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PAPER

A novel biosensor for copper (II) ions based on turn-on resonance light scattering of ssDNA templated silver nanoclusters†

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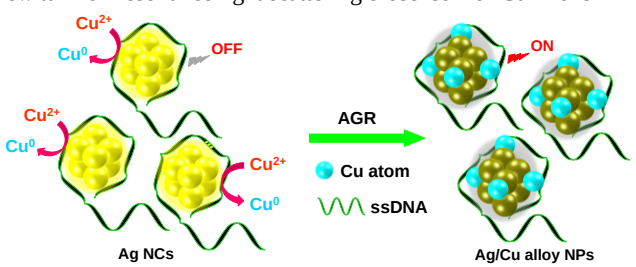
A new ultrasensitive biosensor for copper (II) ions was developed based on turn-on resonance light scattering (RLS) of ssDNA templated silver nanoclusters through anti-galvanic reduction (AGR). In our experimental assay, ultra-small size fluorescent C-rich ssDNA templated silver nanoclusters (C-rich ssDNA-Ag NCs) exhibit direct reduction of copper (II) ions to elemental copper (Cu^0) and the resulting Cu^0 formed copper nanoparticles on the surface of C-rich ssDNA-Ag NCs spontaneously. The process produced Ag/Cu alloy nanoparticles with larger diameter, turning-on RLS signal of C-rich ssDNA-Ag NCs. As the concentration of Cu^{2+} ions increased, the RLS signal of C-rich ssDNA-Ag NCs gradually enhanced. The present method achieves the detection of Cu^{2+} ions in a linear range of 5×10^{-9} M to 7.5×10^{-7} M, with detection limit of 2 nM. Moreover, RLS spectrum, TEM image, fluorescence spectrum, X-ray photoelectron spectroscopy (XPS), and MALDI-TOF mass spectrometry were carried out for investigating the mechanism of the biosensor. The present strategy of constructing biosensor based on AGR of metal nanoclusters paves a new way to design novel RLS probe.

Introduction

Metal nanoclusters (NCs), consisting of several to tens of metal atoms, have attracted a great deal of attention due to their unique physical, electrical, and optical properties.[1] Especially, when the sizes of metal nanoclusters approach the Fermi wavelength of conduction electrons, they exhibit a unique fluorescence property due to quantum confinement.[2] Metal nanoclusters have emerged as a new class of promising optical probes for constructing biosensors because of their ultrasmall size, excellent photostability, tunable fluorescence, good biocompatibility, and low toxicity.[3] Recently, there have been a few reports about developing the sensors for Hg^{2+}, Cu^{2+}, Ag^{+} and As^{3+} using gold nanoclusters (Au NCs) or silver nanoclusters (Ag NCs). Copper, one of the most abundant transition metals in the human body, plays vital roles in many fundamental biological processes. However, Cu^{2+} ions can cause serious damage to the liver and kidney at high concentrations.[4] Besides, excess amounts of Cu^{2+} ions can also induce a series of environmental problems.[5] Therefore, it is of great significance to develop an assay for high-sensitively detecting Cu^{2+} ions.

Resonance light scattering (RLS), one kinds of elastic light scattering, occurs when the incident beam is close to its molecular absorption.[6] Pasternack firstly established the RLS technique to study the aggregations of porphyrins.[7] Subsequently, Huang et al. first utilized RLS technique for quantitative analysis and achieved the determination of biomacromolecules.[8] In decade years, RLS has been applied to the study and determination of nucleic acids,[9] proteins,[10] small molecules,[11] metal ions,[12] drugs,[13] and so on. With the rapid development of nanotechnology, great attentions have been focused on the use of metal nanoparticles as RLS probes.[14] Recently, it is reported that noble metals can reduce non-noble metal ions, which is opposite to the classic galvanic theory.[15] The new chemical process is named as anti-galvanic reduction (AGR), which provides a facile and powerful route to fabricate kinds of nanomaterials. Within our knowledge, no study has been reported to introduce the growth of metal nanoclusters induced by AGR for designing RLS probe for detecting Cu^{2+} ions.

