophobic interactions. (B) *Ex vivo* fluorescence imaging of normal urothelium and tumor tissues treated with P1-FITC, RAP, or TRAP and the corresponding H&E-stained sections. (C) Fluorescence imaging of the tumor boundary after the same treatments. TP, targeting peptide; RAP, self-assembly peptide; TRAP, TP-RAP conjugate; FITC, fluorescein isothiocyanate; H&E, hematoxylin and eosin; CD44v6, cluster of differentiation 44 variant 6. Reproduced with permission from reference[53]. Copyright 2023, Advanced Materials.

engineering to enhance the binding strength between cells and bubbles and achieves rapid and specific CTC recognition and suspension enrichment through the TCO-Tz click reaction. In addition, the system possesses anti-contamination, 3D culture, and single-cell phenotypic analysis capabilities.

Li et al.[70] constructed a deformable affinity interface for the selective capture of CTCs from whole blood by combining MUC1-targeting aptamer drugs with flexible M13 viral nanofibers and immobilizing the nanofibers on the surface of magnetic beads *via* click reactions (**Figure 4A**). This flexible structure significantly enhanced the multivalent binding ability and improved the CTC capture efficiency (>90%), while effectively reducing the non-specific adhesion of leukocytes (**Figure 4B**). In clinical breast cancer samples, this method achieved a molecular typing accuracy of 91.07%, which was significantly better than traditional methods, demonstrating the strong potential for early cancer diagnosis and precise typing.

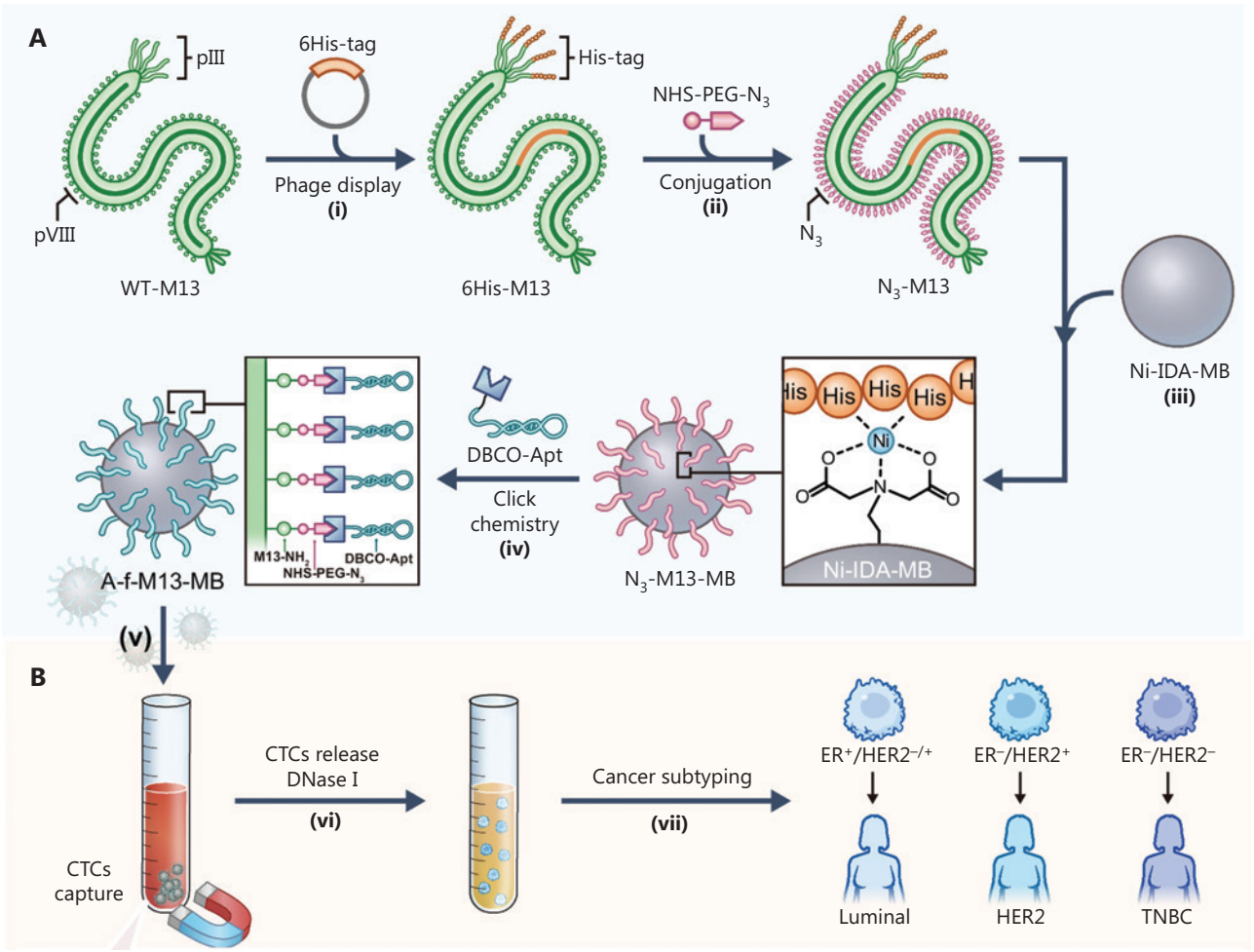


Figure 4 Engineering of aptamer-conjugated M13 viral nanofibers for capture and molecular profiling of breast cancer circulating tumor cells (CTCs). (A) Engineering of M13 viral nanofibers: Schematic depicting the stepwise functionalization of wild-type M13 phage (WT-M13). (i) Genetic engineering introduces a 6xHis tag at the N-terminus of the pIII minor capsid protein, generating 6His-M13. (ii) Chemical modification attaches an azide (N_3) moiety to the sidewall by reacting NHS-PEG-N_3 with the N-terminal amine of the major pVIII protein, producing N_3-M13. (iii) The N_3-M13 nanofiber is immobilized onto Ni-IDA-grafted magnetic beads (Ni-IDA MB) in an end-on orientation *via* the affinity interaction between Ni^{2+} and the 6xHis tag, yielding N3-M13-MB. (iv) A DBCO-labeled aptamer (DBCO-Apt) is conjugated to the azide groups on N_3-M13 *via* a click reaction while immobilized on the beads, forming the final capture complex termed aptamer-flexible-M13-MB (A-f-M13-MB), highlighting the flexibility of the M13 scaffold. (B) Workflow for CTC capture and profiling and the process for isolating and characterizing breast cancer CTCs from patient whole blood. (v) A-f-M13-MB selectively captures target CTCs, which are then isolated using a magnet. (vi–vii) Captured CTCs are released by treatment with DNase I and subsequently subjected to immunofluorescence staining for profiling of clinically relevant surface protein biomarkers, including estrogen receptor (ER) and human epidermal growth factor receptor 2 (HER2). Based on staining patterns, $ER^+/HER2^+$ or $HER2^-$ cells are classified as luminal subtype, $ER^-/HER2^+$ cells as HER2-positive subtype, and $ER^-/HER2^-$ cells as basal-like subtype. CTC, circulating tumor cell; WT-M13, wild-type M13 bacteriophage; 6xHis, hexahistidine tag; NHS-PEG-N_3, N-hydroxysuccinimide-polyethylene glycol-azide; Ni-IDA MB, nickel-immobilized metal affinity chromatography-grafted magnetic beads; DBCO, dibenzocyclooctyne; Apt, aptamer; A-f-M13-MB, aptamer-flexible-M13-MB; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2. Reproduced with permission from reference[70]. Copyright 2024, Nature Communications.

