Click Chemistry

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Visual Detection of Copper(II) by Azide- and Alkyne-Functionalized Gold Nanoparticles Using Click Chemistry**

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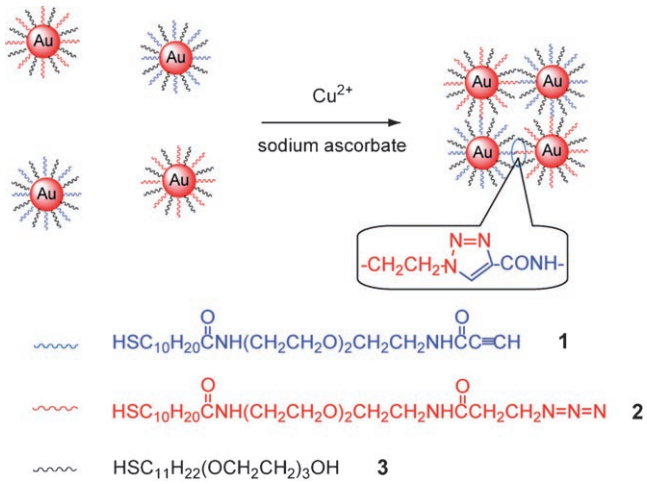
We report a method for the detection of Cu^{2+} ions by azide- and terminal alkyne-functionalized gold nanoparticles (Au NPs) in aqueous solutions using click chemistry.[1] The catalyst, Cu(I), was conveniently derived from the reduction of Cu(II) in the presence of sodium ascorbate. This method allows the naked eye, without the aid of any advanced instrument, to assay for the presence of Cu^{2+} ions by the aggregation of Au NPs as a result of the Cu(I)-catalyzed conjugation between the two functional groups.

Copper is a transition metal essential for life but also highly toxic to organisms, such as certain algae, fungi and many bacteria and viruses.[2] In recent years, copper has been suspected of causing liver damage in children.[3] The analysis and measurement of copper in environmental and biological samples have become increasingly important.

Several methods exist for the detection of Cu^{2+} ions, for example, those based on organic fluorophores[4] or chromogenic sensors,[5] quantum dots,[6] atomic absorption spectroscopy,[7] inductively coupled plasma mass spectroscopy,[8] absorbance spectro-photometry,[9] peptides[10] and voltammetry.[11] The color changes associated with the aggregation of metal nanoparticles has led to the development of a number of assays for a variety of target species.[12,13] Colorimetric methods can be convenient and attractive in many applications because they can be easily monitored with the naked eye, without the aid of any advanced instruments. The extinction coefficient of 13 nm-diameter gold nanoparticles is $2.7\times10^{8}\,\text{M}^{-1}\,\text{cm}^{-1}$, several orders of magnitude more than those of traditional organic chromophores.[14] As a result, colors arising from nanoparticles at nanomolar concentrations can be observed by the naked eye, allowing sensitive detection of small amounts of analytes. Since Cu(I) is used as a catalyst in the cycloaddition reaction between azides and alkynes in click chemistry based on Huisgen's reaction,[15] the amount of copper needed for its completion is typically small. Therefore, a method that can visualize the progress of the reaction using the aggregation of Au NPs might also be useful for the detection of trace amounts of Cu(II) (by detection of Cu(I)). Because the azide/alkyne functional groups and their conjugation are highly selective and are essentially inert to most biological molecules, oxygen, water, and the majority of common reaction conditions in chemical synthesis, and are tolerant of a wide range of solvents, temperatures, and pH values, we reasoned that an assay based on such chemistry may find myriad uses.[16–21] Our method for the detection of Cu^{2+} ions relies on the Cu(I)-catalyzed 1,3-dipolar cycloaddition of alkynes and azides on the surface of functionalized Au NPs, that results in the aggregation of Au NPs (Scheme 1).

We synthesized azide- and terminal alkyne-functionalized thiols, **1** and **2**, and prepared gold NPs coated with these



Scheme 1. The detection of Cu^{2+} ions using click chemistry between two types of gold NPs, each modified with thiols terminated in an alkyne (**1**) or an azide (**2**) functional group.

functional groups by ligand-exchange reactions using these thiols and citrate-stabilized Au NPs (Scheme 1). To keep the Au NPs stably dispersed, we employed a commercially available thiol, **3** ($HS(CH_2)_{11}(OCH_2CH_2)_3OH$), as a stabilizing agent along with **1** and **2**.[22] The azide- and alkyne-functionalized Au NPs were purified by repeated centrifugation and redispersion in H_2O/*t*BuOH, to obtain a deep reddish purple solution. The mixture of the two kinds of Au NPs (1.1×10^{-5} M) exhibits a characteristic plasmon absorption band at 529 nm, and sizes of approximately 14 nm (see Supporting Information, Figure S1).