As part of our continuing studies in silver nanoclusters,[16] a new turn-on resonance light scattering biosensor for Cu^{2+} ions



Scheme 1 Schematic illustration of the turn-on resonance light scattering biosensor for Cu^{2+} ions based on anti-galvanic reduction of C-rich ssDNA templated silver nanoclusters.

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was proposed based on the growth of C-rich ssDNA templated Ag NCs (C-rich ssDNA-Ag NCs) induced by AGR (Scheme 1). In this contribution, our present approach is conceptually different from the previous RLS methods which rely on determining the aggregation of metal nanoparticles.[17] C-rich ssDNA-Ag NCs exhibit direct reduction of Cu^{2+} ions to elemental copper (Cu^0) and the resulting Cu^0 formed copper nanoparticles on the surface of C-rich ssDNA-Ag NCs spontaneously. The process forms the Ag/Cu alloy nanoparticles with larger diameter, turning on the RLS signal of C-rich ssDNA-Ag NCs, which allowed the quantitative determination of Cu^{2+} ions. The proposed RLS biosensor exhibited good performances such as ultrasensitivity, excellent selectivity, low cost and facile fabrication. Compared with the developed assay for detecting Cu^{2+} ions by Ag NCs before,[16a] the present approach has higher sensitivity and wider linear ranges. Moreover, the present strategy of designing RLS biosensor based on the AGR of metal nanoclusters opens up a fresh insight of designing novel RLS probe.

Experimental section

Materials

Silver nitrate ($AgNO_3$, 99.99%) and sodium borohydride ($NaBH_4$, 98%) were purchased from Alfa Aesar and used without further purification. The DNA oligonucleotides were purchased from Sangon (Shanghai, China). All other reagents were of analytical reagent grade. The water was purified through a Millipore system. Unless otherwise noted, experiments were carried out in 10 mM Tris/HCl buffer solution (pH 7.0).

Apparatus

The resonance light scattering spectra were measured with a model LS-55 spectrofluorometer (Perkin-Elmer, USA). Fluorescence measurements were performed by a FLS-920 picosecond fluorescence lifetime spectrometer (Edinburgh Instruments, UK). XPS measurements were implemented on a Thermo ESCALAB 250 instrument configured with a monochromated $Mg_{K\alpha}$ (1486.8 eV) 150 W X-ray source, 0.5 mm circular spot size, a flood gun to counter charging effects, and the analysis chamber base pressure $< 1 \times 10^{-9}$ mbar; data were collected with FAT = 20 eV. The take-off angle, defined as the angle between the substrate normal and the detector, was fixed at 0 º. The samples were dropped on the surface of the silicon substrate by natural evaporation. All binding energies were calibrated using either the $Au_{4f7/2}$ peak (84.0 eV) or the C_{1s} carbon peak (284.6 eV). HR-TEM images were taken with a JEM-2011 high-resolution transmission electron microscopy operating at 200 kV. The as-prepared Ag nanoclusters were dried on carbon-coated copper grids by slow natural evaporation. Mass spectra were obtained on a MALDI-TOF-MS (Bruker Daltonics Inc. BIFLEX III) with a 3-HPA matrix in linear mode.

Preparation of ssDNA templated silver nanoclusters

Silver nanoclusters (Ag NCs) were synthesized according to the reported method with minor modification.[18] The ssDNA sequence (C-rich ssDNA, 5'-$(CCCTAA)_3CCCTA$-3', 23 bp) was chosen as the template for fabricating Ag NCs. Briefly, certain volume of $AgNO_3$ solution was introduced into aliquot volume of DNA solution in 10 mM Tris/HCl buffer solution (pH 7.0). The mixture solution was kept at 0 ℃ for 15 minutes. Then, the mixture was reduced by quickly adding $NaBH_4$ (freshly prepared before using) under vigorously shaking for 2 min. Final concentrations were 15 μM for the ssDNA template, 90 μM for $AgNO_3$ and 90 μM for $NaBH_4$. The reaction mixture was kept in the dark at 4 ℃ for another 3 hours before the determination.