Multimodal imaging for diagnosis and therapy guidance

Multimodal imaging combines two or more imaging techniques to overcome the limitations of single-modal imaging (e.g., the low sensitivity of MRI and low spatial resolution of PET). Click chemistry enables modular integration of multiple imaging agents and a single therapeutic agent into one system. Specifically, by pre-modifying different imaging modules into

complementary pairs through click reactions and utilizing signals from the TME, the two modules can undergo rapid, efficient, and bio-orthogonal covalent crosslinking *in situ* at the lesion site, synchronously enhancing various imaging modalities[71,72]. This strategy does not require pre-chemical coupling, allows for the arbitrary adjustment of module ratios, and offers advantages, such as simple preparation, synergistic signal amplification, low background, and prolonged retention, thereby providing a universal platform for the construction of intelligent multimodal probes.

For example, Pang et al.[73] constructed a bio-orthogonal diagnosis and therapy-integrated molecular platform based on a novel FRET pair (PDO-Tz). Covalent linking of the fluorescent quencher group, tetrazine (Tz), with the fluorophore, PDO, allows the release of TCO-caged drugs within tumor cells *via* an IEDDA reaction, simultaneously activating the fluorescent signal and releasing the active drug. This IEDDA reaction demonstrated up to 44-fold fluorescence enhancement in A549 cells and mouse models, enabling real-time imaging of tumors and visualization of drug release. In addition, Dong et al.[74] designed an activatable bimodal probe using tumor acidity-mediated click orthogonal reactions. This probe consists of acid-responsive iron oxide nanoparticles (cDIOs) with dibenzocyclooctyne (DBCO) and aggregation-induced emission (AIE) molecules (AATs) with azide groups, which undergo covalent cross-linking in an acidic tumor environment, achieving an approximately 12.4-fold enhancement in NIRF signals and an approximate 2.8-fold increase in the MRI r_2 relaxation rate, while prolonging the probe retention time in tumors and significantly improving the imaging contrast and time window.

Miao et al.[75] triggered the *in situ* generation of TCO on the tumor cell membrane using alkaline phosphatase (ALP), then carried out a bio-orthogonal IEDDA reaction using an imaging probe modified with Tz. This IEDDA reaction resulted in the construction of a dual-modal probe for PET/NIRF or BL/NIRF, achieving up to 25-fold enhancement in radioactive probe uptake, a 4.5-fold increase in the fluorescence signal and a TBR exceeding 7.0 in ALP-positive tumors. This dual-modal probe significantly improved imaging sensitivity and specificity. Consequently, this dual-modal probe was successfully applied for preoperative PET imaging and intraoperative fluorescence-guided resection in mouse tumors.

Click chemistry for tumor therapy

Click chemistry contributes to tumor therapy by improving the targeting, efficacy, and safety of therapeutic systems[76]. Click chemistry is widely used in chemotherapy, phototherapy, immunotherapy, and gene therapy to construct targeted drug delivery systems, stimuli-responsive release systems, and synergistic therapeutic platforms.

Chemotherapy

Chemotherapy is the most common tumor treatment. However, the efficacy is limited by poor targeting and severe side effects[77-80]. Click chemistry addresses these issues by conjugating chemotherapeutic drugs with targeting ligands to enhance tumor accumulation and constructing stimuli-responsive drug delivery systems to control drug release in the TME[81,82]. For example, Man et al.[83] constructed a four-core Cu(I) complex (AFt-Cu_4) nanoparticle (NP) wrapped in desferrioxamine, utilizing the acid-responsive release and CuAAC catalytic activity to *in situ* click synthesize resveratrol analogs within tumors. This process achieved an efficient and low-toxicity synergistic treatment with a tumor inhibition rate of nearly 90% and induced immunogenic cell death (ICD) that activates T cells, significantly inhibiting distant metastasis.

Another study by Yao et al.[84] coupled ALP-triggered molecular self-assembly with bio-orthogonal reactions of Tz/trans-cyclooctene to cascade NapK(Tz)YpF. The self-assembly first dephosphorylated and formed fibrous tissue *in situ* under the action of overexpressed enzymes in tumors, then specifically unlocked doxorubicin (DOX) through a reverse electron demand DA reaction, thereby increasing the toxicity of DOX toward cancer cells 228-fold and enhancing selectivity against normal cells by approximately 50-fold, significantly inhibiting tumors in a nude mouse HeLa model.

Wang et al.[85] designed a carbonic anhydrase IX (CAIX)-targeted peptide (P1-DBCO) based on the intrinsic resistance characteristics of patients with renal cell carcinoma (RCC) to chemotherapy drugs, then introduced an azide peptide [P2-N3] (**Figure 5A**). These two proteins interact on the tumor cell membrane surface to form a new hydrophobic-enhanced peptide (P3) *in situ* through click chemistry. P3 subsequently self-assembles into aggregates and stably anchors to the membrane (**Figure 5B**), utilizing the principle of membrane tension disturbance to create transient pores and significantly increasing the influx of DOX. Consequently, the *in vitro* IC_{50} was reduced by 3.5-fold and the *in vivo* tumor suppression rate increased by 3.2-fold, achieving a sensitization effect for RCC chemotherapy (**Figure 5C**).

Photothermal therapy (PTT) or photodynamic therapy (PDT)

Click chemistry is used to conjugate phototherapeutic agents with targeting ligands or other therapeutic agents to enhance targeting and achieve synergistic therapy. PTT uses photothermal agents (e.g., AuNPs, black phosphorus, and carbon nanotubes) to convert NIR light into heat, thereby inducing tumor cell necrosis. Click chemistry improves PTT efficacy by increasing the accumulation of photothermal agents in tumors[86-89]. For example, Zhang et al.[88] co-encapsulated a Ni-bis(dithiolene) NIR photothermal agent (TPE-Ni) with an amino-alkyne click reagent (OPYO) in thermosensitive liposomes to construct TPE-Ni/OPYO NPs.

