Trends in Analytical Chemistry 58 (2014) 90–98

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

journal homepage: www.elsevier.com/locate/trac

Review

Fluorescent metal nanoclusters: From synthesis to applications

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ARTICLE INFO

Keywords:
Bioconjugation
Bioimaging
Biosensing
Cellular targeting
Detection
Fluorescent metal nanocluster
Fluorescent probe
In vivo
Optical imaging
Synthesis

ABSTRACT

Fluorescent metal nanoclusters (NCs) are a class of emerging fluorescent materials. They have excellent photostability and biocompatibility with sub-nanometer size and are easy to synthesize. Taking advantage of these features, fluorescent metal NCs have been involved in exciting developments of analytical methods for fluorescent biosensing and bioimaging. In this review, we first summarize the approaches to synthesis and bioconjugation for fluorescent metal NCs (Ag, Au, Cu and Pt). We then highlight their applications as fluorescent probes for metal ions, small molecules, nucleic acids, and protein detection. We also summarize the use of metal NCs in cellular and *in-vivo* targeting and imaging. Finally, we envision the various prospects for research on fluorescent metal NCs in the future.

Contents

1. Introduction

Fluorescent metal nanoclusters (NCs) as a class of emerging fluorophores have attracted great interest from researchers because of their excellent features, such as biocompatability, photostability, subnanometer size and ease of synthesis. Metal NCs represent the missing link between metal atoms (exhibiting distinct optical properties) and nanoparticles (NPs) (exhibiting plasmons), and display molecule-like behavior. In bulk metal, the conduction band has no energy gap separating it from the valence band, so electrons do not suffer from a barrier and move freely. The scattering of the electrons is determined by the electron mean free path. In metal NPs, the size is comparable to or smaller than the electron mean free path, where the motion of electrons becomes limited by the size of the NP and interactions are expected to be mostly with the surface. This gives rise to surface-plasmon resonance effects [1]. In metal NCs, the size of metals is

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http://dx.doi.org/10.1016/j.trac.2014.02.011
0165-9936/

further reduced to around 1 nm or less, down to a few atoms, and the continuous band structure becomes discontinuous and is broken up into discrete energy levels. They are not conductive and plasmonic. Interaction with light still exists, but similar to organic dye molecules, via electronic transitions between the energy levels, resulting in light absorption and emission. This optical property gives them potential as fluorescent probes in fluorescent biosensing and bio-imaging, like quantum dots and fluorophores.

This review focuses on the synthesis of fluorescent metal NCs (Ag, Au, Cu and Pt) and their applications as fluorescent probes for metal ions, small molecules, nucleic acids and protein detection. We also include the use of metal NCs in cellular and *in-vivo* targeting and imaging.

2. Synthesis of fluorescent metal nanoclusters

Generally, metal NCs are formed by the reduction of metal ions in the presence of suitable reducing agents. However, under this condition, metal NCs are strongly prone to interact with each other and aggregate irreversibly to reduce their surface energy, resulting in large NPs. Thus, a proper stabilizing scaffold is necessary for the production of metal NCs. Furthermore, the nature of the scaffold is responsible for not only their sizes, but also their fluorescence. We therefore give a summary of the synthesis of fluorescence metal NCs (Ag, Au, Cu and Pt) based on different kinds of scaffold.

2.1. DNA oligonucleotides

As the scaffold for preparing metal NCs, DNA oligonucleotides have been widely used with Ag, Au, and Cu. In particular, the high affinity of silver ions to cytosine bases on single-stranded DNA favors DNA oligonucleotides as an excellent template for the synthesis of fluorescent AgNCs. The first DNA-templated AgNCs were reported in early 2004 by Dickson and co-workers [2]. In 2007, they further prepared water-soluble, near-infrared (NIR)-emitting DNA-encapsulated AgNCs with bright, photostable emission at the single-molecule and bulk levels. More importantly, no blinking on experimentally relevant time scales (0.1 to >1000 ms) complemented the shortcomings of quantum dots (QDs) [3]. These excellent properties of DNA-templated AgNCs greatly motivated more research on them. Various DNA sequences were selected to stabilize fluorescent AgNCs. As shown in Fig. 1, a DNA microarray was developed for high-throughput selection of DNA oligonucleotides for AgNC encapsulation. Based on this method, five optimized single-stranded DNA sequences were selected for the creation of AgNCs with tunable fluorescence emissions throughout the visible and NIR [4]. The fluorescent properties of DNA-stabilized AgNCs are influenced by not only the DNA sequence and structure, but also the environment around them. The fluorescent emissions of DNA-scaffolded AgNCs were sensitive to the sequence and the secondary structure of the DNA, and isolated, hairpin-based AgNCs exhibited fluorescence with steady dipole radiation patterns and intermittency [5].

