USES OF CLICK-CHEMISTRY IN CELL PENETRATING PEPTIDES FOR THERANOSTICS

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INTRODUCTION

Cell-Penetrating Peptides (CPPs) refers to one kind of small peptide that is able to easily cross cell membranes and enter into cells. They can be utilized to transport bioactive compounds that are bonded covalently, which could if not, be unable to penetrate cells[1]. CPPs are commonly composed of lipophilic and cationic amino acids, which allow them to interact with both the hydrophobic and negatively charged regions of cell membranes[2]. Due to this characteristic, they have been utilized to transport a range of cargos, such as siRNA, peptides and small proteins. CPPs are a viable choice for cellular research and the development of therapeutic targeting of intracellular molecules due to their capacity to efficiently distribute biologically active cargos. The discovery of CPPs was initially made by the examination of naturally occurring proteins. The mechanism behind the cell penetration of CPPs are widely debated and the subject of ongoing research. However, the main theories can be broadly grouped into two categories: energy dependent endocytic pathways and energy-independent direct entry pathways. These adaptable peptides are easy to make, modify and analyze, yet are able to deliver noncovalently or

covalently conjugated bioactive cargos, such as large plasmid DNA or small chemical drugs, inside cells. This is primitively achieved through endocytosis, which allows for high levels of targeting of tumors, gene silencing, or gene expression. The key features of CPPs are their low toxicity to cells, the ability internalized by a wide range of cell types, efficacy that varies depending on the dosage and the ability to transport a diverse range of cargos without size or type limitations[3]. Though CPPS are frequently nonselective and passive, they can be chemically modified or functionalized to create effective delivery vectors that target specific tissues or cells. Developing clinically effective systemic delivery systems require careful attention to both the design of the delivered cargo and the CPP employed to transport it. However, one of the major challenges in using CPPs for these applications is their ability to target specific cells or tissues in the body. To address this challenge, various techniques have been used to modify CPPs, including Click chemistry. The modifications made using click chemistry can include attaching diagnostic or therapeutic agents to the CPPs, which can then be used to target specific cells or tissues in the body. These modified CPPs possess the potential to be utilized in a variety of theranostic applications, such as imaging, drug delivery, and gene therapy.

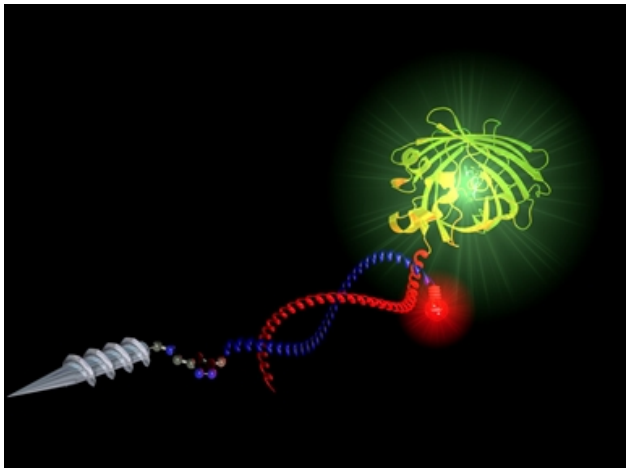

Fig 1 : Cell-penetrating peptides

CLICK-CHEMISTRY

The Huisgen cycloaddition [4,5], which is commonly referred to as 'click-chemistry', is a chemical process that results in the formation of five-membered heterocycles, specifically triazoles, by the reaction between dipolarophiles and 1,3-dipoles. The initial Huisgen reaction lacked specificity and necessitated high temperatures and pressures, rendering it impractical for widespread use. However, in 2002, researchers discovered that using small amounts of copper as a catalyst allowed for fast, effective and selective azide-alkyne cycloaddition under normal temperature conditions, in an organic solvent. Subsequently, it was found that the reaction can also be conducted in polar media, such as alcohols or water[6]. This resulted in a noteworthy rise in the utilization of. Huisgen cycloadditions in various fields including inorganic chemistry, Biochemistry, polymer chemistry and organic synthesis

Click reactions typically involve the bonding of carbon to heteroatoms and are highly exothermic, making the additions irreversible. Azides are easy to introduce, resistant to water and oxidation, compatible with numerous functional groups, while also exhibiting high reactivity towards others. For in vitro and in vivo applications, azides are biologically orthogonal and not found in natural species. The success of click chemistry heavily relies on the presence of triazoles as an essential component, as their stable bonds, similarities to amide bonds, and possible biological properties elevate their significance beyond just serving as a synthesizable linker.