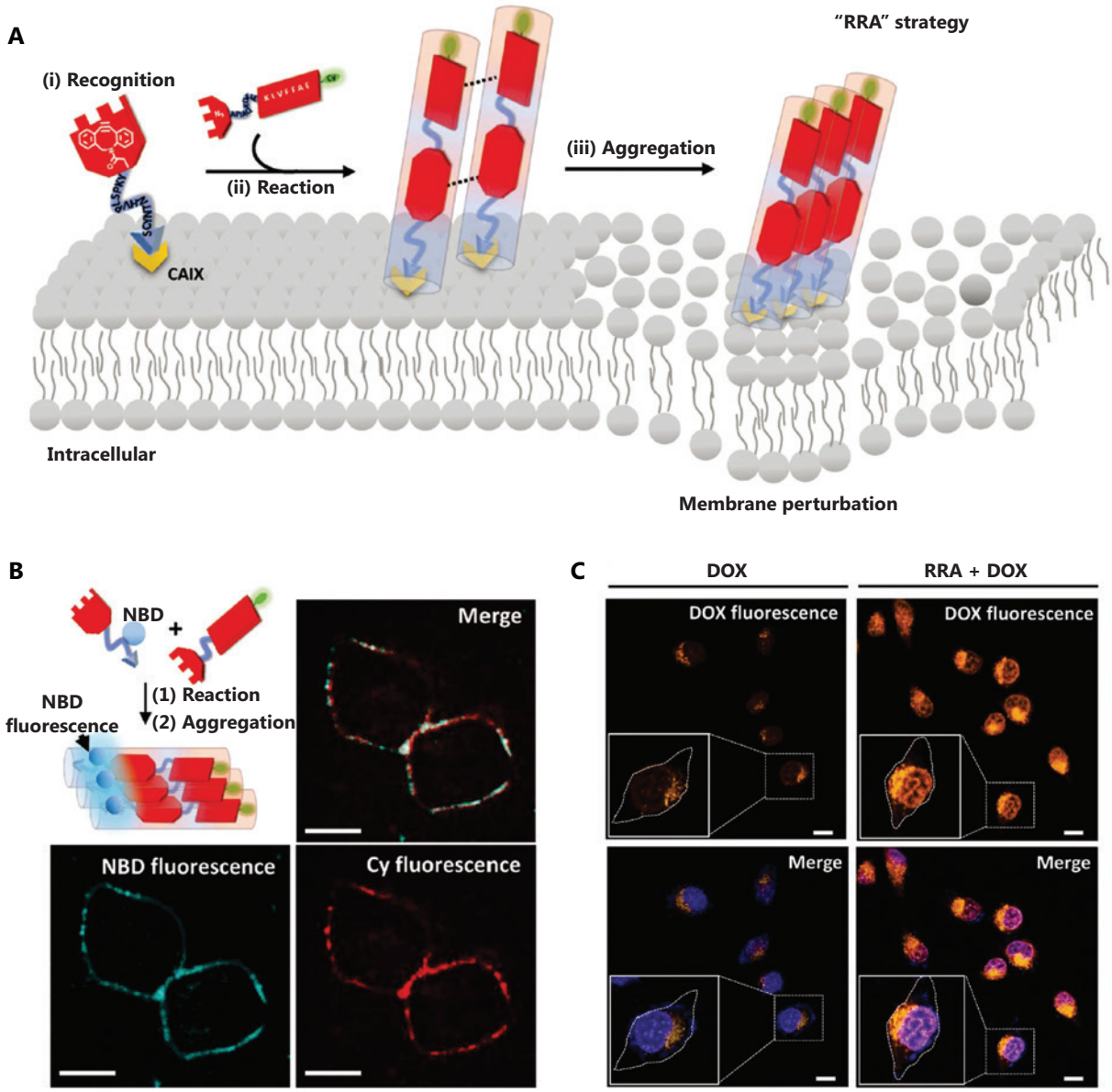


Figure 5 Targeted peptide assembly on renal cancer cells induces membrane perturbation and enhances drug delivery. (A) Schematic of the cell-level process. P1-DBCO specifically recognizes renal cancer cells by targeting carbonic anhydrase IX (CAIX). Subsequent addition of P2-N_3 enables a click reaction with P1-DBCO, forming the monomeric peptide P3 directly on the cell membrane. Concurrently, P3 monomers self-assemble into supramolecular structures, leading to membrane perturbation. (B) Confocal microscopy images of SK-RC-52 cells treated with NBD-labeled P1-DBCO alone or the RRA mixture (NBD-labeled P1-DBCO: P2-N3 = 1:1) for 15 min. Blue fluorescence indicates NBD localization. (C) Confocal images of SK-RC-52 cells with or without RRA pretreatment, followed by treatment with doxorubicin [DOX (100 nM)] for 1 h. CAIX, carbonic anhydrase IX; DBCO, dibenzocyclooctyne; DOX, doxorubicin; N3, azide; NBD, nitrobenzoxadiazole; P1, targeting peptide; P2, assembly peptide; P3, P1-P2 conjugate; RRA, reaction mixture of P1-DBCO and P2-N3. Reproduced with permission from reference[85]. Copyright 2019, Advanced Materials.

Under 940 nm laser irradiation, these particles quickly heat up (≈65°C), triggering a phase change that releases OPYO and initiating a bio-orthogonal click reaction with amino acids inside the tumor cells, achieving *in situ* chemical cross-linking. This process directly ablates solid tumors by blocking protein function and synergistically induces ICD,

resulting in the complete eradication of primary tumors and a 68% suppression rate of distal tumors.

PDT uses photosensitizers (e.g., porphyrins and phthalocyanines) to generate ROS upon light irradiation, thereby inducing tumor cell apoptosis. Click chemistry has been used to conjugate photosensitizers to targeting ligands to reduce off-target ROS generation[90-92]. Chu et al.[93] designed a BODIPY photosensitizer quenched with tetrazole and a BCN-modified EGFR-targeting peptide as two independent components, which only undergo the IEDDA click reaction in cancer cells that simultaneously highly express biotin receptors and EGFR, resulting in the *in situ* release of quenching and recovery of ROS generation ability, achieving precise PDT activation and significantly enhancing treatment specificity and efficacy.

Jiang et al.[94] achieved the *in situ* construction and dissociation of a drug warehouse driven by click reactions by co-assembling the photosensitizer, Ce6, with pH-responsive DBCO-modified nanoparticles. Jiang et al.[94] utilized ROS under light exposure to trigger TK cross-linking breakage, enabling the precise delivery of PDT and hypoxia-activated prodrugs to different regions of the tumor and significantly enhancing the effectiveness and penetration of PDT.

To address the limitations of poor targeting in PDT and PTT and the aggregation-caused quenching (ACQ) effect that hampers the therapeutic effects, Cui et al.[95] designed a drug comprising BODTPE and DBCO. DBCO undergoes a click reaction after the surface azide groups are pretreated, anchoring the NPs to tumor cell membranes. This not only achieves precise targeting but also provides strong fluorescence and AIE effects in the NIR-II region, resulting in enhanced therapeutic effects after ROS production during aggregation with a photothermal conversion efficiency of 54.7%.

