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DNA-templated copper nanoparticles: Versatile platform for label-free bioassays

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ABSTRACT

Label strategies are the mainstream techniques and gain great success for the sensitive quantification of biomolecules. However, they sometimes suffer from the inconveniences of time-consuming labeling, low stability of bioconjugates, and the function disorder of labeled-biomolecules. Recently, DNA-templated Cu nanoparticles (NPs) exhibit great potential for label-free fluorescent bioassays, thanks to the advantages of outstanding fluorescent properties, low toxicity, low price, good biocompatibility, and more importantly, instantaneous reaction, and facile integration with nucleic acid-based signal amplification and target-recognition strategies. In this review, we aim to discuss major developments and applications of DNA-CuNPs-based label-free assays for biomolecules. The DNA structures were manipulated for amplified sensing, multimode sensing, and versatile applications including cancer cell analysis, polyacrylamide gel electrophoresis, logic circuit, genotyping etc. Emerging analytical strategies were also discussed in detail. We address the advantages, applicable situations, and limitations of DNA-CuNPs-based label-free strategies and propose suggestions for future developments.

1. Introduction

A sensitive technique for biomolecules (nucleic acids, enzymes, proteins, etc.) analysis possesses an important role in the elucidation of molecular mechanism and early diagnosis for many human diseases, since even a few biomolecules are often sufficient to initiate pathophysiological processes and affect the biological functions of cells [1,2]. Label strategies are the state of art techniques for the sensitive quantification of biomolecules, which have gained great success in vast biological and clinical studies. The target biomolecules or the bio-recognition molecules are tagged with an optical, electric, electrochemical, radio isotope, or metal stable isotope tag [3,4]. The change of the signal intensities of the tag, indicates the binding between the capture and the target biomolecules. In spite of the great success of label strategies, they often suffer from the limitations associated to: (1) the need of sophisticated and labor- and time-consuming label processes; (2) the low reproducibility and low stability of labeled bioconjugates; and (3) the interference on the function of the biomolecules involved after label processes [5,6].

Lacking of detectable moieties, the sensitive and label-free quantification of biomolecules is often hard to realize. Recently, DNA-templated metal nanoparticles (NPs) exhibit great potential for label-free fluorescent bioassays, thanks to the advantages of outstanding fluorescent properties, ultrafine size, good biocompatibility, low toxicity, and more importantly, facile integration with nucleic acid-based signal amplification and target-recognition strategies [7,8]. Among DNA-template metal NPs, copper NPs (CuNPs), including double stranded DNA-templated CuNPs (dsDNA-CuNPs) pioneered by Mokhir et al. [9] and poly(thymine)-templated CuNPs (polyT-CuNPs) pioneered by Wang et al. [10] and Shao et al. [11], are becoming excellent functional biochemical probes for label-free bioassay. Their formation is derived from the clustering of Cu(0), which is produced from chemical reduction of Cu(II) on DNA backbones. Because of the quantum-confinement effect, CuNPs are excitable by a λ_{ex} at 340 nm and emit a fluorescence with max λ_{em} at around 600 nm. As shown in Table 1, the *in-situ* production of fluorescent DNA-templated CuNPs is efficient and finished in several minutes under ambient conditions, which is more convenient and significantly faster than the procedure of other fluorescent metal NPs, such as AgNPs [12], AuNPs [13], and

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Table 1
The comparison of different DNA-templated fluorescent metal nanoparticles.

Metal NPs	Sequence-dependence	Reaction time	Stokes shift	Quantum yield	Raw material price/$ kg^{-1}	Toxicity	Invention year	Ref.
Cu NPs	dsDNA	10 min	~260 nm	11.2%	7 (Cu)	Low	2010	[9,15,16]
	PolyT ssDNA	5 min	~275 nm	11.2%			2013	[10,17]
Ag NPs	Cytosine-rich DNA	12 h	~100 nm	17%	600 (Ag)	Medium	2004	[18,19]
Au NPs	–	46 h	~258 nm	2.2%	43000 (Au)	Low	2012	[20,21]
Au/Ag NPs	Cytosine-rich DNA	5 h	~110 nm	4.5%	600 (Ag)-43000 (Au)	Medium	2011	[22]
PbS NPs	–	82 h	~89 nm	–	3.1 (Pb)	High	2013	[23]
CdS NPs	–	1.5 h	~205 nm	–	3.1 (Cd)	High	1992	[24]

PtNPs [14] (several hours or several days are usually required). Furthermore, the large Stokes shift is favorable for the relieving of background interference from complicate biological matrixes. The facile and fast formation of CuNPs endows a substantial basis for their wide applications in versatile nucleic acid-related label-free bioassays.

Despite intriguing properties and some excellent relevant reviews [5–7,25–27], to the best of our knowledge, there has been no comprehensive review focusing on this emerging technique to date. Therefore, this review focuses on major applications and developments of DNA-CuNPs-based label-free bioassays, with emphasis on methodological innovations. Fig. 1 highlights important developments over the past few years in the field. In 2010, Mokhir et al. [9] presented the first report of dsDNA-CuNPs, while Wang et al. [10] and Shao et al. [11] pioneered the polyT-CuNPs in 2013 independently. Subsequently, the DNA-CuNPs of high fluorescence were successfully applied in the sensitive and label-free detection of nucleic acids, enzymes, proteins, small biomolecules, and metal ions. The DNA structures were also intricately manipulated for amplified sensing, multimode sensing, and versatile applications including cancer cell analysis, polyacrylamide gel electrophoresis, logic circuit, genotyping etc. Emerging analytical strategies, such as electrochemical assay, surface plasmon resonance (SPR) assay, and inductively coupled plasma mass spectrometry (ICPMS) assay, were also discussed in detail. The advantages, limitations, and personal suggestions for future developments are addressed.