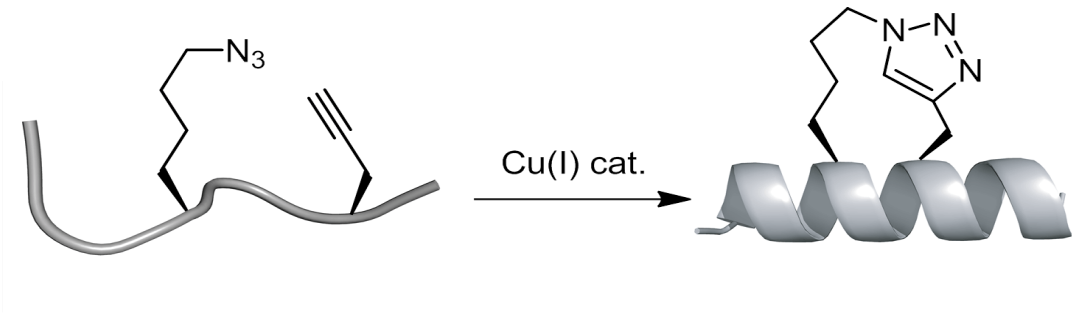

Figure 2: Stapled peptides by click-chemistry

The widespread use of copper azide-alkyne cycloaddition (CuAAC) can be attributed to the distinctive characteristics of azides and the resulting triazoles. CuAAC entails the creation of a rigid five-membered heterocycle 1,2,3-triazole ring, which serves as an isostere of the peptide bond. It emulates the planarity of the amide moiety while being less susceptible to hydrolytic cleavage. Thus, this approach is suitable for the ligation [7] of small molecules, oligonucleotides[8], and peptide cargos to enable additional functionalization, including the integration of CPPs[9].

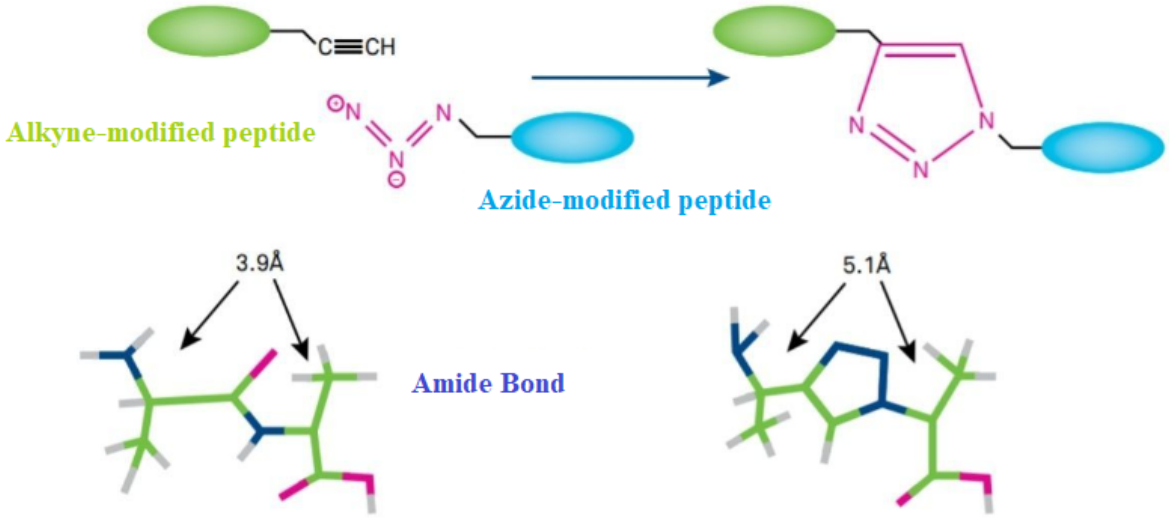

Figure 3: Clicking of an Alkyne functionalized peptide with an azide-modified substrate in the presence of Cu(I) to form a triazole-linked complex

CELL PENETRATING PEPTIDES AND CLICK-CHEMISTRY

Click chemistry provides several alternatives for protein / peptide modification, and can be integrated with other methods to develop intricate structures and functional multi-component

systems. There are several ways to carry out this chemistry, such as transforming peptides to azido derivatives after synthesis to click with substrates with clickable alkynyl groups or using azide or alkyne-containing amino acids during peptide synthesis for inter- or intramolecular click reactions. The use of building blocks with clickable moieties is crucial in constructing cyclic peptides, side chain-modified peptides, interside-chain peptide chimeras, and peptide-small molecule conjugates. Solid-phase resin modified with clickable groups is another approach for generating modified / clickable peptides. Click chemistry is compatible with diverse protected amino acid side chains utilized in peptide synthesis.