Immunotherapy

Immunotherapy activates the immune system to attack tumors. However, the efficacy of immunotherapy is limited by low response rates and immune-related adverse events (irAEs)[96-99]. Click chemistry is used to construct immunotherapeutic systems that enhance immune activation and reduce irAEs, such as bispecific antibodies, immune checkpoint inhibitor (ICI) conjugates[100], and cancer vaccines[72,101-103]. For example, Bai et al.[104] first anchored DSPE-PEG-N_3 to the endothelial membrane of the tumor-draining lymph nodes (TdLNs) through percutaneous injection, then administered DBCO-modified rapamycin micelles (Rap@PAG-DBCO). This procedure allowed the two components to achieve drug *in situ* capture and enrichment through a click reaction, significantly restoring T cell killing function and enhancing the effectiveness of immunotherapy.

Yang et al.[105] used SPAAC to construct a cancer vaccine by first delivering ovalbumin (OVA, a model antigen) into dendritic cells (DCs) with fluoroalkane-grafted polyethyleneimines, followed by the conjugation of glycopolymers with a terminal group of dibenzocyclooctyne (DBCO) onto the DCs (**Figure 6**). The G-DCV vaccine enhanced antigen presentation by DCs and induced a strong OVA-specific $CD8^+$ T cell response. In a melanoma model, the vaccine efficiently inhibited tumor growth when combined with immune checkpoint blockade inhibitors.

In addition to the design and synthesis of vaccines and immunotherapeutics, click reactions can be used to synthesize supramolecular assemblies *in situ* for immune regulation[106-109]. Xiao et al.[110] constructed a nanofiber (TP-AP) *in situ* by coupling the DBCO-modified PD-L1 targeted peptide (TP) with an azide self-assembling peptide (AP) through a reagent-free click reaction (**Figure 7A**). Due to the small size and dual properties of deep penetration and assembly retention (AIR), TP-AP penetrated 121 μm deep into the tumor in 4T1 and CT26 models (**Figure 7B**), extended PD-L1 occupancy by 3.2-fold, and enhanced tumor suppression effect by 1.5-fold compared to antibodies but with lower toxicity (**Figure 7C**).

Mamuti et al.[111] constructed a peptide nanoagonist (PVA-CD40) with multivalent interfaces *in vivo* using a reagent-free click reaction utilizing the CD40 anchor peptide (AAP) and assembly driving peptide (ADP). This structure induces the oligomerization of CD40 receptors and continuously activates downstream NF-κB signaling on the surface of DCs through hydrophobic collapse and β-sheet self-assembly. This process achieves long-term retention in lymph nodes (>120 h) and simultaneously inhibits the growth of primary and distant tumors.

Targeted protein degradation therapy

Targeted protein degradation therapy utilizes bifunctional molecules, such as PROTAC and LYTAC, to hijack endogenous ubiquitin-proteasome or lysosomal systems to catalytically clear pathogenic proteins[112-114]. Introducing click reactions into targeted protein degradation therapy allows for the *in situ*, reagent-free connection of target protein ligands and E3 ligands in the pathologic microenvironment[115-117], thereby achieving modular, rapid destruction of target proteins and reduced toxicity in the body[118-121]. Wang et al.[122] triggered a Cu(I)-catalyzed azide-alkyne cycloaddition reaction using two peptide precursors (PPOI and PE3) in a tumor cell environment with high GSH. This process resulted in the generation of Nano-PROTAC monomers that can self-assemble into β-sheet helical nanofibers (**Figure 8**). This stable approach significantly enhanced protein–protein interactions and circumvented the hook effect. Nano-PROTAC achieved dose-dependent, long-lasting (72 h) protein degradation with an EGFR degradation rate of 95% and an AR degradation rate of 79%. Furthermore, Nano-PROTAC inhibited tumor growth by 81.4% and 58.6% in A549 and LNCap tumor-bearing mouse models, respectively, while ensuring safety.

Zhang et al.[123] constructed a signal aptamer chimera (SApt) that does not rely on LTR by coupling the YXXØ lysosomal sorting signal derived from LAMP-2a to a targeting membrane

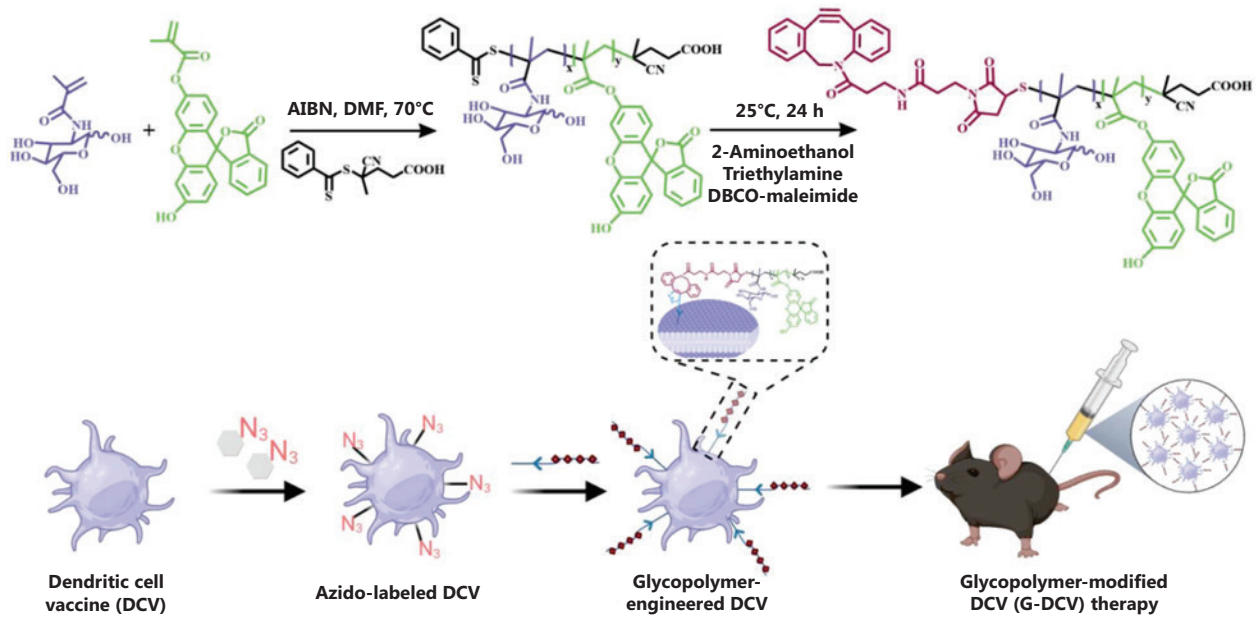


Figure 6 Construction and mechanism of glycopolymer-engineered dendritic cell (DC) vaccines for enhanced T cell activation. This schematic illustrates the synthesis and functional application of glycopolymer-modified DC vaccines (G-DCV). The preparation involves a two-step bio-orthogonal strategy. DCs are first metabolically engineered to display azide-modified glycans, followed by conjugation of dibenzocyclooctyne (DBCO)-functionalized glycopolymers *via* copper-free click chemistry, resulting in G-DCV. The engineered glycopolymers on the DC surface serve as multivalent ligands that specifically facilitate and strengthen dendritic cell adhesion to T cells. This enhanced interaction promotes immunological synapse formation, leading to improved antigen presentation and co-stimulatory signaling, thereby significantly augmenting the activation and proliferation of antigen-specific T cells to potentiate the adaptive immune response. DC, dendritic cell; G-DCV, glycopolymer-engineered dendritic cell vaccine; DBCO, dibenzocyclooctyne. Reproduced with permission from reference[105]. Copyright 2024, Angewandte Chemie International Edition.

protein *via* click chemistry, achieving targeted recognition and sorting guidance for dual-functional integration. This strategy effectively inhibited tumor growth with no significant toxicity, thereby realizing the precise treatment of membrane proteins.