The resonance light scattering detection of Cu^{2+} based on AGR of C-rich ssDNA-Ag NCs

Certain volume of the C-rich ssDNA-Ag NCs solution was added into a test tube. Then, a series of solutions of Cu^{2+} ions were pipetted into the test tubes by using microsyringes before resonance light scattering measurement. The RLS spectrum was then measured by scanning simultaneously the excitation and emission monochromators ($\Delta\lambda$ = 0.0 nm) from 250 to 700 nm with the excitation and emission slits 5 nm.[19] Based on the spectra, RLS intensities were obtained at the maximum peak. Spectrum curves were made based on the data collected on the first minute after adding the solution of Cu^{2+} ions.

Selectivity measurement

The selectivity of this sensing system for Cu^{2+} was evaluated by measuring the RLS response of the system to other common cations (Na^+, K^+, Ag^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, Ni^{2+}, Mn^{2+}, Co^{2+}, Cu^{2+}, Hg^{2+}, Fe^{3+}, and Al^{3+}). The salt solutions above were mixed with Ag NCs solutions before the determination. However, as for the detection of Cu^{2+} or Hg^{2+} ions, EDTA solution was mixed with metal ions for several minutes and then introduced Ag NCs solutions.

The fluorescence measurements of the C-rich ssDNA-Ag NCs upon the reaction with Cu^{2+} ions

A certain concentration of copper ions solution was pipetted into the C-rich ssDNA-Ag NCs solution by using microsyringes before fluorescence measurement. Fluorescence spectra were made based on the data collected on the first minute after the addition of cysteine.[20] Spectrum curves were made based on the data collected on the first minute after adding the solution of Cu^{2+} ions.

Results and discussion

Characterization of the as-prepared ssDNA templated silver nanoclusters

Silver nanoclusters (Ag NCs) were synthesized by using a C-rich ssDNA sequence as a template in aqueous solution. Upon the excitation at 468 nm, the prepared C-rich ssDNA-Ag NCs exhibit an emission peak centered at 560 nm (Fig. 1a). CIE1931 Chromaticity Coordinate Calculation illustrates that C-rich ssDNA-Ag NCs emit in the yellow region (Fig. 1b). The chemical nature of prepared silver nanoclusters has been investigated by X-ray photoelectron spectroscopy (XPS). The presence of a sharp peak centered at 368.2 eV, relative to the Ag

