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Surface Modification of Magnetic Nanoparticle via Cu(I)-Catalyzed Alkyne-azide [2 + 3] Cycloaddition

Po-Chiao Lin,[†,‡,§] Shau-Hua Ueng,[‡,§] Sheng-Chieh Yu,[‡] Mi-Dan Jan,[†] Avijit Kumar Adak,[†] Ching-Ching Yu,[†] and Chun-Cheng Lin*[,†,‡]

Department of Chemistry, National Tsing Hua University, Hsinchu, Taiwan and Chemical Biology and Molecular Biophysics, Taiwan International Graduate Program, Academia Sinica, Taipei, Taiwan, Genomic Research Center and Institute of Chemistry, Academia Sinica, Taipei, Taiwan

cclin66@mx.nthu.edu.tw

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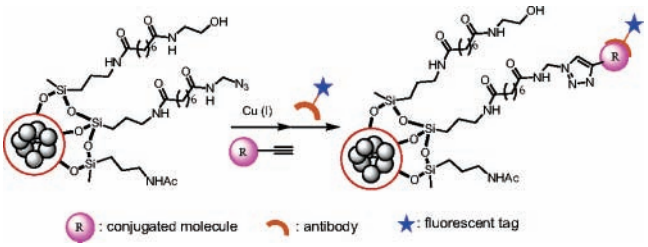
ABSTRACT



The Cu(I)-catalyzed alkyne–azide [2 + 3] cycloaddition has been demonstrated to be an effective and orthogonal conjugation reaction to covalently immobilize biomolecules on magnetic nanoparticles (MNPs). The azido group on the MNP surface provides better conjugation efficiency with alkynated molecules. Moreover, the *C*-terminal alkynated protein was site-specifically immobilized on MNP. The protein binding activity presented by site-specific immobilization is higher than that by random amide bond formation.

Because of the advantage of a larger surface area to volume ratio as well as homogeneity in aqueous solution, biomolecule-coated nanoparticles (NPs) have shown promising applications in complicated biosystems.[1] Through a combination of the unique optical, electronic, or magnetic properties of NPs with various probes, functionalized NPs have become an exploitable field for biosensing and diagnostic applications.[1] NPs have been widely used to elucidate biological phenomena, such as protein−protein interactions[2] and in vivo cell imaging.[3] Among the functionalized NPs, gold NPs (AuNPs) have received most of the attention because of the orthogonal reactivity for strong Au−S bond formation.[4] Previously, we demonstrated that mannose encapsulated AuNPs exhibit specific and efficient recognition with FimH in *Escherichia coli*.[5] Additionally, galactose- and trisaccharide antigen (P^k antigen)-coated AuNPs have been applied to target protein isolation, identification, and epitope mapping by MALDI-TOF MS.[6] However, the separation of AuNP-protein complexes from biomixture is inconvenient owing to the need for centrifugation. Thus, development of a new NP-based affinity probe for rapid and effective isolation would enhance bioseparations.

Various magnetic nanoparticles (MNPs) have been designed as powerful carriers for use in diverse research,[7] such as protein purification,[8] pathogen detection,[8] and antigen

[†] Taiwan International Graduate Program.
[‡] Genomic Research Center and Institute of Chemistry.
[§] Authors equal contribution.

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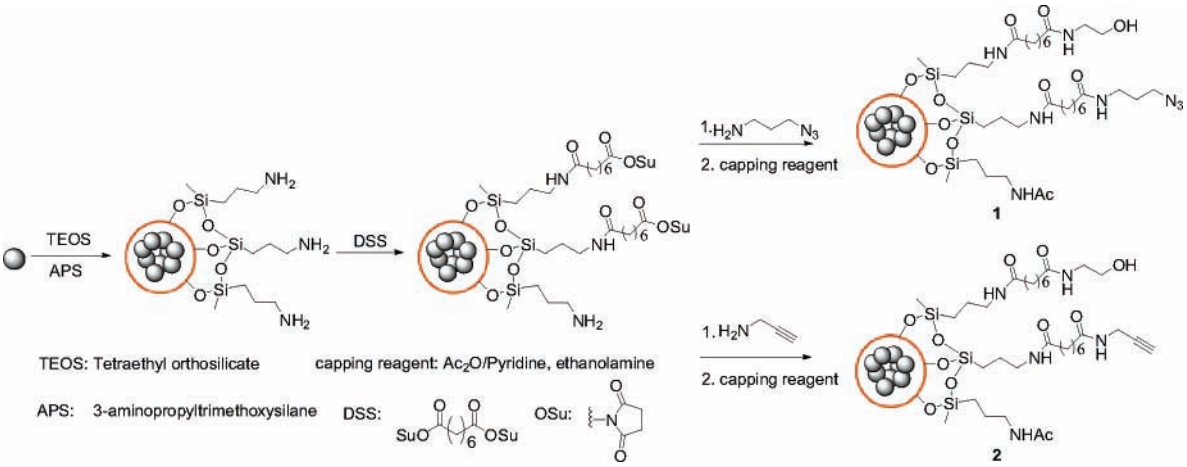
Scheme 1. Fabrication of Azido- and Alkynyl-MNPs