Gene therapy

Gene therapy uses nucleic acids (e.g., siRNA, miRNA, and CRISPR-Cas9) to correct genetic defects or inhibit tumor cell proliferation. However, the efficacy of gene therapy is limited by poor cellular uptake and nucleic acid degradation[124-126]. Click chemistry has been used to construct gene delivery systems that protect nucleic acids and enhance targeted delivery[127-132]. For example, Teng et al.[133] constructed a ClickRNA-PROTAC system that links drug molecules with gene therapy modules through click reactions to achieve tumor-specific protein degradation (**Figure 9**). This system utilizes mRNA encoding SNAP-E3 fusion proteins. After specific expression in tumor cells, target protein ligands carrying azide groups are recruited through click reactions, forming stable protein complexes and inducing ubiquitination and degradation of target proteins. This approach overcomes the traditional dependence of PROTACs on endogenous E3 ligases and significantly enhances the targeting and efficacy of the treatment.

Click chemistry for tumor diagnosis-therapy integration

The ultimate goal of tumor theranostics is to integrate diagnosis and therapy into a single system, enabling real-time monitoring of the treatment response and personalized treatment adjustments. Click chemistry facilitates this integration by conjugating imaging agents and therapeutic payloads to targeting ligands to construct multimodal theranostic systems[134,135].

Multimodal imaging-guided monotherapy

Multimodal imaging combines two or more imaging techniques (e.g., FI/PET and MRI/PTT) to overcome the limitations of single-modal imaging (e.g., the low sensitivity of MRI and low spatial resolution of PET). Click chemistry enables modular integration of multiple imaging agents and a single therapeutic agent into one system.

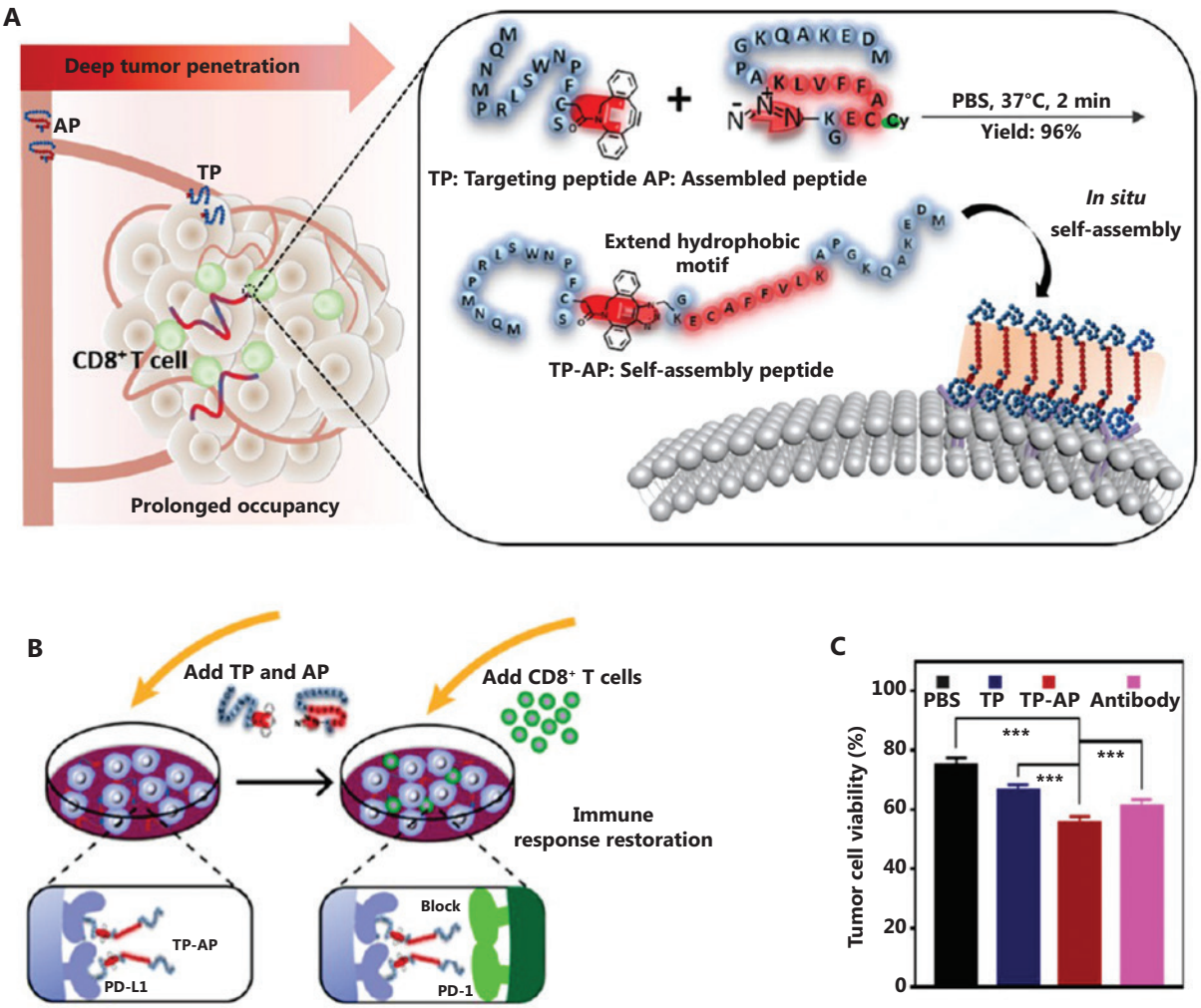


Figure 7 A click-reaction-induced conjugation and self-assembly (CRICB) strategy for enhanced tumor penetration, prolonged PD-L1 blockade, and antitumor immunotherapy. (A) Schematic of the CRICB strategy for tumor-targeted nanofiber formation: The strategy employs a targeting peptide (TP) that specifically recognizes programmed death-ligand 1 (PD-L1) on cancer cells. After TP binding, an assembly peptide (AP) is introduced and reacts with TP *via* reagent-free click chemistry to form the conjugate TP-AP. The extended hydrophobic motif in TP-AP drives its self-assembly into supramolecular nanofibers directly on the tumor cell surface. This CRICB strategy promotes deep penetration and enhanced accumulation of TP-AP within solid tumors, resulting in prolonged PD-L1 occupancy. (B) Immunotherapeutic effect of the CRICB strategy. By sustaining PD-L1 blockade on tumor cells, the TP-AP nanofibers enhance T-cell-mediated antitumor immunity, leading to effective tumor inhibition. (C) *In vitro* cytotoxicity assay. Viability of 4T1-OVA cells after pretreatment with PBS, TP, TP-AP, or an anti-PD-L1 antibody, as measured by the CCK-8 assay. Data demonstrate the specific cell-killing effect induced by the TP-AP conjugate through PD-L1 engagement and immune activation. CRICB, click-reaction-induced conjugation and self-assembly; TP, targeting peptide; AP, assembly peptide; PD-L1, programmed death-ligand 1; CCK-8, cell counting kit-8. Reproduced with permission from reference[110]. Copyright 2020, Acs Applied Materials & Interfaces.