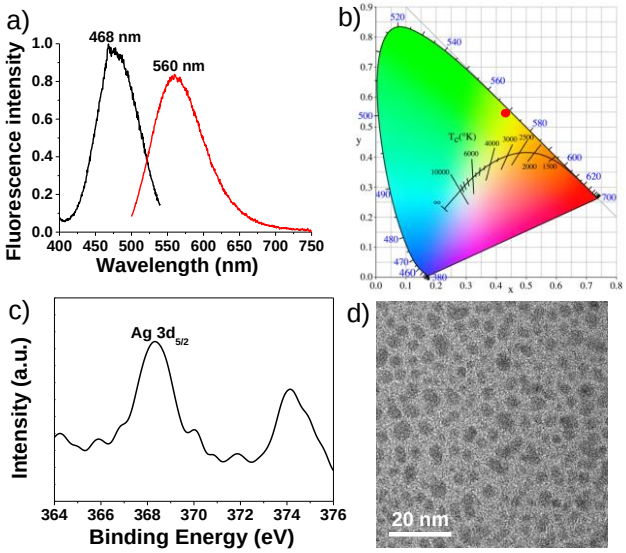

Fig. 1 (a) The excitation (black) and emission (red) spectra of ssDNA templated silver nanoclusters. The template is C-rich ssDNA (5'-(CCCTAA)$_3$CCCTA-3'). The DNA sequence is shown in 5'-3'. (b) The image of CIE1931 Chromaticity Coordinate Calculation of C-rich ssDNA templated Ag NCs (C-rich ssDNA-Ag NCs). (c) Ag3d XPS spectrum of C-rich ssDNA-Ag NCs. (d) TEM image of the synthesized C-rich ssDNA-Ag NCs, scale bar: 20 nm.

$3d_{5/2}$ signal, suggests that it exists the elemental silver in silver nanoclusters (Fig. 1c).[15b] As shown in Fig. 1d, the C-rich ssDNA-Ag NCs are well dispersed and the diameter is about several nanometers by TEM image.

Turn-on resonance light scattering for sensing Cu^{2+} ions based on AGR of the as-prepared C-rich ssDNA-Ag NCs

The resonance light scattering change of C-rich ssDNA-Ag NCs in the presence of Cu^{2+} ions is investigated. As shown in Fig. 2, with increasing the concentration of Cu^{2+} ions, the RLS signals increase gradually. We guess that it is due to the stepwise enlargement of C-rich ssDNA-Ag NCs by taking place anti-galvanic reduction. A good linear relationship between the RLS intensity and the concentration of Cu^{2+} ions is obtained in the range of 5×10^{-9} M to 7.5×10^{-7} M. The limit of detection (LOD) can be calculated by the equation LOD = $3S_0/S$, where 3 is the factor at the 99% confidence level, S_0 is the standard deviation of the blank measurements (n = 8), and S is the slope of the calibration curve. The LOD value calculated was 2×10^{-9} M. A safe limit of Cu^{2+} ions in drinking water has been set to 1.3 ppm (*ca.* 20 μM) by the U.S. Environmental Protection Agency (EPA).[21a] Besides, compared with other methods reported for the determination of Cu^{2+} ions, our present approach has lower sensitivity (Table S1, ESI†).[21]

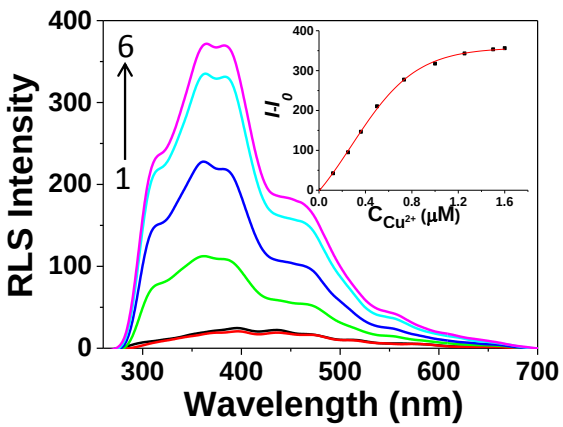

Fig. 2 Resonance light scattering (RLS) spectra of C-rich ssDNA templated silver nanoclusters in the absence and presence of various concentrations of copper (II) ions solution at room temperature. Conditions: 1 (black line), the buffer solution; 2 (red line), C-rich ssDNA-Ag NCs; 3-6: 2 + Cu^{2+} ions (μM): 0.125 (green line), 0.375 (blue line), 0.75 (cyan line), 1.5 (magenta line). The inserted figure reveals the plot of the increment of the resonance light scattering intensity of C-rich ssDNA-Ag NCs versus the concentration of copper (II) ions.