diagnosis.[9] The success of applying MNPs to biology relies on the efficient fabrication of target probes on the particle surface. Amide bond formation has been the most utilized method to immobilize probes on the MNP surface.[10] Alternatively, Shiff's base formation[11] and nucleophilic addition on epoxide[12] also have been commonly used to conjugate solid materials with probe molecules. However, the application of the aforementioned methods in protein conjugation with a solid support results in random covalent bond formation that may reduce the protein activity.[13] While the specific orientation of an immobilized target protein on a solid surface can be achieved by noncovalent interactions, such as biotin− streptavidin[14] and His tag−Ni^{2+} interactions,[15] the immobilized protein may dissociate from the surface during long-term storage.[16] To address this technical issue, we recently showed that Cu(I)-catalyzed alkyne-azide [2 + 3] cycloaddition[17] is an efficient and site-specific orthogonal reaction that covalently immobilizes target protein onto a glass surface. The site-specific immobilized protein showed higher ligand-binding activity when compared with protein immobilized by random amide bond formation.

Because of its high degree of efficiency, complete specificity, and compatibility with water, the Cu(I)-catalyzed azide-alkyne cycloaddition[18] could potentially be applied to both in vitro and in vivo[19] systems, including drug discovery,[20] protein conjugation,[17,21] proteomics research,[22] virus[23] and bacterial[24] surface modification, and fabrication of DNA[25] and sugar microarrays.[26] Thus far the use of Cu(I)-catalyzed cycloaddition to functionalize an MNP surface remains unexplored.[27] In this report, we describe the immobilization of diverse molecules onto the MNP surface by Cu(I)-catalyzed 1,3-dipolar cycloaddition.

To investigate the reactivity difference between soluble or immobilized azide and alkyne, the azido and alkynyl functional groups, respectively, were assembled onto the MNP (silica oxide-coated Fe_3O_4).[9] The *N*-hydroxysuccinimide (OSu)-activated MNP was incubated with 10 mM aqueous 3-azidopropanylamine and monopropargylamine, respectively, for 12 h at 4 °C. The MNP was then capped with Ac_2O/pyridine (1/1) for 3 h followed by ethanolamine (100 mM**)** for 1 h to yield the azido- and alkynyl-modified MNPs (**1** and **2**), as shown in Scheme 1. The capping steps

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were performed to block the nonreacted amine and activated ester on the MNP surface, which should reduce the nonspecific interaction between functionalized MNPs and the target molecule. The modified MNPs were reacted with mannose modified with azido (**3**) or alkynyl (**4**) groups at its reducing end, as shown in Figure 1. Conjugations **5** and

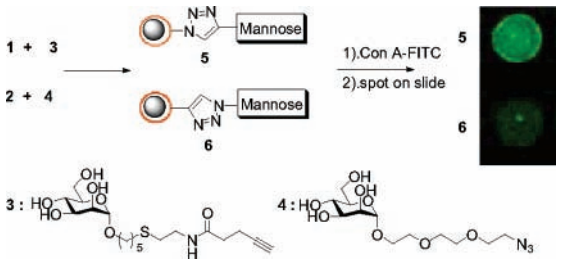

Figure 1. Conjugation of azido- and alkynyl-MNPs with azide- and alkyne-modified mannose.