Multimodal imaging-guided combinatorial therapy

Click chemistry offers a precise, modular platform for integrating multiple functional components directly at the disease site, advancing the development of multimodal imaging-guided combination therapy. This approach ensures imaging agents and therapeutic drugs are co-localized with high fidelity. Complementary information from different imaging modes enhances diagnostic accuracy, while synergistic drug action on the same cellular target improves therapeutic specificity. Moreover, the covalent bonds formed *via* click reactions maintain the integrity of the theranostic system during circulation, preventing premature separation of the components.

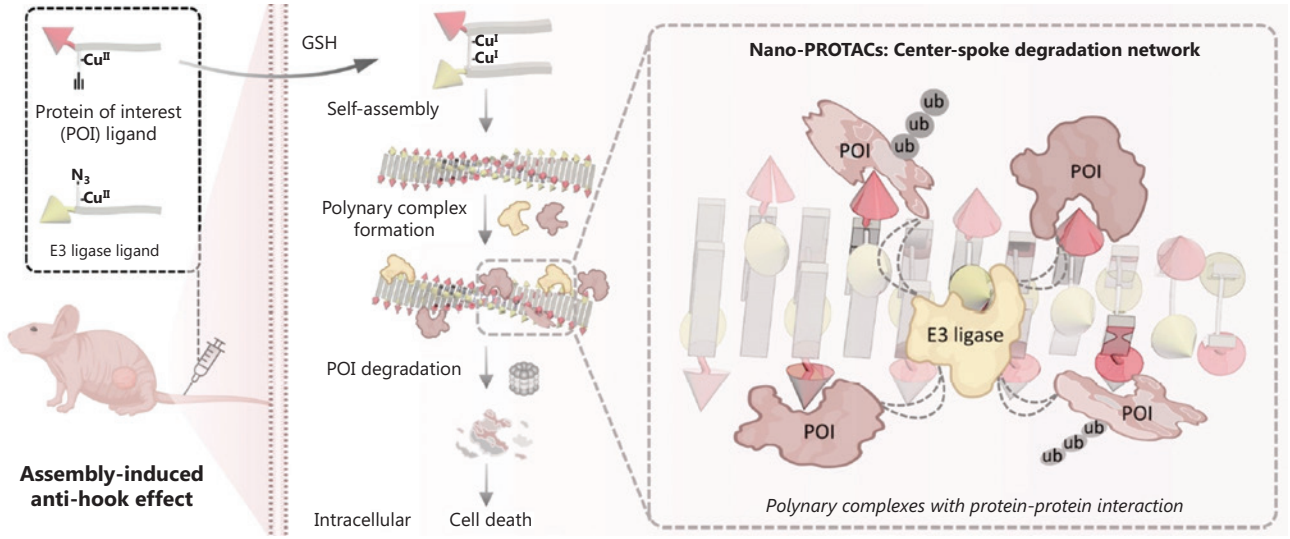


Figure 8 A self-assembling Nano-PROTACs platform for sustained intracellular protein degradation. This schematic illustrates an intracellular click-reaction-driven self-assembly strategy to construct Nano-PROTACs. Two independent peptides targeting a protein of interest (POI) and an E3 ligase, respectively, conjugate inside cancer cells (e.g., *via* high GSH) to form assembly-driving monomers. These monomers rapidly self-assemble into organized β-sheet nanostructures (Nano-PROTACs) within the cytoplasm, displaying multiple binding ligands. PROTACs, proteolysis-targeting chimeras; POI, protein of interest; GSH, glutathione; EGFR, epidermal growth factor receptor; AR, androgen receptor; AIR, assembly-induced retention. Reproduced with permission from reference[122]. Copyright 2023, Angewandte Chemie International Edition.

Combinatorial therapy (e.g., chemotherapy + PTT and PDT + immunotherapy) improves treatment efficacy by targeting multiple tumor cell pathways. Click chemistry enables the integration of multiple therapeutic and imaging agents into a single system, allowing real-time monitoring of synergistic therapeutic effects. A representative study by Wang et al.[72] constructed a multifunctional diagnostic and therapeutic system that targets PD-L1 based on bio-orthogonal click chemistry. Wang et al.[72] first implanted azide "chemical receptors" on the surface of TNBC cells using Ac_4ManNAz, then achieved MR/NIRF dual-modal imaging *via* DBCO-modified PD-L1 antagonist peptide-probe (APP^{Gd}-Cy7). Wang et al.[72] subsequently delivered a pH-sensitive DOX prodrug (APP^{Gd}-DOX) using the same strategy, triggering drug release in the acidic TME and inducing caspase-3-mediated GSDME-dependent cell necroptosis, releasing ICD-related DAMPs. Moreover, the click reaction increased the accumulation of the probe and prodrug at the tumor site 4–15 times, significantly enhancing the PD-L1 blockade effect. *In vivo* experiments showed that this integrated strategy increased CTL infiltration by 3.3-fold and reduced the proportion of Tregs by 65%, ultimately achieving an 86% inhibition rate of tumor growth.

Challenges and limitations

Despite the rapid progress of click chemistry in the integration of tumor diagnosis and treatment, clinical translation still faces multiple bottlenecks. First, the long-term toxicity risk of Cu-catalyzed systems has yet to be eliminated and even with chelating ligands or nanocarriers, trace amounts of free Cu can still cause chronic toxicity, such as liver damage. Therefore, there is an urgent need to develop Cu-free reaction systems with faster kinetics [$k_2 > 10^2\ M^{-1}s^{-1}$] (e.g., modified SPAAC). Specifically, when designing such drugs for CuAAC, even with advanced chelating ligands or nanocarriers meant to capture copper, trace amounts of free Cu(I) can still escape, posing chronic toxicity risks, like liver and nerve damage, which remains a major hurdle for *in vivo* therapies. In contrast, metal-free reactions, like SPAAC, avoid metal toxicity but struggle in dense tumors or fibrotic tissue. The bulky structure of strained cyclooctynes and the slower reaction rates compared to IEDDA can limit how deeply cyclooctynes penetrate and react, possibly causing uneven labeling or incomplete drug attachment at the target site. Second, the phototoxicity bottleneck of light-triggered strategies needs to be addressed because UV light can easily damage DNA and proteins. In addition, the efficiency and biosafety of visible/NIR photosensitizers (iridium complexes and up-conversion nanoparticles) require simultaneous improvement.