The selectivity assay

To investigate the selectivity of our developed biosensor, the RLS changes of the as-prepared C-rich ssDNA-Ag NCs with other common cations such as Na^+, K^+, Ag^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, Ni^{2+}, Mn^{2+}, Co^{2+}, Hg^{2+}, Fe^{3+}, and Al^{3+} were carried out. As shown in Fig. 3, the proposed enhanced RLS assay has high selectivity for determining Cu^{2+} ions against most of metal ions. It is also found that Hg^{2+} ions caused a significant RLS enhancement. Fig. 3 reveals that the chelating agent ethylene diamine tetracetic acid (EDTA) can coordinate with Cu^{2+} ions while it does not coordinate with Hg^{2+} ions.[22] Thus, when the samples include Cu^{2+} ions and Hg^{2+} ions simultaneously, the introduction of the masking reagent EDTA only eliminate the effect of Cu^{2+} ions on the enhanced RLS intensity. The enhancement value of the RLS intensity of ssDNA-Ag NCs (I_a) is induced by only Hg^{2+} ions. When without the addition of the masking reagent EDTA, the enhanced value of the RLS intensity of ssDNA-Ag NCs (I_b) is induced by both Hg^{2+} ions and Cu^{2+} ions together. Therefore, the concentration of Cu^{2+} ions can be determined by subtracting the value of enhanced RLS intensity (I_b - I_a) induced by Hg^{2+} ions. Therefore, our present method is not only used for the detection of transition metal Cu^{2+} ions, but also explored for the determination of heavy metal ions Hg^{2+} ions.

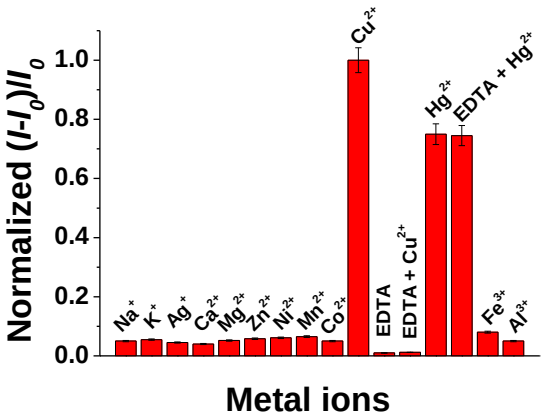

Fig. 3 Effect of different metal ions on the relative RLS ($(I-I_0)/I_0$) of the prepared C-rich ssDNA-Ag NCs. Conditions: The concentrations of metal ions used: Ag^+, 100 mM; K^+, Na^+, 10 mM; other metal ions includes Cu^{2+}, 1 mM; EDTA, 2 mM. The error bars represent the standard deviation of three measurements.

The mechanism of turn-on resonance light scattering of the as-prepared C-rich ssDNA-Ag NCs for sensing Cu^{2+} ions

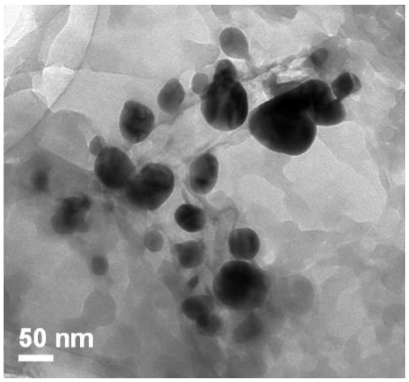

Fig. 4 TEM image of synthesized C-rich ssDNA-Ag NCs in the presence of Cu^{2+} ions solution (1 mM), scale bar: 50 nm.

According to the Rayleigh theory, the enhanced RLS signal mainly origins from the aggregation of light-scattering particles.[6a,23] The change of the intensity of resonance light scattering depends on the varying size of the particles greatly. In other words, larger particles produce stronger light scattering signals. In our assays, upon the reaction with Cu^{2+} ions, it appears the significant increased RLS signals of C-rich ssDNA-Ag NCs (Fig. 2), which suggests that it maybe form the aggregate of particles. Based on the change of resonance light scattering spectra, the stability constant (K_s) calculated is 9.65×10^6 L/mol and stoichiometry (n) is 2.6. However, TEM image reveals that it appears the separate particles with larger diameter (Fig. 4) compared with only Ag NCs (Fig. 1d) instead of the aggregation after the introduction of Cu^{2+} ions. Therefore, we speculate that the present assay maybe is conceptually different from the previous metal nanoparticles-based RLS assays which mainly rely on the aggregate of nanoparticles.

