International Journal of Frontline Research in
Chemistry and Pharmacy
Journal homepage: https://frontlinejournals.com/ijfrcp/
ISSN: 2945-4824 (Online)

(REVIEW ARTICLE)

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Click Chemistry: Innovative strategies and bio-orthogonal applications

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International Journal of Frontline Research in Chemistry and Pharmacy, 2025, 03(02), 001-004

Publication history: Received on 31 October 2025; revised on 03 December 2025; accepted on 06 December 2025

Article DOI: https://doi.org/10.56355/ijfrcp.2025.3.2.0024

Abstract

Click chemistry has become one of the most innovative methods in modern chemical research, largely due to its unparalleled efficiency, modularity, and ease of use. It enables the rapid synthesis of complex molecules and functional materials under mild conditions, is environmentally friendly, and offers high yields and excellent functional group tolerance. Over the past two decades, click chemistry has evolved from classic copper(I)-catalyzed azido-yne cycloaddition reactions to a range of bioorthogonal, ultrafast, metal-free reactions, as well as strain-induced cycloaddition reactions, tetrazine-based reverse Diels-Alder linkages, and sulfur-fluorine exchange reactions. These advances have enabled precise molecular manipulation in biological systems and supported advanced applications in areas such as real-time imaging, targeted drug delivery, metabolic labeling, and pre-targeted radiochemistry.

...Beyond the fields of chemistry and biology, click chemistry is now also fundamental to the work of materials scientists, polymer engineers, and especially nanoparticle experts. Its modular and predictable nature allows for easy transformation into gels, smart materials capable of detecting stimulus-induced shape or functional changes, surface coatings with on-demand integrated electronics, and controllable nanocarriers with diverse structures. Advances in catalyst design optimization, ligand acceleration, and computer technology have not only improved reaction rates but also enhanced stability and biocompatibility. This has enabled the widespread application of this powerful new chemical synthesis tool in industrial and clinical fields. For example, flow chemistry and fluidized bed catalysis have shortened reaction times, significantly increased product yields, and reduced the amount of toxic waste flowing into rivers.

Despite the numerous successes of click chemistry, it still faces challenges such as reagent stability, side reactions, cost, and the development of more environmentally friendly and sustainable processes. Machine learning, computer modeling, and reaction design promise to help address these issues in the future. Therefore, researchers can expect the emergence of next-generation click reagents with customizable reactivity, selectivity for planar structures and spatial orientation, and compatibility in complex biological or material environments. This will make click chemistry a versatile, reliable, and powerful method for molecular construction, opening up unprecedented possibilities for the future of synthetic chemistry, biomedical research, and materials design—a truly new era..

Keywords: Click Chemistry; CuAAC; SPAAC; IEDDA; Bio orthogonal; Bioconjugation

1. Introduction

Click chemistry refers to a set of reactions designed for high yield, selectivity, and simplicity, allowing rapid assembly of molecules from modular building blocks [1]. These reactions tolerate a wide variety of functional groups, operate

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under mild conditions, and minimize purification steps. This design philosophy has transformed drug discovery, chemical biology, and materials science by providing reliable and reproducible synthetic methods [2].

2. Historical Development and Milestones

The concept of click chemistry was formally introduced in 2001, emphasizing efficiency and reliability [3]. The copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC) emerged as a defining reaction, providing regioselective 1,4-disubstituted 1,2,3-triazoles [4]. Subsequently, strain-promoted azide–alkyne cycloaddition (SPAAC) and tetrazine-based inverse-electron-demand Diels–Alder (IEDDA) reactions enabled bio orthogonal applications in living systems[5].

2.1. Timeline of click chemistry evolution:

- 2001 → Concept of click chemistry introduced
- 2002 → CuAAC developed
- 2004 → SPAAC for metal-free bio orthogonal reactions
- 2008 → Tetrazine-based IEDDA reactions introduced
- 2015–2025 → Expanding applications in materials, nanomedicine, and radiochemistry[6]

3. Cu(I)-Catalyzed Azide–Alkyne Cycloaddition (CuAAC)

CuAAC is widely used due to its high regioselectivity and robustness [7]. Copper(I) coordinates to the terminal alkyne, activating it towards nucleophilic attack by the azide, forming a metallacycle intermediate that collapses into a stable triazole [8]. The reaction exhibits high yields with minimal side products, making it suitable for synthesizing functional molecules and libraries [9].

Recent ligand-assisted CuAAC variants improve biocompatibility and reduce copper toxicity, enabling in vivo applications and protein labeling [10].

4. Strain-Promoted Azide–Alkyne Cycloaddition (SPAAC)

SPAAC eliminates the need for copper, using ring strain in cyclooctynes to drive 1,3-dipolar cycloaddition with azides [11]. This enables bioorthogonal labeling in live cells and organisms [12]. Computational and kinetic studies have optimized cyclooctyne derivatives, improving reaction rates and stability for biological use [13]

5. Tetrazine-Based Inverse-Electron-Demand Diels–Alder (IEDDA)

Tetrazine–trans-cyclooctene ligations are ultrafast bio orthogonal reactions used in pretargeted imaging, drug delivery, and metabolic labeling [14]. The reaction proceeds within milliseconds, forming dihydro pyridazines without interfering with endogenous biomolecules [15].

Optimization of substituents balances reactivity with stability, producing tetrazine reagents suitable for in vivo applications [16].

6. Emerging Click Reactions

Beyond CuAAC, SPAAC, and IEDDA, reactions such as sulfur-fluoride exchange (SuFEx) and optimized oxime/hydrazone ligations provide additional orthogonal strategies [17]. Photo-triggered click reactions enable spatiotemporal control for advanced materials and cellular studies [18].

7. Applications in Chemical Biology

Click chemistry facilitates selective labeling of proteins, nucleic acids, glycans, and lipids, preserving biological activity [19]. Applications include metabolic glycan labeling, protein tagging, and in vivo imaging [20].

8. Drug Discovery and Bioconjugation

Click reactions streamline synthesis of molecular libraries, linker attachment, and antibody–drug conjugate (ADC) assembly [21]. They enhance efficiency in medicinal chemistry workflows and enable precise modification of biomolecules [22].

9. Radiochemistry and Pretargeting Strategies

IEDDA reactions are applied in radiochemistry for rapid assembly of radiotracers [23]. Pretargeted imaging reduces background radiation and improves imaging contrast [24].

10. Polymer Chemistry and Materials Engineering

Click chemistry allows controlled synthesis of block copolymers, star polymers, dendrimers, and functional surfaces [25]. Its orthogonality ensures predictable assembly of complex materials for flexible electronics, hydrogels, and responsive coatings.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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