CPPs have recently been conjugated with numerous biomolecules, including as drugs, nucleic acids, and proteins, using click chemistry. CuAAC has been utilized, for instance, to conjugate drugs to CPPs, enabling the delivery of the drugs to cells with precision. Similar to this, CPPs have been conjugated to nucleic acids like siRNA and plasmid DNA using CuAAC, enabling selective transport of these molecules into cells.

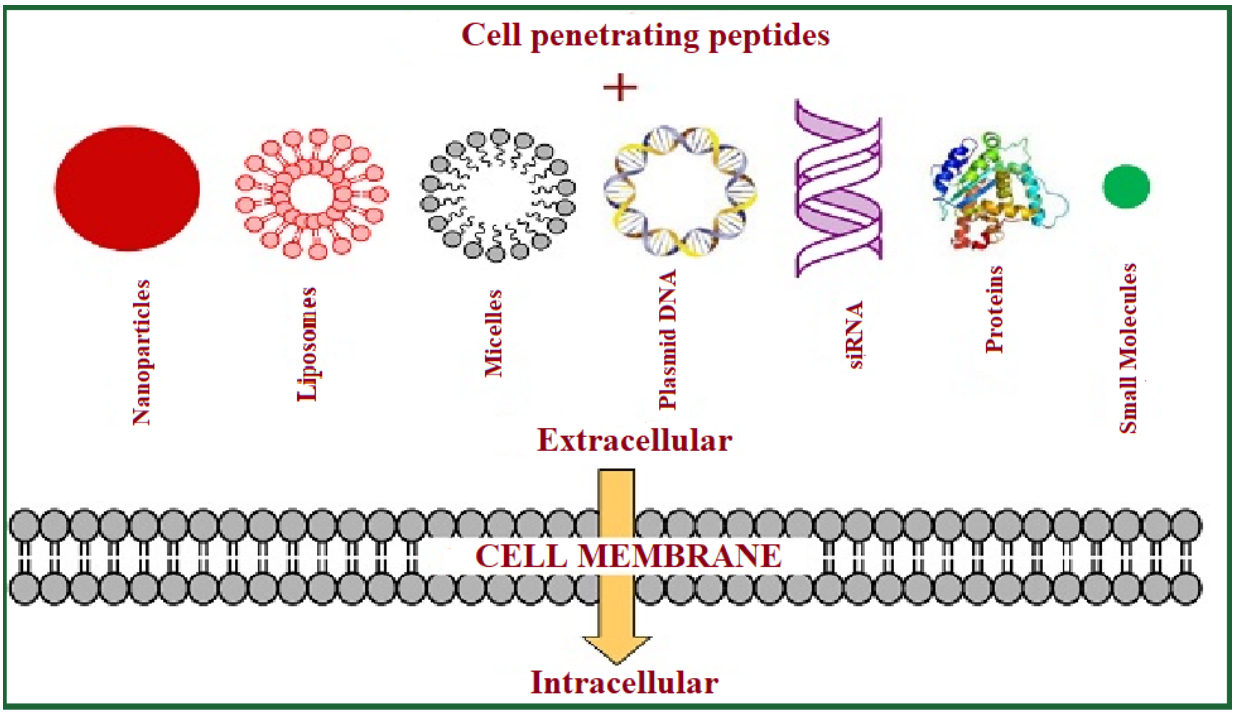

Fig 4: Applications of cell penetrating peptides (CPPs) for intracellular delivery of diverse cargoes.