Furthermore, at the bio-orthogonal level, endogenous nucleophiles (protein thiols and amino acid amines) compete with substrates, significantly reducing the coupling efficiency. For example, the thiol groups of serum albumin can react with maleimides in the reaction with DA, leading to premature release of the drug payload. A more complex high interstitial

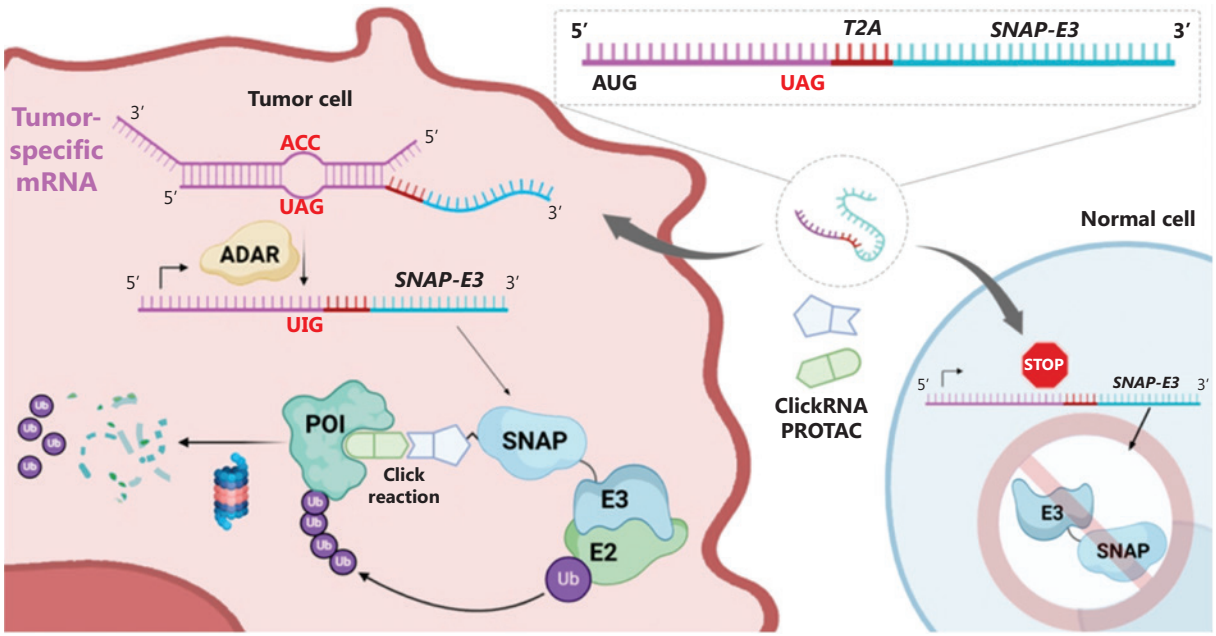


Figure 9 Schematic of the ClickRNA-PROTAC platform for conditional protein degradation. This diagram illustrates a spatially controlled protein degradation strategy. Lipid nanoparticles (LNPs) deliver mRNA encoding a SNAP-tag-E3 ubiquitin ligase (SNAP-E3) fusion protein. Following translation, sequentially administered small molecules [dibenzocyclooctyne-conjugated benzylguanine (BG-DBCO) and an azide-conjugated ligand of the target protein (Ligand-N3)] are assembled onto the SNAP-E3 *via* a SNAP-tag reaction and a subsequent click reaction. This assembly forms a complete PROTAC complex in tumor cells expressing a specific trigger mRNA, leading to ubiquitination and degradation of the protein of interest (POI). In normal cells lacking the trigger, SNAP-E3 is not expressed, preventing complex formation and ensuring specificity. ClickRNA-PROTAC, click-chemistry-based RNA-activated proteolysis-targeting chimera; SNAP-E3, SNAP-tag-E3 ubiquitin ligase fusion protein; LNP, lipid nanoparticle; BG-DBCO, benzylguanine-dibenzocyclooctyne; Ligand-N3, azide-conjugated ligand; POI, protein of interest. Reproduced with permission from reference[133]. Copyright 2024, Journal of the American Chemical Society.

fluid pressure, a dense extracellular matrix, and heterogeneous blood flow perfusion in the TME collectively hinder the effective enrichment of substrates. Therefore, there is an urgent need to design TME-unlocking substrates (e.g., pH-activated azides) to achieve tumor-specific, spatiotemporally controllable click coupling, thus overcoming enrichment reaction bottlenecks. Achieving efficient click-coupling *in vivo* is challenging due to low effective concentrations caused by dilution and diffusion barriers. NP delivery systems address this by co-localizing click partners to boost local concentration. More advanced pre-targeting strategies, such as IEDDA-based approaches, separate targeting from delivery. A slow-clearing targeting agent (e.g., an antibody conjugate) accumulates first and clears from circulation, followed by a fast-clearing effector molecule. The subsequent rapid click reaction then specifically captures the effector at the target site, significantly improving reaction efficiency while minimizing systemic exposure. Beyond these challenges, a deeper evaluation of the long-term biocompatibility, controlled biodegradability, and efficient clearance mechanisms of click chemistry materials and probes remains essential for clinical advancement. These factors critically influence immune responses and chronic toxicity profiles, urging the development of chemically designed degradable linkers and carrier systems that ensure safe metabolic removal after fulfilling their diagnostic or therapeutic roles.

The bio-orthogonal reactivity of click chemistry intermediates, such as azides or strained alkynes, may still risk off-target modifications or immune activation through unintended interactions with biomolecules. To ensure long-term safety, degradable linkers or scaffolds are essential to prevent accumulation in organs like the liver and spleen and to enable efficient clearance. Moving click chemistry from the lab to the clinic faces key hurdles in manufacturing and regulation. Reproducible producing click-functionalized nanomaterials with consistent properties across batches is essential for reliable performance but remains difficult. Scaling up production while preserving the activity of both the click groups and attached biomolecules adds further complexity. Additionally, regulatory frameworks for these complex conjugates are still developing, particularly concerning the *in vivo* stability of the click linkage and potential new impurities.

Despite the abovementioned challenges, the clinical translation of click chemistry in tumor theranostics has achieved preliminary positive progress. **Table 3** summarizes selected

clinical and advanced preclinical studies employing click chemistry strategies, covering areas such as pre-targeted imaging, radioimmunotherapy, intraoperative guidance, and *in situ* theranostics. These examples not only validate the feasibility of applying click chemistry in complex living systems but also provide crucial references for overcoming current bottlenecks and designing next-generation systems with greater clinical potential.