When Cu^{2+} and the reductant (sodium ascorbate) were both added to the mixture of Au NPs at room temperature, the color began to fade and the aggregation of Au NPs occurred, resulting in a clear solution with a precipitate

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(Figure 1). Over time, the solution became colorless, accompanied by the gradual appearance of additional precipitate. UV/Vis spectroscopy showed that the plasmon band at 529 nm moved to 588 nm, similar to the case where the aggregation of Au NPs is induced by the cross-linking of DNA.[23] Within twenty-four hours, aggregation of Au NPs resulting from Cu(I)-catalyzed conjugation became complete. Because the process of this aggregation can be monitored visually, the naked eye alone can judge the presence or absence of Cu^{2+} ions without the aid of any instruments.

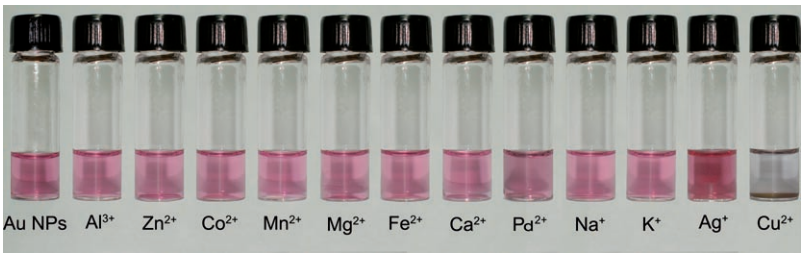

Figure 2. A photograph of the solutions containing the mixtures of functionalized Au NPs with different metal cations in the presence of sodium ascorbate after 24 h. Ion concentration of Al^{3+}, Zn^{2+}, Co^{2+}, Mn^{2+}, Mg^{2+}, Fe^{2+}, Ca^{2+}, Pd^{2+}, Na^+, K^+, and Ag^+ is 0.5 mM. $[Cu^{2+}] = 200$ μM.

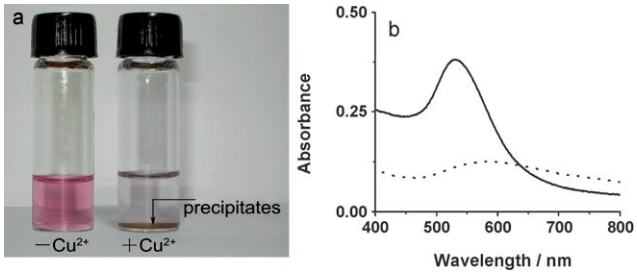

Figure 1. The assay for Cu^{2+} ions by the naked eye (a) Photographs of the solution containing only the mixture of functionalized Au NPs (left) and the same mixture after the addition of Cu^{2+} (right); (b) UV/Vis spectra obtained from solutions of functionalized Au NPs and after 24 h in the presence of Cu^{2+} ions and sodium ascorbate. solid line: Au NPs–Cu^{2+}, dotted line: Au NPs + Cu^{2+}.

To evaluate the minimum concentration of Cu^{2+} ions in aqueous solution detectable by this color change and formation of precipitates, we added Cu^{2+} into the mixture of Au NPs to obtain a Cu^{2+} concentration of 500 μM, 300 μM, 200 μM, 100 μM, 50 μM, 20 μM, and 10 μM, respectively. Sodium ascorbate at five times the concentration of Cu^{2+} was also added. After overnight reaction, the results showed that only when $[Cu^{2+}] \geq 50$ μM, were the changes of color and formation of precipitates obvious, whereas when $[Cu^{2+}] < 20$ μM, distinct changes of the solution could not be observed. We also tested the effects of either increasing or decreasing the concentration of Au NPs in the solutions, but were unable to detect lower concentrations of Cu^{2+} ions. We therefore conclude that the minimum concentration of Cu^{2+} detectable by eye is approximately 50 μM. This lowest detectable concentration cannot be compared with that obtained with the help of advanced instruments, but we believe, however, that this concentration sets the record for the detection of Cu^{2+} ions by the naked eye alone.[24]