6 were obtained by 1,3-dipolar cycloaddition catalyzed by Cu(I), which was generated by reacting $CuSO_4$ (2 mM) with tris(carboxyethyl)phosphine (TCEP, 2 mM) in the presence of triazolyl amine ligand (2 mM)[17,23] in PBS (pH 8.0, 0.1 M) at 25 °C for 12 h. To investigate the immobilization efficiency of mannose onto the MNP, the mannose-coated MNPs were incubated with concanavalin A (Con A)-FITC (25 μg/mL) in HEPES buffer (pH 7.4, containing 1% BSA, 1 mM Ca^{+}, 1 mM Mn^{2+}) at 37 °C for 1 h. The MNP complex (1 μL) was then spotted on a glass slide, and the emission was detected by a microarray fluorescence reader. The emission intensity resulting from azido-MNP was stronger than that for alkynyl-MNP. The results are consistent with the reported reaction mechanism.[28] The cycloaddition reaction started from the activation of alkyne by Cu(I) indicates that reaction is faster when alkyne is in solution-phase owing to the fast diffusion rate (homogeneous reaction).

The azido MNP was then used as the core NP to conjugate with the diverse molecules (**7**−**10**) listed in Figure 2A. These biomolecule-functionalized MNPs (**11**−**14**) could be used in biological studies such as the biotin−streptavidin affinity interaction, flag peptide-tagged protein separation, antigen−antibody recognition, and carbohydrate−lectin detection. In general, the biomolecules on the MNP surfaces were detected using fluorescently labeled antibodies and lectins. The Tn antigen (**11**), flag peptide (**12**), and biotin (**14**) on the MNP surface were detected by *Psophocarpus tetragonolobus*-FITC, antiflag M2 antibody-Cy3, and streptavidin-Cy3, respectively. Because of the unavailability of a fluorescently labeled antibody for the detection of 6-(2,4-dinitro-phenyl-amino) derivative (DNP, **13**), a fluorescently labeled secondary antibody was used to detect the antibody which binds on DNP **13**. On the basis of the glass-slide fluorescence-sensing assays, each of the biomolecule-functionalized MNPs

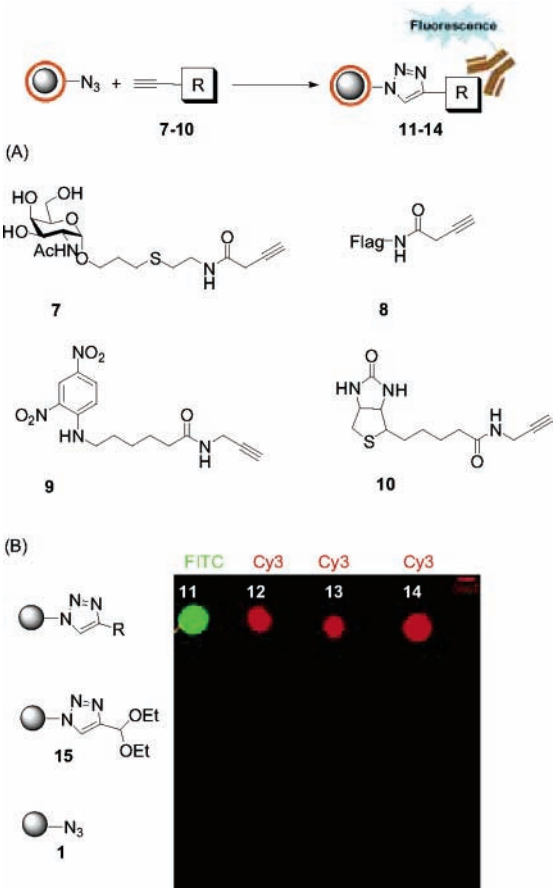

Figure 2. (A) Chemical structures of four azide-modified molecules, including Tn-, Flag-, DNP-, and biotin-alkynes; (B) fluorescence detection of biomolecule-conjugated MNPs with corresponding fluorescently labeled antibodies. Compounds **7**−**10** yield the corresponding compounds **11**−**14**.

exhibited good emission intensities, indicating sufficient immobilization (Figure 2B). To investigate the potential nonspecific interactions caused by the 1,2,3-triazole ring and nonreacted azide, the core triazole-MNP **15** (formed by reacting proparpylaldehyde diethyl acetal with azido MNP) and azido-MNP **1** were incubated with each of the fluorescently labeled proteins used in this study. The resulting negative signals indicated that neither the azide group nor the triazole ring caused nonspecific interactions (Figure 2B).