2. Mechanism of CuNPs formation on DNA templates

DNAs are polynucleotides composed of a monophosphorylated deoxyribose sugar linked to nitrogenated nucleobases: the purines of guanine (G) and adenine (A), and the pyrimidines of thymine (T) and cytosine (C). N^4 different DNA sequences could be made from N nucleotides, providing a 2.86 bits per nm DNA information storage capability [42]. Nucleobases inside DNA connect with each other through base-pairing of T to A and C to G and p-stacking. Base-pairing produces hairpin and loop configurations of single-stranded DNA (ssDNA), or can hybridize with each other to form dsDNA helical structures. The base-pairing feature makes DNA an ideal building block for the formation of highly ordered nanostructures. Complicated 2D objects, 3D objects, and surface patterns, are realized by DNA self-assembly [42–44]. Oligonucleotides of specified sequences of less than 150 bases are easily obtained by automated equipment. Moreover, chemical moieties and modified nucleotides could be facilely added to a given sequence. DNA sequences can be exponentially amplified using commercially available enzymes, primer oligonucleotides, and nucleotide triphosphates. DNA can be also manipulated by enzymes to read, cut, repair, mark, twist, and unwind specific DNA sequences.

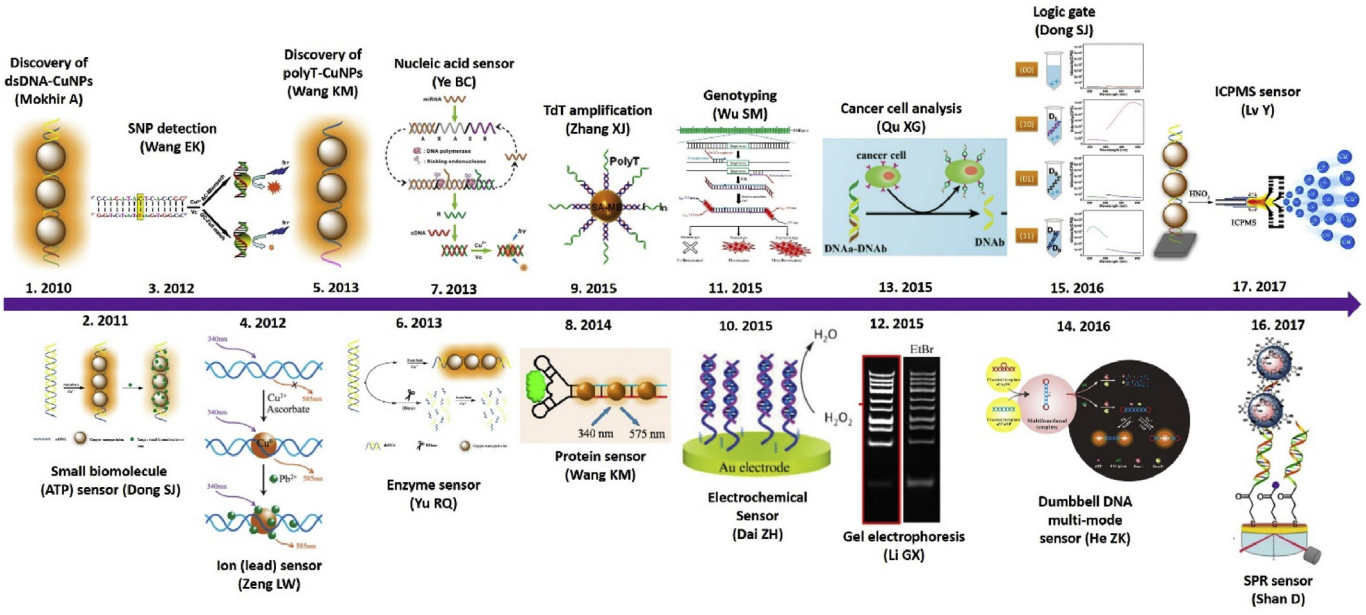

Fig. 1. Historical timeline of label-free analytical strategies using DNA-templated CuNPs. Parts 1 and 5 reproduced with permission from Ref. [9] and ref. [10]. Copyright John Wiley and Sons. Parts 2, 3, 11, 14, and 17 reproduced with permission from Ref. [28], Ref. [29], Ref. [30], Ref. [31], and Ref. [32]. Copyright American Chemical Society. Parts 4, 7, 9, 10, 13, and 15 reproduced with permission from Ref. [33], Ref. [34], Ref. [35], Ref. [36], Ref. [37], and Ref. [38]. Copyright Royal Society of Chemistry. Parts 6, 8, and 16 reproduced with permission from Ref. [39], Ref. [40], and Ref. [41]. Copyright Elsevier B.V. Part 12 reproduced with permission from Ref. [16]. Copyright Springer-Verlag.