We tested the selectivity of this assay for Cu^{2+} ions by using other metal ions in place of copper, including Al^{3+}, Zn^{2+}, Co^{2+}, Mn^{2+}, Mg^{2+}, Fe^{2+}, Ca^{2+}, Pd^{2+}, Na^+, K^+ and Ag^+ at concentrations of 0.5 mM. Only the Cu^{2+} sample induced noticeable aggregation of the mixture of azide-/alkyne-functionalized Au NPs (Figure 2). None of these metal ions interfered with the assay. No color changes (except for Ag^+) or aggregation could be observed even after several months. Even when the concentrations of these ions increased to 500 times the concentration of Cu^{2+}, that is, at 0.1M, we still found no distinct color change or precipitates in the mixture of functionalized Au NPs. The presence of the reductant is also crucial for the assay of Cu^{2+} ions: when adding only copper sulfate or only sodium ascorbate to the mixture of Au NPs, we saw no change of color in the solution even after 24 h. (see Supporting Information, Figure S2). When Ag^+ ions and the reductant were simultaneously added in the assay system under the same conditions, the color of the Au NPs became redder. This result may be ascribed to the surface electron effect of the silver ion on Au NPs.[25] No precipitates formed, however, in the case of Ag^+.

To test whether the specificity in the detection of Cu^{2+} is compromised by complex mixtures of other cations, we used any combination of four different types of cations from the following pool of cations, Al^{3+}, Fe^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, Ca^{2+}, Co^{2+}, Na^+, and K^+, in the assay. The Au NPs were stably dispersed in mixtures of these cations with the presence of excess sodium ascorbate. Au NPs began to precipitate, however, when the solution of Cu^{2+} was added to the mixture. The total concentration of non-copper cations was 200 μM. We believe that the highly selective nature of the click chemistry was responsible for the high selectivity of the assay for Cu^{2+}. This result suggests that the detection of Cu^{2+} can be performed in solutions with relatively complex mixtures of other cations.

In conclusion, we have developed a procedure for detecting Cu^{2+} in aqueous solutions using Cu(I)-catalyzed click chemistry between gold NPs. Although an indirect assay, this method is highly specific even in the presence of high concentrations of mixtures of other cations. Aside from the applications that require the detection of copper itself, because of the compatibility of click chemistry with biomolecules, efforts in our group are being made to extend current work to applications in bioassays. We anticipate this methodology to find uses in many scenarios where sensors for Cu^{2+} are required. The ability to detect the presence of Cu^{2+} can be potentially extended to assay for analytes indirectly, without the need for equipment that is typically bulky, thus facilitating miniaturization for "lab-on-a-chip" and related applications.

Experimental Section

Au NPs with average diameters of 14 nm were prepared by the citrate-mediated reduction of $HAuCl_4$. A stirred aqueous solution of $HAuCl_4$ (0.08 M, 1.25 mL) was heated to reflux, and then trisodium citrate solution (38.3 mM, 10 mL) was added quickly, resulting in a change in solution color from pale yellow to deep red. After the color change, the solution was heated under reflux for an additional 30 min and allowed to cool to room temperature. Ligand exchange reactions were performed under stirring at room temperature for 24 h by mixing a certain volume of as-prepared gold colloids with a methanol/water solution containing an excess of terminal alkyne-functionalized thiol **1**, or azide-functionalized thiol **2**. Typically, 500 μL of citrate-stabilized gold sols (1 mM) were diluted by adding Milli-Q water (deionized water purified in a Milli-Q system available from the Millipore Corporation, 5 mL). The basicities of the sols were then adjusted to pH 9 by NaOH (30 μL, 0.5 M in Milli-Q water). Under vigorous stirring, thiol **3** (100 μL, 0.01 M in methanol) and **1** (200 μL, 0.01 M in methanol) were added to the gold sols simultaneously. The mixture was stirred for 24 h and then was centrifuged for 20 min (13000 rpm, Eppendorf centrifuge) to obtain alkyne-functionalized Au NPs. The obtained alkyne-functionalized Au NPs were washed with H_2O/*t*BuOH (3 × 5 mL), centrifuged, and finally redispersed in H_2O/*t*BuOH (1.5 mL). To prepare terminal azide-functionalized Au NPs, similar procedures were used, except that **2** (200 μL, 0.01 M) was added, instead of **1**. The azide-functionalized and terminal alkyne-functionalized Au NPs were mixed to obtain a homogenous dispersion solution.

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