Future perspectives

Promoting click chemistry diagnostic and therapeutic systems into clinical settings should focus on four key aspects. First, precise control, in which acid-sensitive azides, ROS-cleavable alkynes, and other TME-responsive substrates can be used to achieve a selective first unlocking of tumors, followed by light-activated cyclooctyne to realize a temporal and spatial second encryption with two progressive steps to suppress off-target effects. To make precise control possible, chemists should collaborate with materials scientists to develop new responsive bonding systems with clear triggering thresholds and fast response kinetics. Tumor biologists need to provide clinically relevant ranges for TME parameters, such as pH and ROS concentration gradients, to guide the precision optimization of responsive materials. Furthermore, interdisciplinary teams should work together to establish *in vitro* and *in vivo* validation models, conducting thorough assessments of the selectivity and safety of different control strategies. This approach will help inform the development of clinical dosing and irradiation protocols. Second, intelligent design, which involves establishing a substrate structure-kinetics database combined with high-throughput experimental iterations to quickly identify ligand-probe-drug combinations with high tumor accumulation, fast release rates, and optimal S:N ratios in imaging[136]. The success of this strategy requires close collaboration among chemists, informatics specialists, and tumor biologists. It is recommended to establish an open-access, multidimensional database integrating chemistry, biology, and pharmacokinetics, containing accumulation and release data across various tumor models. Materials scientists can design modular probe libraries based on this database, while oncologists perform high-throughput screening using patient-derived organoids or biopsy samples, with the resulting data feeding back into the design cycle for continuous optimization, which are all aimed at accelerating the discovery of clinically viable combinations. Third, a personalized closed loop is adopted that relies on modular coupling to instantaneously assemble customized diagnostic and therapeutic modules based on tumor markers, such as HER2 and FR, coupled with liquid biopsy CTC counting to dynamically adjust the dosage and formulation. The same chemical platform can also conveniently modify nanorobots and organ chips for active targeting verification *via* magnetic/acoustic control and toxicity evaluation, reducing reliance on animal models and forming a comprehensive design-manufacturing-evaluation translational pathway[137]. Advancing this closed-loop system toward clinical application requires a well-defined interdisciplinary workflow. Clinical oncologists would identify patient-specific biomarkers and monitor CTCs, based on which

Table 3 Click chemistry-based tumor theranostic strategies in clinical trials

Strategy/candidate name	Core principle & click reaction type	Target/indication	Clinical stage & key progress
SQ3370	Intratumoral tetrazine + intravenous TCO-doxorubicin prodrug, activated *via* IEDDA click	Advanced solid tumors	Phase I/IIa completed; first-in-human validation, safe delivery of 12–15× doxorubicin dose
CAPAC® IV Strategy	Intravenous TCO-antibody fragment + tetrazine prodrug, systemic IEDDA click therapy	NSCLC, gastric cancer	Late preclinical; planned entry into clinical trials in 2026
^{225}Ac pre-targeted RIT	TCO-anti-CA19.9 antibody + ^{225}Ac-tetrazine, pre-targeted IEDDA for α-radiotherapy	CA19-9 expressing tumors (e.g., pancreatic cancer)	Phase I ongoing; evaluating safety/feasibility of α-particle therapy
^{64}Cu pre-targeted imaging	TCO-anti-CA19.9 antibody + ^{64}Cu-tetrazine probe, pre-targeted IEDDA for PET	CA19-9 expressing tumors (e.g., pancreatic cancer)	Phase I ongoing (imaging component)
Tagworks platform	BCN-antibody + tetrazine-payload, click-to-release *via* specific mechanism	Various solid tumors (preclinical models)	Phase I initiated; testing universal click-release platform
PD-L1 pre-targeted PET	TCO-anti-PD-L1 antibody + ^{18}F-tetrazine probe, pre-targeted IEDDA for immunoPET	PD-L1 positive tumors	Phase I; for imaging tumor immune microenvironment

chemists rapidly synthesize corresponding modular click-chemistry building blocks. Materials scientists and bioengineers then collaborate to develop suitable nanorobotic or organ-on-a-chip platforms for functional validation and toxicity prediction of individualized treatment regimens. We recommend establishing a cross-disciplinary translational team that starts with clinical questions, reverse-engineers material and chemical solutions, and iterates rapidly through microfluidic organ-on-a-chip testing, ultimately accelerating the development timeline for personalized therapeutics. Fourth, AI-enabled click chemistry design, machine learning, and computational screening are now actively shaping the development of click-reactive probes and bio-orthogonal reaction pairs[138]. By predicting critical properties, such as reaction kinetics, substrate selectivity, and *in vivo* stability, AI models significantly shorten the optimization cycle for new theranostic agents[139]. One practical example includes the use of deep learning to design tetrazine derivatives that exhibit both faster reaction rates and improved tumor-targeting profiles[140]. Looking ahead, coupling AI-driven design with high-throughput experimental validation is expected to fast-track the creation of next-generation click chemistry tools, further enabling tailored theranostic strategies.

To realize this vision, we must establish a systematic interdisciplinary framework. A Click Chemistry Theranostics Alliance should be formed, bringing together chemists, oncologists, materials scientists, and translational experts for regular workshops on needs alignment and data sharing. The alliance would jointly develop standardized protocols and evaluation metrics covering chemical design, *in vitro* testing, animal models, and early clinical trials, ensuring data comparability across stages. Finally, we encourage cross-disciplinary translational projects with clear clinical objectives, defined development pathways, and concrete division of responsibilities, accelerating the translation of click-chemistry theranostics from lab-to-clinic.

Conclusions

Click chemistry, with flexibility, efficiency, and biocompatibility, is a powerful core tool for constructing integrated systems for tumor diagnosis and treatment[141]. Classical reactions, such as CuAAC, DA/rDA, SPAAC, and IEDDA, have demonstrated mature molecular imaging specificity, tumor treatment efficacy, and synchronization of diagnosis and treatment. However, *in vivo* reaction efficiency, long-term biosafety, and large-scale clinical translation remain unresolved bottlenecks[142]. Future research must focus on fourfold synergy to promote the realization of stimulus–response-type reactions to precisely unlock the TME, AI-assisted high-throughput design to quickly optimize the relationship between substrate, kinetics, and pharmacodynamics, modular coupling to support personalized formulations that match patient heterogeneity in real time, and integration with emerging technologies, such as nanorobots and organ-on-chip, to complete closed-loop verification both *in vivo* and *in vitro*[143]. The complex intersection of chemistry, materials science, and oncology will accelerate the transition of click chemistry diagnostic and therapeutic systems into clinical applications, providing a new scalable paradigm for precision oncology[144].

Beyond oncology, click chemistry offers a promising platform for translation in several other clinical areas[145-148]. For example, in infectious disease imaging, click chemistry enables the rapid assembly of pathogen-specific probes. Bioorthogonal tissue engineering strategies leverage precision in regenerative medicine. Furthermore, personalized therapies for autoimmune or metabolic disorders may benefit from the modular design of click chemistry. Click chemistry provides a versatile toolkit for advancing precision medicine across a broad spectrum of medical specialties given the bio-orthogonality, modular nature, and functional diversity[149-151].

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Conflict of interest statement

No potential conflicts of interest are disclosed.

Author contributions

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Da-Yong Hou and Ni-Yuan Zhang contributed data or analytical tools.
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