Unlike AgNCs, AuNCs had few reports on their synthesis with DNA oligonucleotides as the scaffold, possibly coming from the weak association between the negatively-charged DNA and the commonly-used precursor $AuCl_4^-$. A 23-mer single-stranded DNA (5′-GAGGCGCTGCCYCCACCATGAGC-3′, Y = C, A, G, and T) was used to stabilize AuNCs in a weakly acidic aqueous solution, with dimethylamine borane (DMAB) as the reductant [6].

For CuNCs, they are commonly scaffolded by double-stranded DNA (dsDNA) and formed through the reduction of Cu^{2+} ions by ascorbate. CuNCs were formed in the presence of dsDNA at a low concentration of $CuSO_4$. Their sizes were proportional to the number of base pairs in the dsDNA template and they were not formed in the case of ssDNA. NC formation was highly sensitive to single nucleotide mismatches, which could be applied in biological and medical areas [7].

Recently, Wu et al. found that fluorescent CuNCs could also be synthesized with ssDNA as the scaffold. By comparing various DNA structures, such as homopolymer DNA, hairpin DNA and pristine DNA, they concluded that the thymine base in an ssDNA template played a dominant role in the formation of fluorescent CuNCs. However, the selective mechanism of CuNC growth is still unclear [8].

2.2. Peptides and proteins

Peptides and proteins as the scaffolds for preparing fluorescent metal NCs favor obtaining good, biocompatible and easily functionalized NCs. In 2007, Dickson et al. realized intracellular production of fluorescent AgNCs on the basis of a nucleus-bound silver-binding protein, nucleolin. After incubation of cells with 100 mM silver nitrate, fluorescent AgNC formation was initiated and used to stain the cells by photoactivation at ambient temperature. Also, in order to produce fluorescent AgNCs *in vitro*, these authors designed short peptides as the scaffolds incorporating the specific amino acids most prevalent in nucleolin. Fluorescent peptide-AgNCs were prepared successfully and applied for cellular imaging [9].

Compared with AgNCs, protein-stabilized AuNCs are more common, and a pioneering approach was proposed by Ying's group in 2009 [10]. They reported a one-pot route of synthesis to prepare fluorescent AuNCs with bovine serum albumin (BSA) as the template (Fig. 2). The BSA-AuNCs consisted of 25 gold atoms and gave an intense red emission (640 nm) when excited at 480 nm. The reason for BSA being an excellent scaffold for fluorescent AuNCs might come from the combination of Au-S bonding with the protein (via the 35 Cys residues in BSA), and the steric protection due to the bulkiness of the protein. Inspired by this report, researchers tried to explore other proteins for preparing AuNCs. Trypsin and lysozyme were also tried for AuNC formation [11,12].

Arakawa et al. [13] presented pH-dependent synthesis of pepsin-mediated AuNCs with fluorescent emissions (blue, green and red) from Au_5 (Au_8), Au_{13}, and Au_{25}, respectively. The pH of the reaction solution played an important role in determining the size of AuNCs. The different charges on the pepsin molecule at different pH values could affect the structural nature and the strength of interaction between the pepsin chains and the gold surface or gold ions, leading to the formation of AuNCs with different sizes at different pH values.

Peptide-templated AuNCs were reported by Jiang and co-workers [14]. In order to give a response to protein post-translational modification (PTM) enzymes, fluorescent AuNCs were prepared with the substrate peptide of the target enzymes. They chose two PTM enzymes, histone deacetylase 1 (HDAC 1) and protein kinase A (PKA), as the models. Substrate peptide 1, CCIHK (Ac), and substrate peptide 3, CCLRRASLG, were designed for HDAC 1 and PKA, respectively. These two peptide-templated AuNCs each displayed excellent fluorescence emissions and the fluorescence intensities were specifically quenched by HDAC 1 and PKA, respectively.

BSA was the scaffold for not only AuNC formation, but also synthesis of fluorescent CuNCs. Different fluorescent properties of BSA-CuNCs were obtained using a different approach. BSA-CuNCs reduced by NaOH displayed maximum excitation and emission wavelengths at 325 nm and 410 nm, which came from Cu_5 and Cu_{13} cores. The fluorescence quantum yield (QY) was calculated to be 15% using kynurenine as the reference [15]. When hydrazine hydrate ($N_2H_42H_2O$) was used as the reducing agent, BSA-CuNCs presented a red color emission (620 nm) and had a QY of 4.1% with