To further investigate the formation mechanisms of metal nanoparticles, XPS of C-rich ssDNA-Ag NCs in the absence (Fig.1c) and presence (Fig. 5) of Cu^{2+} ions were carried out. XPS of synthesized C-rich ssDNA-Ag NCs display the binding energies of 368.2 eV and 374.2 eV (assigned to $Ag3d_{5/2}$ and $Ag3d_{3/2}$, respectively), which indicates that it is neutral for silver element in synthesized C-rich ssDNA-Ag NCs. Upon the reaction with Cu^{2+} ions, it appears new binding energy located at 932.6 eV (assigned to $Cu2p_{3/2}$), which reveals that it must exist copper

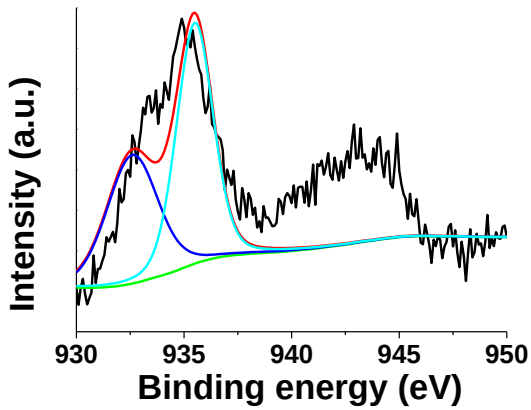

Fig. 5 Cu2p XPS spectrum of synthesized C-rich ssDNA-Ag NCs in the presence of Cu^{2+} ions solution (1 mM).

element in nanoparticles. The Ag/Cu atom ratio indicated by XPS analyses is 1.04:1. Besides, fluorescence spectra of C-rich ssDNA-Ag NCs in the absence and presence Cu^{2+} ions give the strong evidence for occurrence of the reaction. Fig. S1 shows that the maximum emission peak of ultra-small C-rich ssDNA-Ag NCs located at 560 nm disappears after the introduction of Cu^{2+} ions. Thus, we believe that fluorescent C-rich ssDNA-Ag NCs reduce Cu^{2+} ions to Cu^0, producing non-fluorescent Ag/Cu alloy nanoparticles with larger size, inducing the enhanced RLS signal. The conclusion is consistent with other spectrum assays above.

To further verify the reaction between C-rich ssDNA-Ag NCs and Cu^{2+} ions, MALDI-TOF mass spectrometry was also measured. In the mass spectrum of C-rich ssDNA-Ag NCs (6821 m/z, Fig. S2, ESI†), the most abundant species detected are [C-rich ssDNA + Ag_2] (7037 m/z, Fig. S3, ESI†) and [C-rich ssDNA + Ag_3] (7143 m/z, Fig. S3, ESI†). The results are consistent with the simulated results reported.[24] After the reaction between C-rich ssDNA-Ag NCs and Cu^{2+} ions, it appears the bimetallic nanoparticles peak [C-rich ssDNA + Ag_2Cu_1] (7126 m/z, Fig. S4, ESI†). Thus, it can be concluded that it must take place the replacement reaction between silver element of C-rich ssDNA-Ag NCs and Cu^{2+} ions, which is consistent with the results of XPS.

Determination of copper (II) ions in tap water samples

To assess the applicability, the proposed approach was used in the detection of Cu^{2+} ions in tap water samples. Meanwhile, the atomic absorption spectroscopy (AAS) was also performed to verify the reliability of the new method by determining the same samples. Table 1 suggests that our present assay for sensing Cu^{2+} ions based on turn-on RLS signal of C-rich ssDNA-Ag NCs exhibits higher accuracy in measuring Cu^{2+} ions in real samples than the traditional method AAS. Therefore, our developed assay can be used further for detecting the real sample.