Meldal and colleagues [10] made head-to-tail cyclic peptides through copper-assisted ring closure reactions. The process involved incorporating alkyne and azide containing amino acids in standard Fmoc- base SPPS. The results obtained indicate that the reaction has a high degree of selectivity and the ability to efficiently cyclize either protected or unprotected peptides using click chemistry.[11] This chemistry has been shown to be effective in closing tetrapeptide rings, which was challenging because of the risk of oligomerization and ring strain that hampers cyclization.[12]

In recent years, researchers have been investigating the use of click chemistry to modify CPPs for a variety of thernaustics applications. For instance, scientists have combined CPPs with targeting molecules like antibodies, aptamers, or small molecules using CuAAC. This reduces the uptake by healthy cells and enables the CPPs to penetrate particular cells—such as cancer cells—selectively. It has been demonstrated that using this strategy will increase the effectiveness and specificity of medicines including anticancer medications, siRNA, and plasmid DNA. Another strategy is to combine CPPs with imaging agents like fluorescent dyes or radionuclides via click chemistry in order to monitor the uptake of the CPPs in vivo. The pharmacokinetics and biodistribution of the CPPs can be learned from this, which can help to optimize their therapeutic application.

The addition of biorthogonal groups to CPPs using CuAAC is a significant click chemical application in the field of CPP chemistry. Biorthogonal groups are functional groups that can be selectively identified and responded with by other biomolecules without interfering with the organism's native biochemistry. As a result, researchers can utilize. CuAAC to add biorthogonal groups to CPPs, which they can then employ as probes to investigate the location and function of CPPs within the cell

Other click chemical reactions have also been applied in CPP chemistry in addition to CuAAC. For instance, the Staudinger ligation is a click chemistry reaction that makes it possible to conjugate CPPs with phosphine-containing molecules in an effective and selective manner. To enable CPPs to preferentially attach to other biomolecules, phosphine groups have been added via this procedure.

The progress achieved in the field of click chemistry opens up possibilities for synthetic techniques that can convert peptides with interesting functional leads into more stable and structurally streamlined forms.

EXAMPLES OF CLICK-CHEMISTRY MODIFIED CPPS FOR THERANOSTICS

Penetration is one of the most widely used CPP for delivering bioactive cargo into the cytosolic region. It has been demonstrated in the delivery of a cyclic peptide inhibitor called G7-18NATE, targeting Grb-7 to breast cancer cells, leading to a reduction in cell proliferation and migration.[13,14] Furthermore, the combination of G7-18 NATE and penetration was tested in mouse models of pancreatic cancer, resulting in a decrease in Grb7 interaction with focal adhesion kinase and a decrease in tumor spread

Similarly, the TAT peptide, which is a highly efficient cell-penetrating peptide and contains a nuclear localization sequence [15,16], was discovered during research on the HIV TAT transactivator protein. TAT has been modified with a small molecule drug to create a modified CPP that can deliver the drug to specific cells or tissues in the body, making it a useful theranostic agent.

Click chemistry can also be utilized for the cellular delivery of a bicyclic intracellular target by creating a bicyclic peptide inhibitor of the cancer target Grb7 and coupling it to FITC-labeled

derivatives of the traditional CPP Penetration, as well as the equivalent with a further nuclear localization signal (NLS).

The study of peptide-based drugs has extensively utilized click chemistry. It has been utilized to introduce a substitute for disulfide and amide bonds because of their structural closeness. They are very beneficial for modifying peptides to improve their metabolic stability. Its selective and efficient nature, combined with its mild reaction conditions , make it an ideal method for connecting groups such as functional groups and peptide fragments, as well as for performing peptide cyclisation (between head-to-tail or side chain).The addition of triazoles can enhance or mimic the secondary structure of the peptide .Conjugation with functional groups or structures, such as carbohydrates , polyethylene glycol, or radiolabelling/photolabeling agents, can effectively enhance peptide functionality. Click reactions have the potential for the combination of diagnostic and therapeutic agents in a single molecule.

Despite the potential of click chemistry modified CPPs for therapeutics, there are still challenges that need to be addressed. Such as the poor stability of CPPs in vivo, can limit their use in therapy. Researchers have been exploring ways to improve the stability of CPPs, such as by modifying their primary structure or by conjugating them with protective groups. In addition, Poor pharmacokinetics of CPPs can limit their bioavailability and efficacy. To confirm the safety and efficacy of click-chemistry modified CPP in different disease models In vivo studies are needed. Although click chemistry is a promising technique in cell penetrating peptide drug research, it has not been widely used for creating current clinical drugs.

CONCLUSION

To sum up, click chemistry is an effective tool for altering CPPs for theranostic applications. In order to transport both therapeutic and diagnostic compounds to particular cells or tissues in the body, CPPs modified through click chemistry have the potential to be used as theranostics agents. CPPs modified via click chemistry have a wide range of uses, and additional study is required to realize the full potential of this technology.

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