To further examine the application of alkyne-azide [2 + 3] cycloaddition in biomolecule-NP conjugation, we chose the enhanced green fluorescent protein (EGFP) with an alkyne at its C terminus as a target protein. The alkynated EGFP was prepared by the expression protein ligation method[17] and then reacted with azido, alkynated, or aminated MNPs using the same reaction conditions described above. The EGFP-reacted azido-MNP exhibited the strongest fluorescence signal at 530 nm, whereas the EGFP-reacted aminated-MNP showed no signal, indicating a lack of nonspecific adsorption (see SI, Figure S1). Thus, we demonstrated that Cu(I)-catalyzed [2 + 3] cycloaddition

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constitutes an efficient and orthogonal surface chemistry for site-specific immobilization of a protein on an MNP.

We next examined the activity of a protein immobilized on the MNP surface. Maltose binding protein (MBP) was covalently attached to MNPs at its C terminus by site-specific [2 + 3] cycloaddition or at its amino group−containing residues by random amide bond formation. Notably, both the reaction temperature and azide linker affected the conjugation efficiency for triazole formation (see Figures 3A and S2). Longer linker **16** and room temperature (25 °C) led to more efficient immobilization of MNP. On the basis of the Bio-Rad protein assay, comparable amounts of MBP were immobilized on MNPs via the site-specific [2 + 3] cycloaddition and random amide bond formation methods. The maltose binding activity of MBP-MNP was then evaluated by incubation with biotinylated maltose and subsequent visualization with fluorescent streptavidin-Cy3. Using the same protein amount of MNP complex, site-specific immobilization produced approximately twice the fluorescence signal compared with random immobilization (Figure 3D). As shown in Figure 3 panels B and C, the X-ray crystal structure of MBP[29] reveals that the C terminus is distant from the binding site and that 7 of the 41 amino group-containing residues are located near the binding site. The structure suggests that site-specific immobilization of MBP through its C terminus should minimally impact its native structure and thus would have little effect on maltose binding activity.

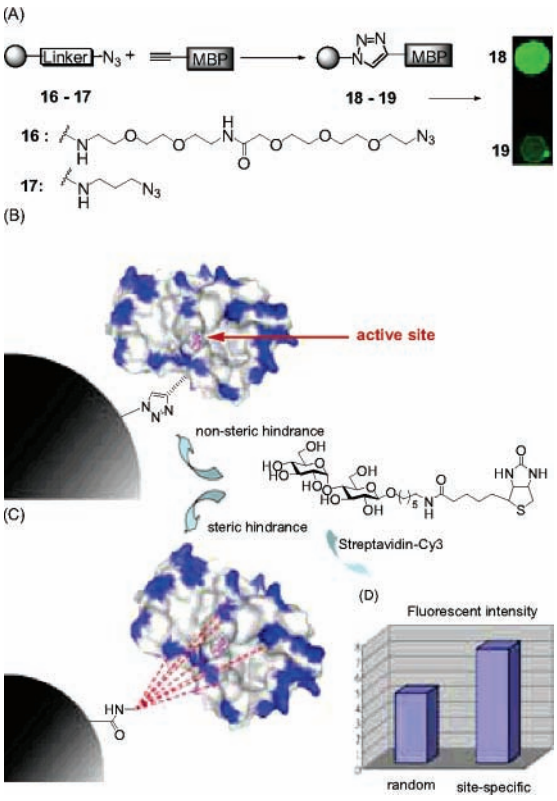

Figure 3. Maltose binding protein (MBP)-conjugated MNP (A) linker effect on Cu(I) catalyzed [2 + 3] cycloaddition; (B) schematic representation of site-specific immobilization, or (C) random amide bond formation; (D) maltose binding activity of MBP was determined as fluorescence intensity upon binding with biotinylated maltose. Blue color represents the amino group-containing residues.

In conclusion, the Cu(I)-catalyzed alkyne-azide [2 + 3] cycloaddition has been demonstrated as an efficient surface reaction that covalently immobilizes biomolecules onto MNP. The azido group on the MNP surface provides a better conjugation efficiency with various alkynated molecules. Moreover, site-specific immobilization of a target protein on the MNP maintained protein activity. Taking advantage of the unique magnetic property of MNPs, their large surface area to volume ratio and lack of nonspecific binding, biomolecule-conjugated MNPs can serve as sensitive probes for application in complex biosystems.

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Supporting Information Available: Experimental section, describing the syntheses of compounds **1**−**19**, glass-slide fluorescence sensing assay, and MBP binding assay. This material is available free of charge via the Internet at http://pubs.acs.org.

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