Table 1 Recovery measurements of Cu^{2+} in local tap water samples.

Tap water Sample	Original Cu^{2+} concentration (nM)	Added Cu^{2+} concentration (nM)	Found by our proposed biosensor[a] (nM)	Found by AAS[a,b] (nM)
1	ND[c]	100	98.5	97.5
2	ND[c]	200	205	210
3	ND[c]	500	525	528

[a] Average values are from six independent measurements.
[b] AAS, Atomic Absorption Spectroscopy.
[c] ND is none detected.

Conclusions

In contrast to the conventional principle for designing RLS probe based on the aggregation of metal nanoparticles, the present work proposed an ultrasensitive metal nanoclusters-based RLS assay for sensing heavy metal Cu^{2+} ions. The developed biosensor utilizes a combination of the advantages of anti-galvanic reduction of C-rich ssDNA templated silver nanoclusters and the sensitivity and selectivity of resonance light scattering technique. After the introduction of Cu^{2+} ions, it occurred the progressive

growth of Ag/Cu alloy nanoparticles instead of Ag NCs through the anti-galvanic reduction. The growth process resulted in the significant enhancement of RLS intensity of C-rich ssDNA-Ag NCs. The proposed method is applicable for fast detection of Cu^{2+} ions, which is particularly suitable for an ultra-trace amount of Cu^{2+} ions. Moreover, this study provides a strategy for the development of new RLS biosensor for analytes. For example, as thiolate-protected gold nanoclusters (Au NCs) reduce Ag^{+} ions through anti-galvanic reduction, a new RLS biosensor for sensing Ag^{+} ions can also be proposed based on AGR of Au NCs. We are working on this project further.

Acknowledgments

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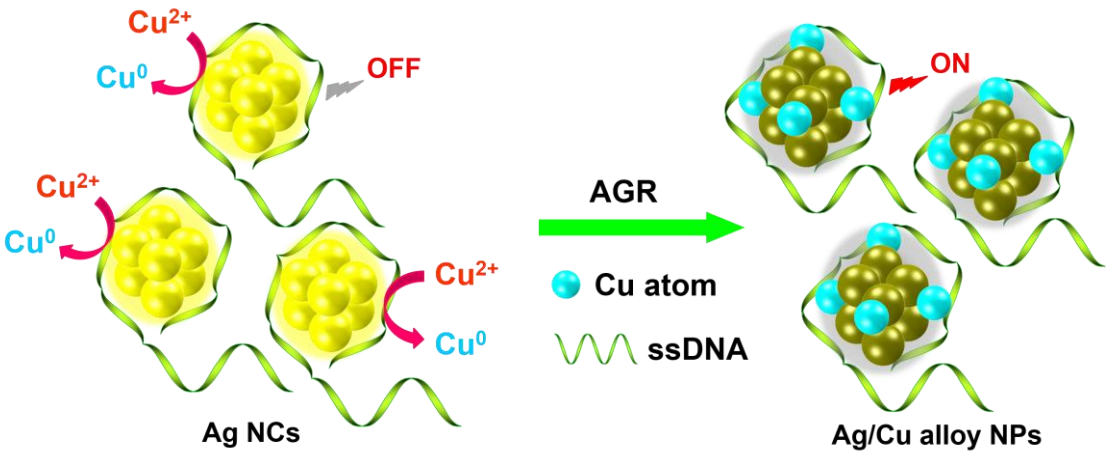
Graphical abstract

for

A novel biosensor for copper (II) ions based on turn-on resonance light scattering of ssDNA templated silver nanoclusters



A new ultrasensitive biosensor for copper (II) ions was first developed based on turn-on resonance light scattering (RLS) of ssDNA templated silver nanoclusters through anti-galvanic reduction (AGR).